A COMMERCIALLY VIABLE CONTROLLED ENVIRONMENT AGRICULTURE (CEA) SPINACH PRODUCTION SYSTEM

Final Report

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Project manager:
Ed Kear

Prepared by

CORNELL UNIVERSITY
DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL ENGINEERING
108 RILEY-ROBB HALL
ITHACA, NY 14853-5701

Project Director: Louis D. Albright, Professor

Project Associates: David S. de Villiers, Robert W. Langhans, Leslie Katzman, Corey Johnson, Manzo Uchigasalci

New York State Electric and Gas Corporation
John R. Zabliski, Senior Engineer, Business Customer Service

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ABSTRACT

Hydroponic spinach is not produced commercially in the United States today. Consumer demand is high for clean, fresh spinach of consistent quality, and the greenhouse product (particularly when locally grown) has definite advantages over the field-grown product in terms of quality, shelf life, consistency, cleanliness and potential value-added features. Two primary problems have prevented commercial hydroponic spinach production in this country: uneven and inconsistent seed germination, and frequent early onset of the root rot disease, *Pythium aphanidermatum*. This project report details two methods to assure consistent seed germination, one for dehulled seeds and one for intact seeds. Each method is based on careful control of the water content and temperature of the germination medium. The primary focus of the work was on baby leaf spinach. A floating hydroponic system was developed wherein plant density, nutrient solution quantity, photoperiod, light integral and temperatures (root and aerial) were optimized. A method to re-grow leaves following a first harvest was shown to increase overall productivity. Root disease was controlled through a very stringent protocol of cleaning between crops. Costs of commercial production were estimated by analogy to the commercial-scale lettuce production facility that has been operated by Cornell for the past several years.

KEYWORDS

Hydroponic, spinach, germination, disease, *Pythium*, greenhouse

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SUMMARY

The project of developing a commercially viable CEA spinach production system was divided into 6 tasks, which are the subjects of the 6 chapters of this report. The first two tasks were considered the most difficult: to develop reliable methods for seed germination and seedling production, and to solve the disease problem that has obstructed commercial greenhouse production in the past. The remaining tasks were to develop and optimize the biomass production system, to evaluate cultivars, to examine market potential for different categories of hydroponically grown spinach, and to analyze cost of production.

Research into the disease problem led to a protocol for continuous production of large-leaf spinach in a deep-flow pond system. The system requires disease free seedlings for transplant and respacing 9 days after seeding, cooling of the pond nutrient solution to 20°C to limit rate of growth of pathogens, and treatment of the solution to keep the concentration of pathogens to an acceptable level. This protocol has been successful on a bench-scale production level but is not yet tested on a commercial scale. Results suggest a protocol similar to that for large-leaf spinach will prove feasible for disease control in production of baby-leaf spinach.

Research into seed germination and seedling production led to two new methods to produce seedlings rapidly, synchronously, and with high percentage germination using either intact seed or de-hulled seed. For de-hulled seed, the recommended method is to plant seed into a soil-like medium with precisely controlled moisture content; after 48 hours incubation at 25°C, flats are ready for flotation. This method also works well with good quality intact seed. For problematical intact seed, the seed is imbibed and then partially dried under controlled conditions before planting.

The biomass production research of this project focused on methods to produce baby-leaf spinach. Factors that were examined were: the effects of timing of harvest, plant density, photoperiod, and temperature (of both the root and shoot zone) on plant morphology, and productivity and quality of the commercial portion of the harvest. Recommendations based on the results are: a crop cycle of 14 to 16 days with a daily light integral of 17 moles m^{-2} (dependent on cultivar and desired leaf size), a plant density of 1500 plants m^{-2}, root zone and aerial temperatures between 20°C and 25°C (dependent on cultivar), and that the photoperiod be long enough to trigger bolting, which increases productivity. We investigated repeated harvesting of the same spinach stand and concluded significant benefits could be derived from a second cutting. Because of the very high plant density required in baby-leaf production and the large area harvested daily, flat filling, seeding, and harvesting need to be mechanized. For logistical and safety reasons we recommend a pond system be used, but to facilitate continuous treatment of the solution we recommend the volume of nutrient solution be kept as small as is feasible.

Numerous commercial cultivars were examined and informally compared through the course of the research. In critical experiments in the seedling and crop production phase, two successful cultivars of contrasting growth habit (Alrite and Eagle) were compared in order to cover the range of plant response likely to be encountered in the spinach crop.

The market potential of hydroponically grown spinach was evaluated in consultations with an industry expert. Demand for spinach is high and the greenhouse product has undeniable advantages over the field product in terms of quality, shelf life, potential value-added features, and year-round reliability of supply, but it became apparent that a different marketing system is needed for the greenhouse product from that used for the field crop because of differences in the methods of production. The CEA product is produced locally and packaged on site, and may be wholesaled directly to institutional customers and market chains. The appropriate price comparison between CEA and field products is after the field product has undergone long-range transport, washing and packaging. In this comparison, the CEA product compares favorably.

Cost of production for spinach was based on detailed analyses of cost of production for lettuce in a commercial-scale greenhouse we have operated for five years, and on a doctoral dissertation projecting

costs of lettuce production in different locations with different climates and cost structures (particularly in terms of electricity and property taxes). Under the same daily light integral used for lettuce (17 moles m^{-2}), spinach achieves the same light use efficiency and whole plant productivity as lettuce. The main differences between lettuce and spinach production are when and where labor is required and in the degree of automation necessary. For the baby spinach crop, much more daily seeding is required than for lettuce, but there are no transplant and respacing steps. The methods of harvest are radically different. If the full possibilities of automation are incorporated into spinach production we estimate the cost of production will be only a little less than for lettuce in terms of whole plant biomass. Because the commercially useful part of the spinach plant constitutes only 50% of the whole plant, it means the cost of the saleable part must be nearly twice that of lettuce. However, the retail price of baby spinach is approximately three times the retail price of lettuce when sold by the pound ($ 6 versus $2). We estimate the cost of production of baby spinach at a favorably located production facility, packaged and ready for wholesale distribution, is less than $3, which is half the current retail price.

ABBREVIATIONS AND ACRONYMS

ATC	air temperature condition (experimental treatment)
CC	commercial cut
CEA	controlled environment agriculture
DO	dissolved oxygen
DW	dry weight
DW/FW	ratio of dry weight to fresh weight
EC	electrical conductivity
FW	fresh weight
HACCP	Hazard Analysis Critical Control Point
MC	moisture content – usually ratio of water to dry matter in the medium
PA	*Pythium aphanidermatum*
PAR	photosynthetically active radiation
PD	plant density as plants per square meter
PU	"pop-up" or seedling in which the root did not penetrate the medium
Rep	replicate
RO	reverse osmosis
SU	sample unit
T1, T2, T3	1^{st} true leaves, 2^{nd} true leaves, 3^{rd} true leaves
T50	time elapsed from seeding to 50% emergence
T90	time elapsed from seeding to 90% emergence
Zos	zoospore
Zoss	zoospores

CHAPTER 1. THE ROOT DISEASE PROBLEM

Task 1: Develop means to limit and prevent the root disease problem in hydroponically grown spinach.

INTRODUCTION

Most leafy salad crops under consideration for hydroponic production present no problem with root disease. Spinach is an exception, being particularly vulnerable to the ubiquitous water-mold *Pythium aphanidermatum* (PA). This is also known to cause damping off of seedlings of many species in wet soil. PA was positively identified as the main pathogen attacking spinach roots in hydroponic systems at Cornell.

PA flourishes in the presence of spinach roots in hydroponic systems; it finds the roots by means of mobile single-cell propagules called zoospores. Once these have landed upon the roots, hyphae penetrate the root interior leading to destruction of root function and wilting and death of the above ground part of the plant. At the same time as the attack on the roots is continued internally, the colony periodically releases additional zoospores into the nutrient solution that spread infection further. If the first wave of attack does not destroy the root system, successive waves likely will. Under certain circumstances, PA invasion can result in extremely rapid plant-kill. In one experiment in our program, healthy 12-day old seedlings of several cultivars of spinach were placed into a deep flow hydroponics pond from which an infected crop had recently been harvested. After only 5 days, every single transplant had collapsed, never to recover.

Within this project, basic research was performed to determine the effects of timing of infection, pathogen concentration in the nutrient solution, and temperature of the nutrient solution on spinach crop productivity. As a result, a protocol for continuous production of large-leaf spinach in a pond growing system was determined. The feasibility of continuous production of baby-leaf spinach in pond systems is less certain, because baby plants are more vulnerable to attack on their root systems. Further research is in progress to determine the cost-effectiveness of various treatment systems and growing systems for disease control in the baby spinach crop.

CHARACTER OF THE DISEASE ORGANISM

PA is a fungus-like organism in a group known as water molds. Taxonomy is unsettled at the kingdom level. It is considered to be a Protista in the five kingdom scheme, but the Protista themselves are sometimes divided into several kingdoms. The phylum is Oomycota, the class Oomycetes, order Peronosperales and the family is Pythiaceae. The genus Pythium is closely related to the genus Phytopthera which is responsible for potato blight.

When food sources are plentiful, the primary means of dispersal of PA is through the zoospore (zos) whose sole function appears to be spreading the species to new hosts. Zoospores (zoss) are asexually produced propagules equipped with flagella (2 each) that permit self-propulsion in ponds and soil solutions. Spinach evidently emits a chemical that is attractive to zoss, which are able to use their flagella to follow a vector of this chemical to its source.

Zoss are short-lived organisms with small energy reserves; if they do not find a suitable food source, after less than an hour they lose their flagella and encyst. However, they can survive in a liquid medium for several days encysted (or dry conditions for a few hours); in encysted form they may still be passively transported in circulating nutrient solution, in splashed water, on hands, or in dirt and soil in pots. Should they encounter a favorable host, they will extrude hyphae and commence colonization.

Zoss are produced in vast numbers from the basic vegetative stage of PA, mycelium, which supports development of sporangia and oospores, both of which produce vesicles that contain numerous zoss. Oospores are primarily involved in sexual reproduction and dormancy.

PA can also be spread by vegetative structures. Under the right humidity and temperature conditions, mycelia threads grow outwards in all directions, forming mats on surfaces and three-dimensional lattice structures in the air by which the pathogen can spread laterally and vertically to new plants or plant parts. In circulating nutrient solution, fragments of mycelia and broken-off infected plant material are other means of dispersal.

Pythium also has a deeply dormant survival mode, the encysted oospore, which can endure for long periods of time (many months) in very dry conditions, and even longer in moist soil; presumably, long distance transport of these propagules is possible in high-wind conditions.

BASIC RESEARCH

The problem of root disease in spinach caused by PA was addressed directly in the doctoral work of Leslie Katzman (supported in part by NYSERDA under this grant), and is reported in her doctoral dissertation (Katzman, 2003). Key findings are presented below. Katzman analyzed the microorganism content of a hydroponic pond nutrient solution after it had developed and demonstrated lethality for spinach, and isolated virulent strains of PA and several other organisms from the mix of microorganisms in the nutrient solution. She developed techniques for reliably producing a supply of virulent strains of PA zoss in known quantities to be used in subsequent research. The above account of the pathogen was abstracted from her dissertation.

Effect of Time of Inoculation With Pythium

Katzman first examined the effect of time of introduction of PA zoss in the spinach growth cycle on subsequent plant growth. Tubs in which plants were grown hydroponically were inoculated with zoss to achieve a concentration of 2500 zoss ml^{-1}, on day 1, day 9, day 14, or day 21 of an experiment in which the plants were harvested on day 28. The results in terms of dry biomass of shoots produced are shown below.

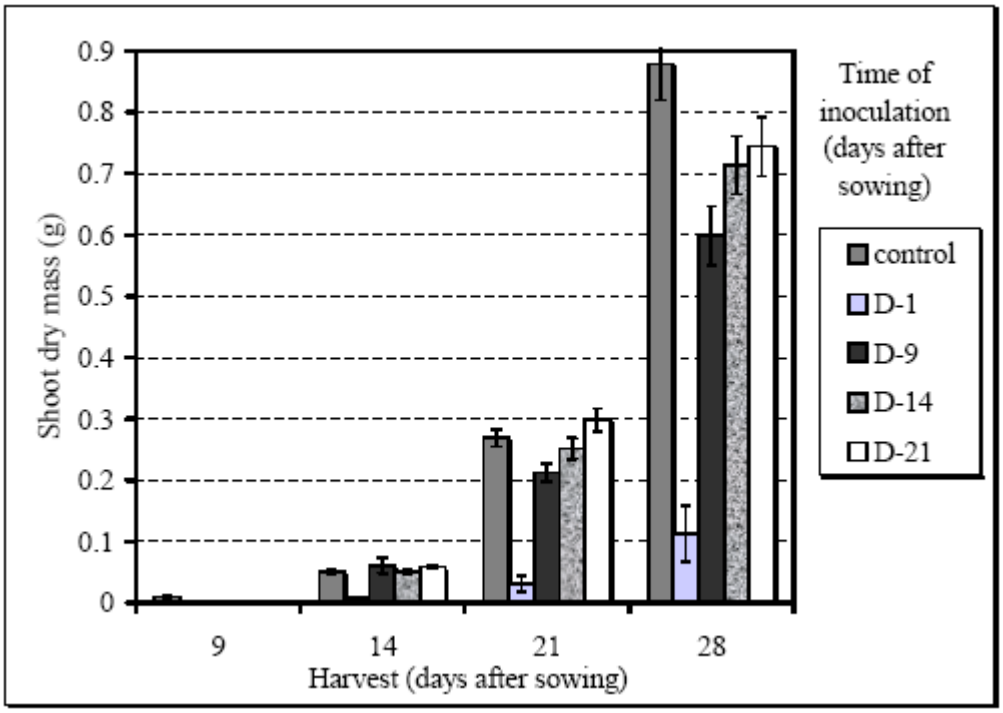

Figure 1-1: Influence of time of inoculation on shoot dry mass of spinach. Means and standard errors of shoot dry mass of plants inoculated with *P. aphanidermatum* zoospores on different days (1, 9, 14, or 21) after sowing and harvested 9, 14, 21, and 28 days after sowing.

A trend is apparent such that the earlier plants were exposed to the pathogen, the larger the effect of the pathogen in terms of reduction in final shoot biomass. It can be seen plants exposed to PA from the start (D-1) did extremely poorly, achieving just 13% the weight of control plants. In fact, 68% of the plants died in this treatment. Plants inoculated on day 9 managed to achieve 68% the weight of controls; those inoculated on days 14 and 21 achieved 81% and 85% respectively. Root and shoot conditions were rated and the findings corresponded to the findings for weight.

The amount of inoculum used on each inoculation date was the same. If the amount of root tissue invaded were the same on each occasion, proportionately less of the root would have been colonized in successively later inoculations because the root system was progressively larger. One suspects that in the case of later

inoculations, not only was there less time for damage to occur before harvest, but that less critical damage occurred because it was spread out over a larger root system.

Populations of zoss free in the nutrient solution were measured on days 1, 9, 14, 21 and 28 in all conditions using a filtration and plating technique. The pattern was for the highest concentration to occur just after inoculation (as one would expect), and for the concentration then to fall to very low levels a week later.

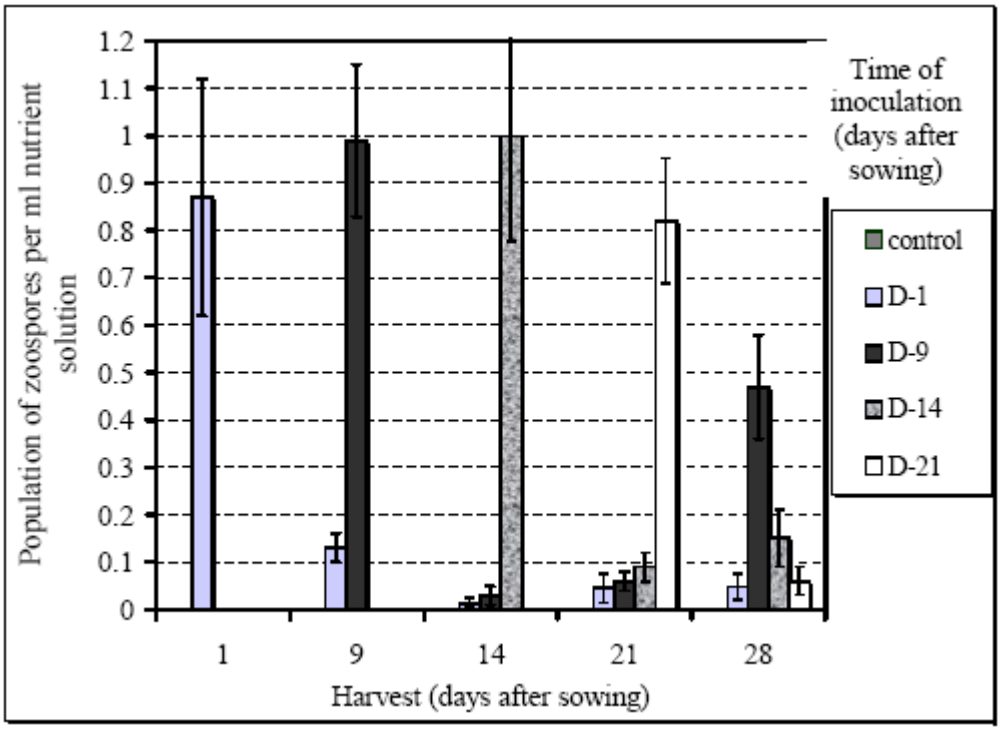

Figure 1-2: Influence of time of inoculation on populations of zoospores in nutrient solution. Means and standard errors of zoospores per ml detected in nutrient solution for harvests at 1, 9, 14, 21, and 28 days after sowing. Treatments varied the timing of inoculation of spinach plants with *P. aphanidermatum*: days 1, 9 ,14, and 21 after sowing.

It may be significant that in the day-9-inoculation condition, the zos population in the solution eventually recovered strongly, after a period of 19 days (day 28, the end of the experiment). This rebound in population was the result of a concerted release of zoss produced in or on the infected roots, and likely would have resulted in a secondary round of infection and damage had the crop been allowed to continue growing. In the day-14-inoculation condition, the free zos population also appeared to be rising after 14 days; however, at this point the experiment was terminated, so the full extent of recovery was not determined.

The manner in which infection spreads after initial inoculation and the reproductive cycle in infected roots is of great interest. It is possible zos production and release shows endogenous periodicity, or is triggered

by host-plant developmental stage or food resource scarcity. If this is the case, knowledge of it could be used to advantage in disease control.

Effect of Introduction of Biosurfactants

In this phase of the study, Katzman tested the effect of adding biosurfactants to the nutrient solution on PA suppression. It may be said that although biosurfactants were effective in killing PA, they also stunted the spinach; consequently, this line of investigation was dropped.

Effect of Concentration of Infecting Agent

Katzman investigated the effect of different concentrations of PA on shoot growth of spinach when introduced into the nutrient solution at time of transplant (day 9). The concentration series ranged from 2.5 ml^{-1} to 25,000 ml^{-1} by factors of ten (2.5, 25, 250, 2500, 25000). An uninfected control was also included.

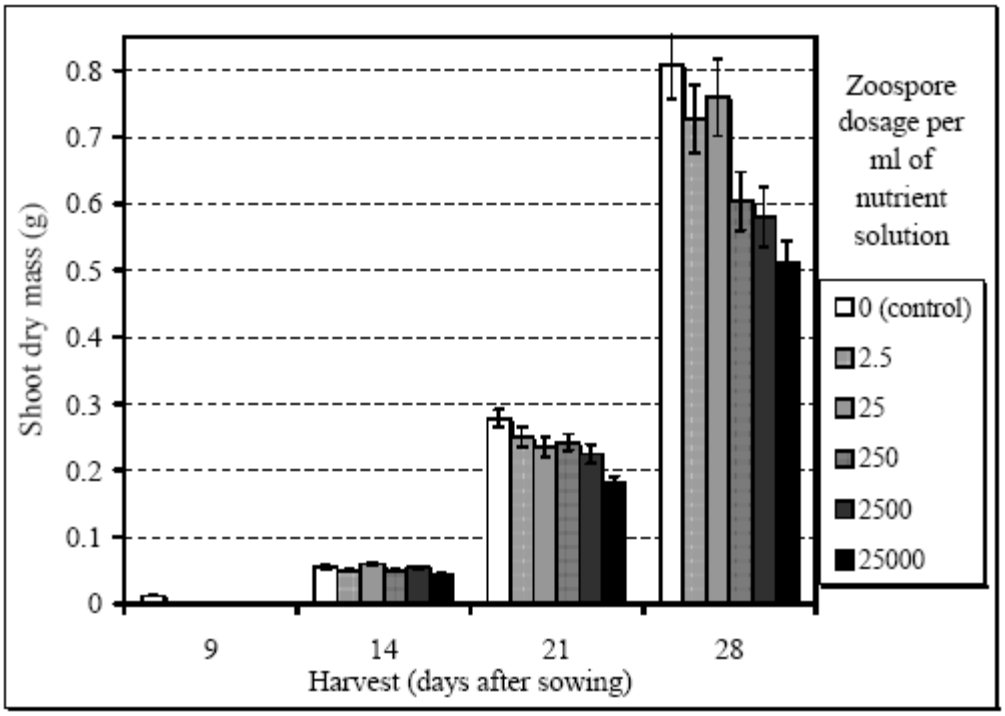

Figure 1-3: Effects of P. *aphanidermatum* zoospore dosage (2.5 to 25,000 in 10-fold increments) on mean shoot dry mass and standard error of spinach harvested 9, 14, 21, and 28 days after sowing.

A clear trend can be seen for increased doses of PA to have had progressively greater detrimental effects on shoot biomass, an effect that became more apparent as the plants aged, indicating it is worthwhile reducing pathogen concentration to a low level. Concentrations up to 25 ml^{-1} had only rather small and statistically insignificant effects on biomass, even after exposure for 19 days. Concentrations more than 250 ml^{-1} had large, significant effects, with weight deficits of more than 25%.

Populations of zoss free in the nutrient solution were measured in all conditions on days 1, 9, 14, 21 and 28 with results as shown below.

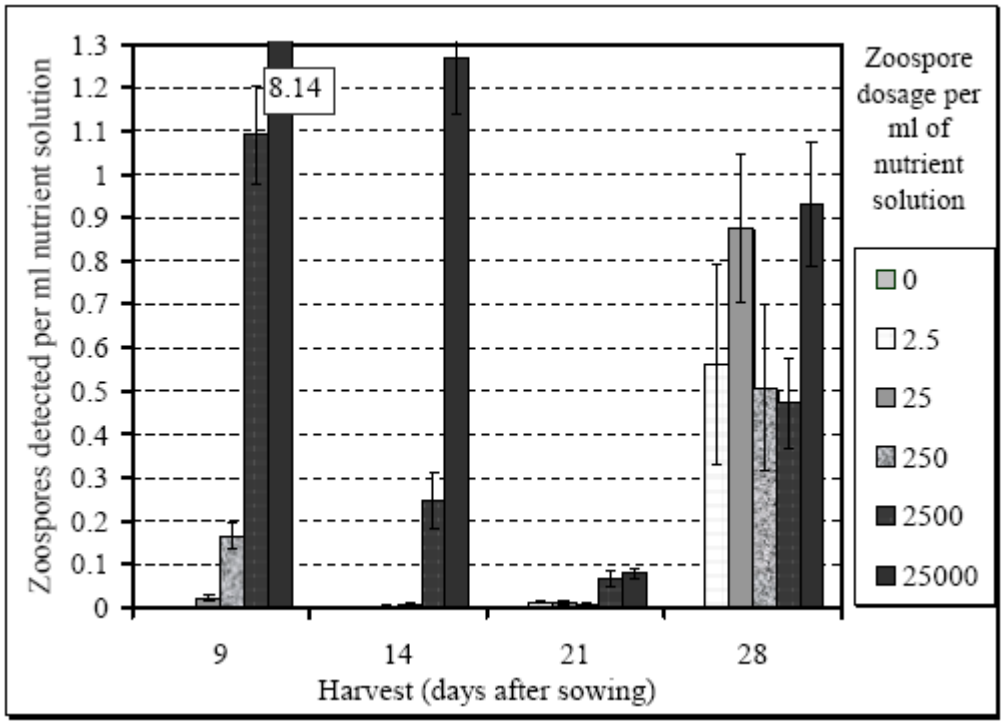

Figure 1-4: Effects of *P. aphanidermatum* zoospore dosage (2.5 to 25,000 in 10-fold increments) on mean zoospore population per ml of nutrient solution for harvest at 14, 21, and 28 days after sowing.

The concentrations of zoss recovered from the nutrient solution just after inoculation was highly correlated with the target inoculation concentration, as expected, after which it fell over time. By day 21, zos concentration was very low in all conditions. Interestingly, the concentration built up again, reaching relatively high levels in all conditions by day 28, and thus considerably exceeding the initial concentration in the 2.5 and 25 ml^{-1} conditions. The pattern for the 2500 ml^{-1} condition was nearly identical to that in the previously described experiment for the day-9 inoculation, as it should have been since it was in effect a replication.

These results suggest several things: First, in the higher concentrations it took two weeks for the zos population to drop to very low levels, suggesting some of the original zoss remained viable in the nutrient solution for this duration, though probably incapable of independent mobility. It suggests zoss may not always be quite as short-lived as generally thought. Second, there appears to have been only a very low level of release of zoss, if any, over the two weeks following inoculation; this is clearly true for conditions with inoculation rates of 2.5, 25, and 250 ml^{-1}, and most likely true for high concentration conditions as

well. Third, production and release of new zoss appears to have occurred at the same time after inoculation, regardless of initial inoculation concentration. Under the conditions of this experiment, the zos reproductive cycle appeared to follow a time schedule. One may speculate it took this long for critical conditions to be reached in the PA colonies in the plants. If zos landings were spatially dispersed in the initial infection, most invasions would expand from point sources and be similar with respect to crowding and food resource availability under different inoculation rates. The uniformity of timing of secondary zoss release may alternatively be synchronized with plant developmental stage, as Katzman suggested.

There were no statistically significant differences in concentrations of free zoss on day 28 among the inoculation conditions. They were all significantly different from the control and from the previous week's level. More information is needed to interpret why this was the case, when one would expect more zos production from the more heavily colonized plants.

Effect of Root Zone Temperature

Katzman's final series of experiments focused on the effect of root zone temperature on disease spread in the roots, and effects of root disease and temperature on shoot growth and quality. Healthy seedlings were transplanted on day 9 into either clean nutrient solution, or solution inoculated with zoss to a concentration

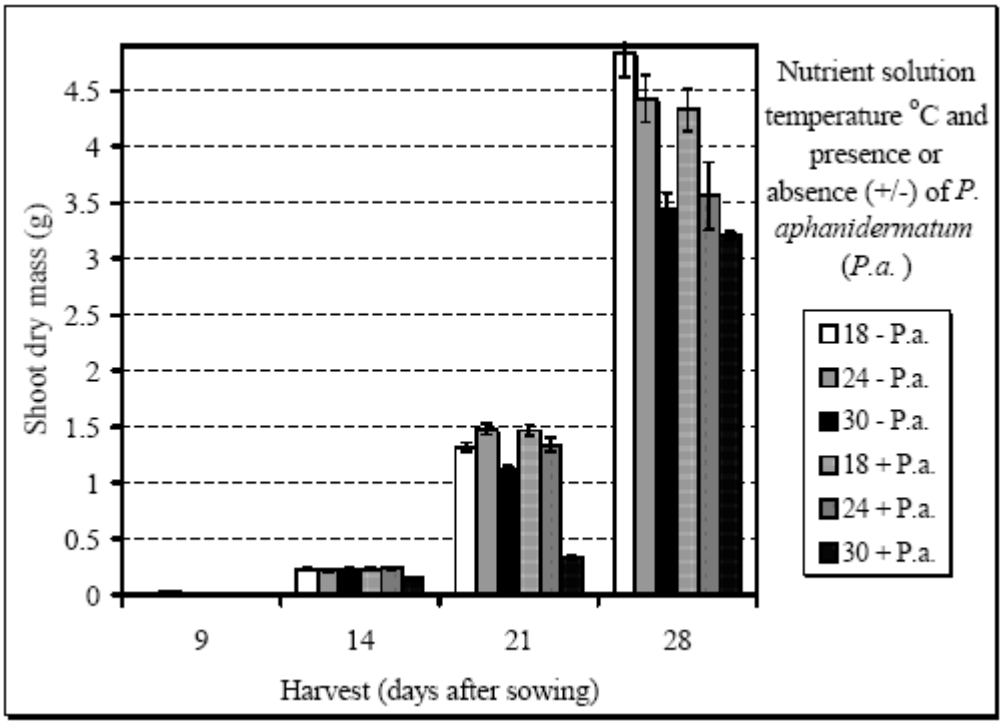

Figure 1-5: Influence of nutrient solution temperature and inoculation with *P. aphanidermatum* on shoot dry mass. Means and standard errors of dry mass (g per plant) for harvests at 9, 14, 21, and 28 days after sowing. Treatments varied the nutrient solution temperatures (18, 24, and 30°C) and presence or absence (+/-) of *P. aphanidermatum (P.a.)*.

of 2500 ml^{-1}. Solution temperatures examined were 18, 24, or 30°C. Harvests were made on days 9, 14, 21, and 28. Findings with respect to dry weight of shoots are shown in the Figure 1-5 below.

The trends seem to indicate a fall-off in biomass with each temperature increase, and a fall-off in biomass with PA inoculation, at least in the day 28 harvest. However, due to high variability in plant size, few of the possible comparisons among shoot weights in the 18 and 24°C temperature conditions were actually statistically significant. Infected and non-infected plants were not different at either temperature, and there was no statistical difference between plants grown at 18 and 24°C in terms of biomass. Plants grown at 30°C, on the other hand showed significant differences between diseased and non-diseased conditions, and in comparison to plants grown at both lower temperatures. The full results of the statistical analysis are shown in Table 1-1 below.

Comparison	Harvest day (days after sowing)		
Temperature °C +/- *P.a.*	Harvest 14	Harvest 21	Harvest 28
18 –*P.a.* vs. 24 –*P.a.*	NS	NS	NS
24 –*P.a.* vs. 30 –*P.a.*	NS	**	**
18 –*P.a.* vs. 30 –*P.a.*	NS	*	**
18 –*P.a.* vs. 18 + *P.a.*	NS	NS	NS
24 –*P.a.* vs. 24 + *P.a.*	NS	NS	*
30 –*P.a.* vs. 30 + *P.a.*	**	**	**
18 + *P.a.* vs. 24 + *P.a.*	NS	NS	NS
24 + *P.a.* vs. 30 + *P.a.*	**	**	**
18 + *P.a.* vs. 30 + *P.a.*	**	**	**
18 –*P.a.* vs. 24 + *P.a.*	NS	NS	**
18 –*P.a.* vs. 30 + *P.a.*	**	**	**
24 –*P.a.* vs. 18 + *P.a.*	NS	NS	NS
24 –*P.a.* vs. 30 + *P.a.*	**	**	**
30 –*P.a.* vs. 18 + *P.a.*	NS	**	*
30 –*P.a.* vs. 24 + *P.a*	NS	**	NS

* The mean difference is significant at the 0.05 level.
** The mean difference is significant at the 0.01 level.

Table 1-1: Tukey's HSD comparisons of mean dry shoot mass (g per plant) between plants grown in different nutrient solution temperatures (18, 24, and 30°C) in the presence or absence (+/-) of *P. aphanidermatum (P.a.)*.

While there were no significant differences in shoot dry weights of plants grown in inoculated solution at 18 and 24°C on either day 21 or day 28, there were striking differences in appearance and quality of the leaves and in root disease symptoms in these conditions. These effects showed as early as day 21. Symptoms of deterioration in leaf quality resulting from presence of PA in the nutrient solution are shown for harvests on days 14, 21, and 28 in figures 1-6, 1-7 and 1-8 respectively. See below.

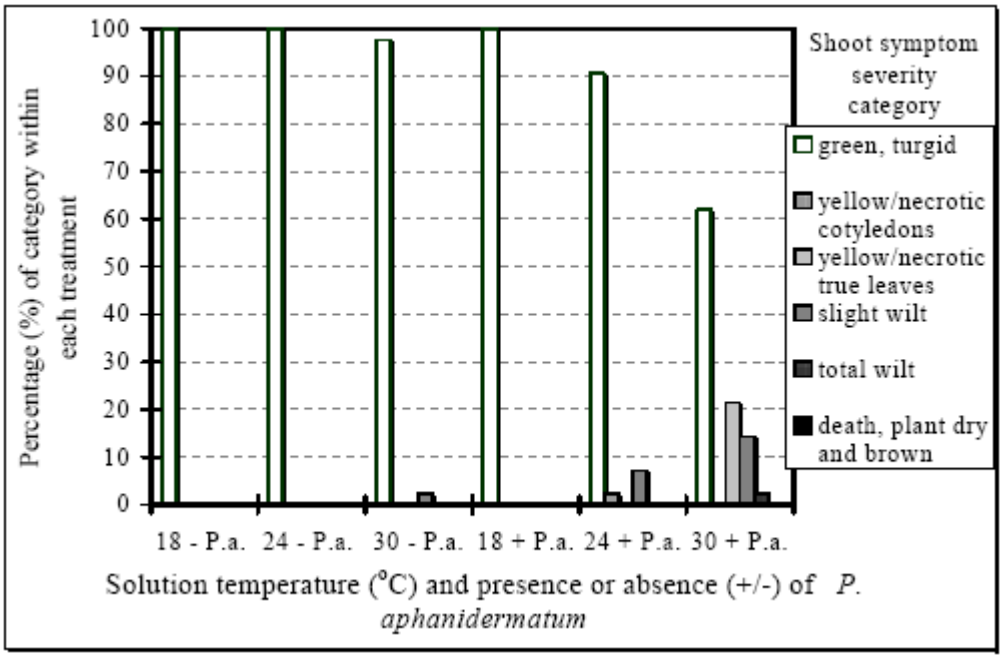

Figure 1-6: Influence of nutrient solution temperature (18, 24, and 30°C) and the presence or absence (+/-) of *P. aphanidermatum (P.a.)* on severity of spinach shoot symptoms for harvest day 14.

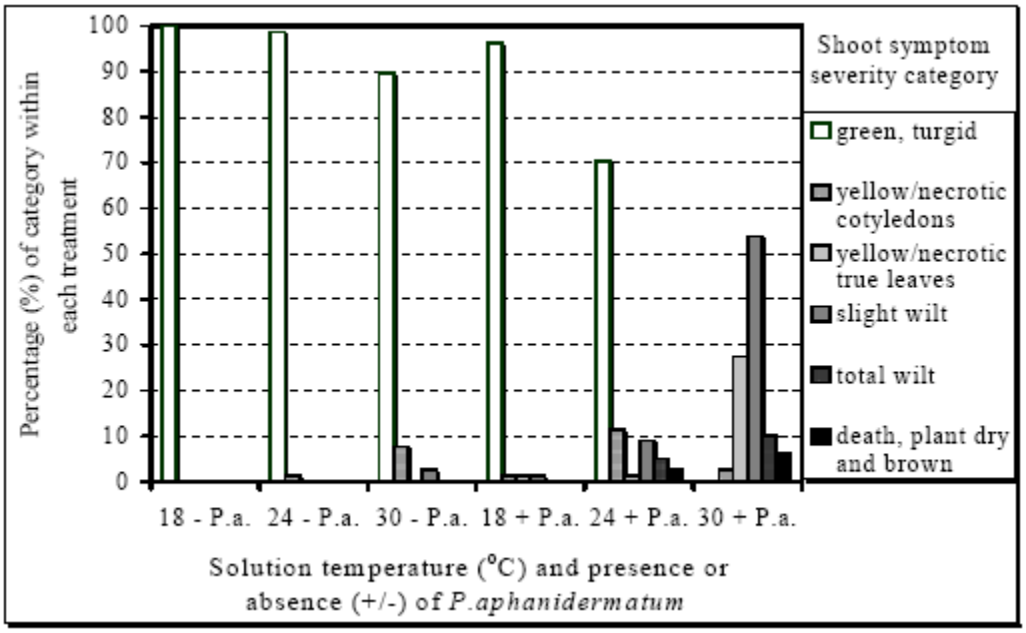

Figure 1-7: Influence of nutrient solution temperature (18, 24, and 30°C) and the presence or absence (+/-) of *P. aphanidermatum (P.a.)* on severity of spinach shoot symptoms for harvest day 21.

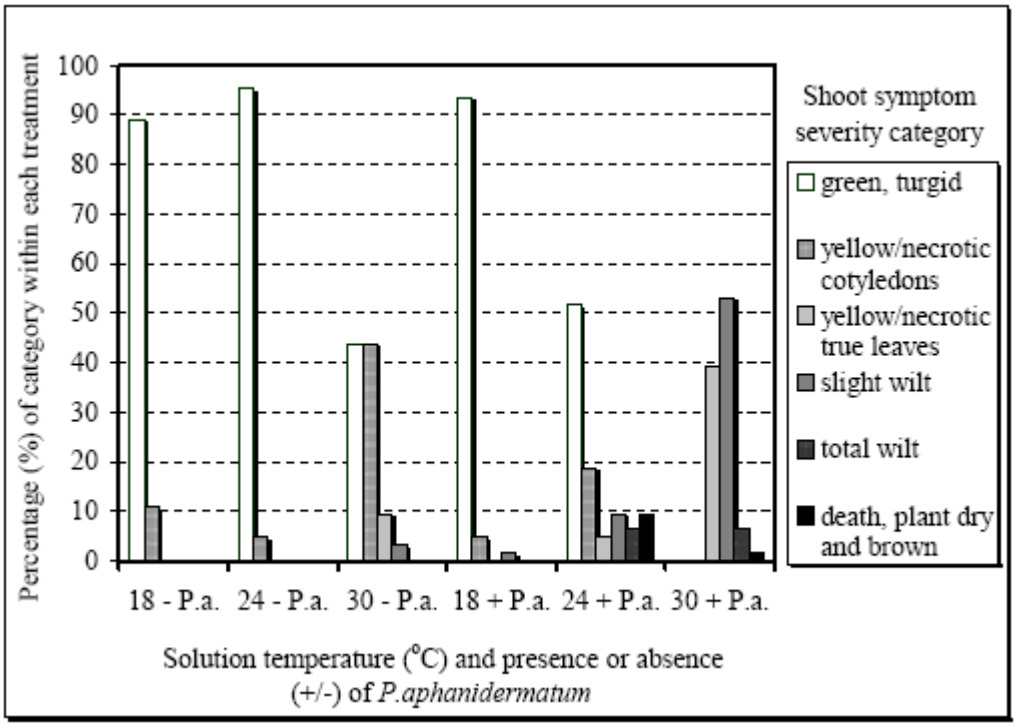

Figure 1-8: Influence of nutrient solution temperature (18, 24, and 30°C) and the presence or absence (+/-) of *P. aphanidermatum (P.a.)* on severity of spinach shoot symptoms for harvest day 28.

By day 21, 30% of leaves in the 24°C inoculated (+ P.a.) condition were defective in some way, compared to only 3% in the 18°C + P.a. condition, and by day 28, 50% were defective in the 24°C + P.a. condition compared to only 6% in the18°C + P.a. condition.

Effects of PA infection were found in the roots in more advanced and exaggerated form than were manifested in leaf appearance, as reflected in Figures 1-9, 1-10, and 1-11, for harvests on days 14, 21, and 28 respectively. See below.

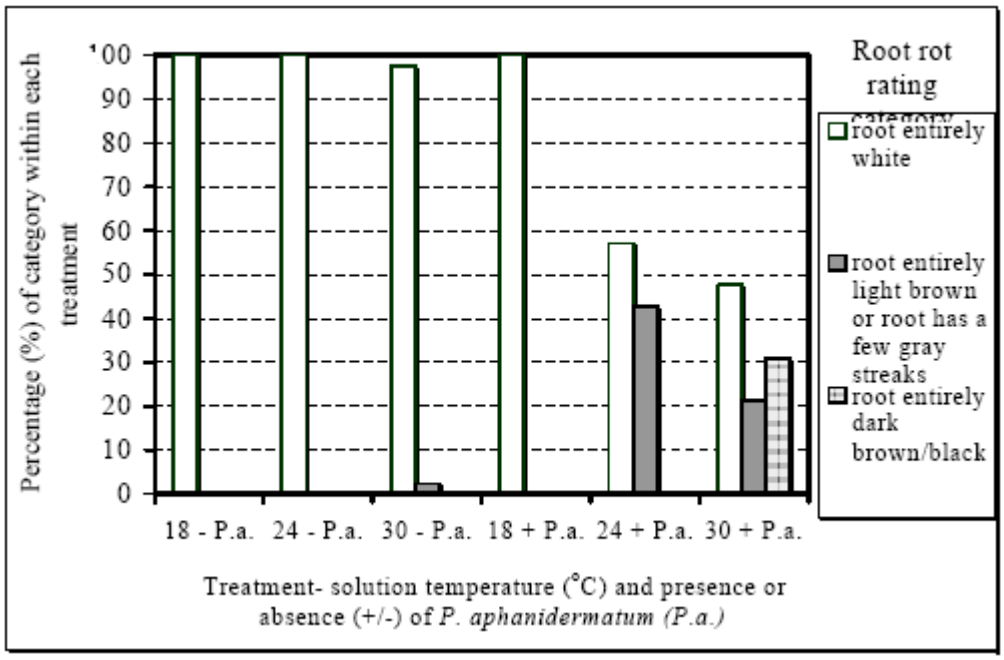

Figure 1-9: Influence of nutrient solution temperature (18, 24, and 30°C) and the presence or absence (+/-) of *P. aphanidermatum (P.a.)* on root rot rating for spinach harvested on day 14.

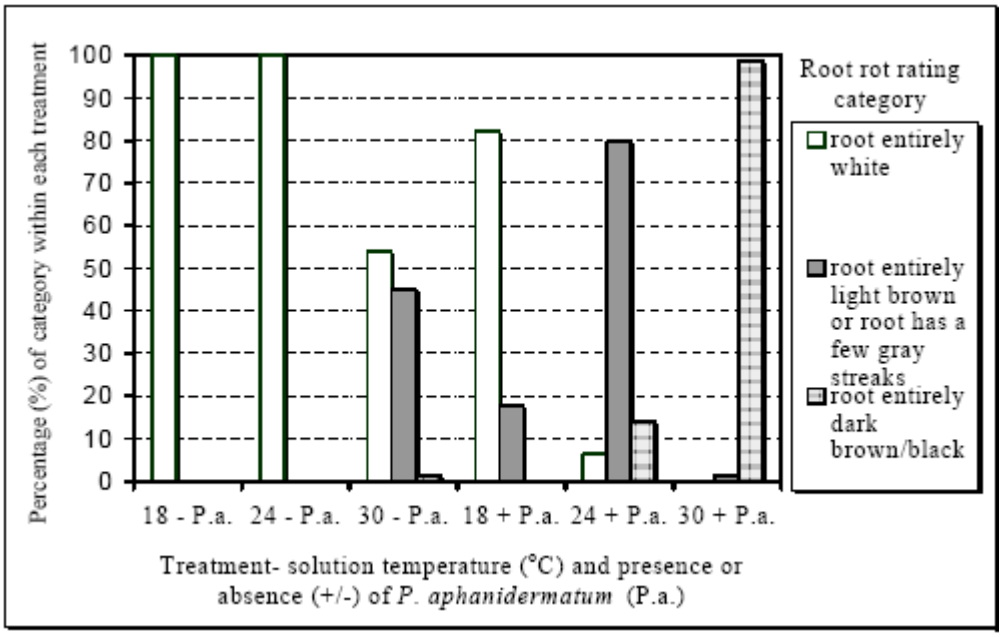

Figure 1-10: Influence of nutrient solution temperature (18, 24, and 30°C) and the presence or absence (+/-) of *P. aphanidermatum (P.a.)* on root rot rating for spinach harvested on day 21.

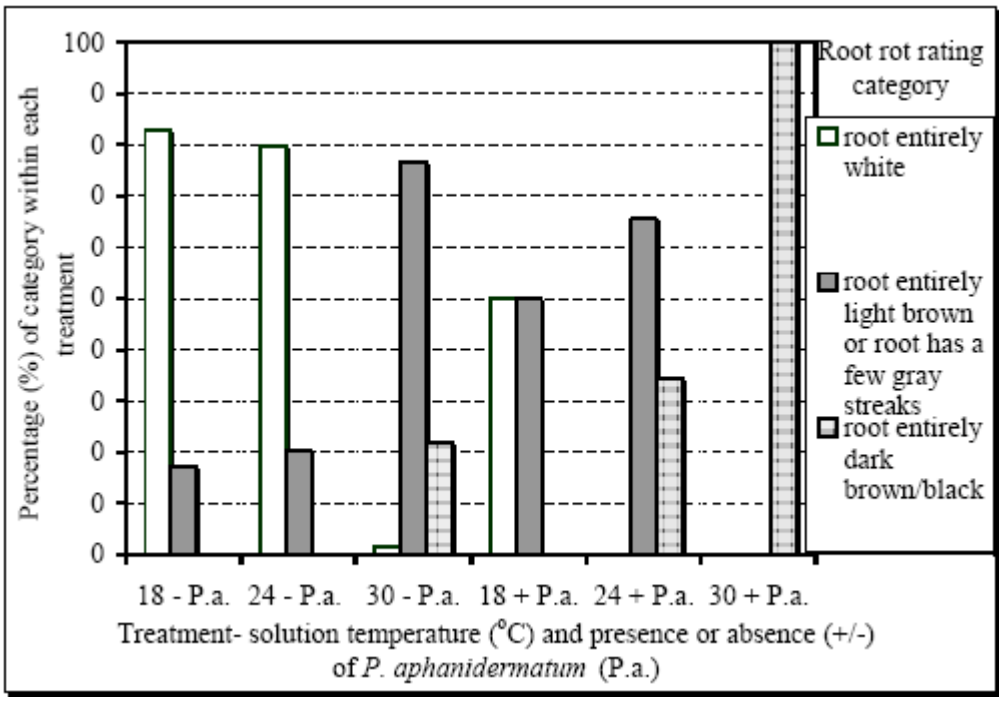

Figure 1-11: Influence of nutrient solution temperature (18, 24, and 30°C) and the presence or absence (+/-) of *P. aphanidermatum (P.a.)* on root rot rating for spinach harvested on day 28.

It can be seen by day 28 none of the roots of plants in the 24°C inoculated condition were white any longer, and in the 18°C condition only 50% were still white. Thus, the disease was progressing rapidly in the roots in all temperature conditions if they had been inoculated, despite not always being highly evident in the shoot dry weight measures, and the higher the temperature the worse the symptoms.

Katzman went on to look at temporal development of sporangia and oospores on the roots (sporangia produce additional zoss beyond the original inoculation). Findings were consistent with the prior data.

The concentration of free zos populations in the nutrient solutions over time is shown in Figure 1-12 below. We were interested to see if there was a pattern of secondary release of zoss from the plants similar to that shown in earlier experiments. Such a cycle was evident in the 30°C condition but not the 24 or 18°C conditions.

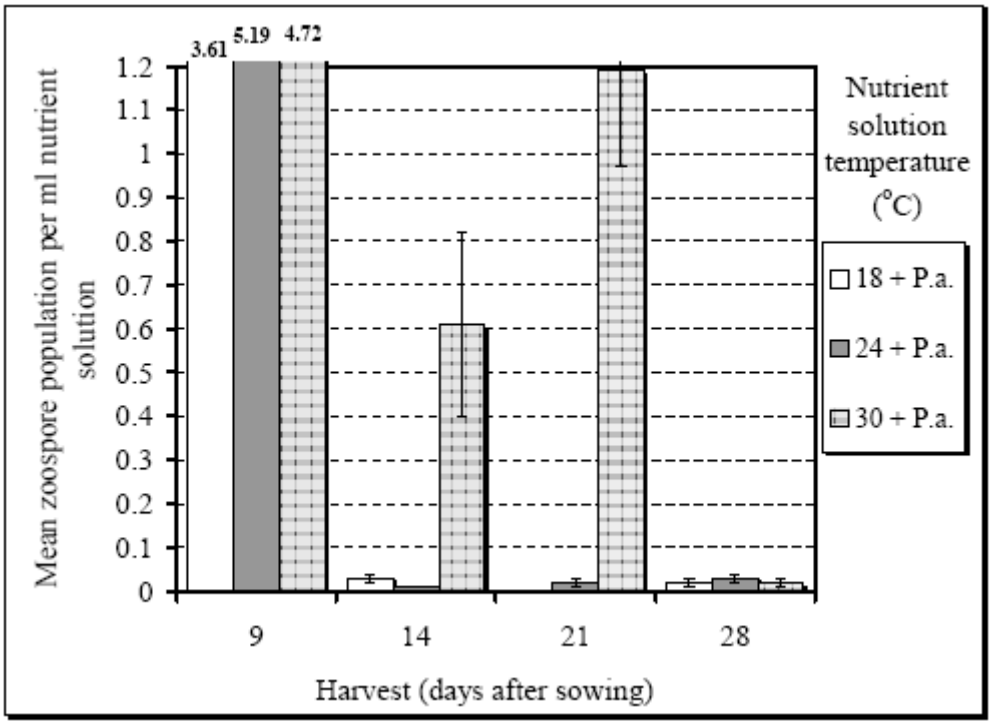

Figure 1-12: Influence of temperature and *P. aphanidermatum* on populations of zoospores in the nutrient solution. Means and standard errors of zoospores per ml of nutrient solution for harvests at 9, 14, 21, and 28 days after seeding. Treatments varied the nutrient solution temperatures (18, 24, and 30°C).

In the case of the 30°C treatment, it appears that the onset of the recovery of the zos population in the nutrient solution was earlier than in the previous studies reported, in which the temperature was lower; the cycle from infection to release was reduced from 19 to 12 days (day 9 to day 21). Free zos population in the 30°C condition then dropped, suggesting either exhaustion of resources or start of another cycle. (Katzman reported a powerful effect of temperature on zos production from mycelium.)

In the 24°C and 18°C conditions, there was no evidence of a major release of zoss, despite the rapid progress of the disease in the roots documented above. In the case of the 24°C condition, a build up of zoss in the solution on day 28 was expected based on the previous experiments described, but in the case of the 18°C temperature it is plausible that the build up to release was slowed enough by the lowered temperature that the experiment was insufficiently long to witness the release. Quite possibly, release was also simply delayed beyond the end of the experiment for the 24°C condition. It should be noted this experiment was conducted with different apparatus than used in the experiments previously described, with a number of changes that may have influenced timing of zos production by the roots. The more obvious changes were continuous circulation of the nutrient solution through a pump, enrichment with pure oxygen, and positive temperature control of the nutrient solution.

IMPLICATIONS OF THE BASIC RESEARCH FOR DISEASE CONTROL

The first set of experiments on timing of inoculation showed that the later in the crop cycle the infection occurred, the less damage took place, even when high levels of pathogen were introduced as early as day 9 in the crop cycle. Thus, a transplant step is very helpful if seedlings can be kept clean up to time of transplant. On the other hand, it showed that high levels of pathogen are utterly devastating to very young seedlings. The second set of experiments on concentration of pathogen showed that if 9-day old transplants were introduced into a pond environment with pathogen levels below 25 ml^{-1}, no significant decrement in dry biomass production occurred. Secondary damage from propagation into the solution of zoss from what infection did occur was delayed more than two weeks after time of transplant and inoculation, regardless of initial infection severity.

Katzman's third set of experiments showed that if the temperature of the pond solution was kept low, coupled with delayed exposure to pathogens achieved through the transplant step, shoot biomass was not significantly affected by even high levels of pathogen exposure. In these experiments the pathogen level was high (2500 ml^{-1}), and the disease did progress considerably in the root zone. One must suppose that had pathogen concentration started low (e.g. < 25 ml^{-1}), and been prevented from any build up by treatment of the solution, no detectable decrement in performance would have occurred even in the presence of some level of disease, and no large release of secondary zoss would have occurred over the time period plants were in the pond.

The concrete finding of the temperature experiments of potential application for spinach production in ponds is that a root temperature does exist (18°C) at which progress of *Pythium aphanidermatum* disease is greatly slowed. In this cultivar at least, crop growth of uninfected plants was not hindered through use of the lower temperature and may even have been increased. Large-scale release into the nutrient solution of zoss produced by infected roots was also apparently delayed or suppressed by the lower temperature. Katzman's work was mainly directed at a production system with a transplant step, with large spinach plants as the end product. If one assumes spinach seedlings can be protected from PA during their early growth before being transplanted into an infected pond environment and, further, the pathogen level in the infected pond can be kept low through some method of treatment, success in production looks likely even without employing lowered temperature. If one adds temperature control it appears the presence of low levels of PA in the nutrient solution will present no problem for pond production of spinach. However, it must be remembered these findings apply with surety only to systems in which a transplant step is used for growing large spinach plants.

SOME PRACTICAL FINDINGS CONCERNING METHODS OF HYGIENE

Disease control needed to be addressed from an immediate practical perspective in the basic research on disease, and research in the production phase of this project, just in order to conduct the research. Katzman

was able to interchange plus and minus inoculum conditions between large tanks with complicated fixed plumbing by applying stringent methods of disinfection and hygiene in growth chamber research on disease.

Bearing in mind the means of propagation of PA, the hydroponic equipment used for research in the biomass production phase of this project was designed so that in the event of a disease outbreak, it could be easily and completely cleaned and disinfected. Strict procedures were implemented so that transmission between experimental treatments would not occur in the event one of the treatment conditions developing the disease.

Specifically, the hydroponic solution tank/pond was chosen to have smooth, easily scrubbed sides with no joints of crevices, and no plumbing entry and exit points where zoss might safely lodge during cleaning operations. Between experiments, all surfaces were scrubbed and dried rapidly. The pump and all plumbing parts for the recirculation system were made easily removable so that between each experiment they might be hooked into a cleaning system in which a disinfectant was circulated. In this way, inaccessible surfaces could be disinfected between experiments. New nutrient solution was used in each experiment. Sensors for testing temperature, pH and EC of the nutrient solution were treated as a potential source of infection. They were never allowed to make contact with the main bulk of solution in case they carried infection and were only ever used on samples of solution. Solution samples were drawn using a dedicated piece of equipment. The samples of solution were then discarded after coming in contact with sensors.

These precautions permitted successful research in a greenhouse range at all times of year. In only one condition of one experiment did any striking disease outbreak occur. As much as possible these precautions should be incorporated into a commercial production facility, at the very least to facilitate recovery from any disease outbreak.

APPLICATION OF DISEASE CONTROL METHODS TO PRODUCTION SYSTEMS

Introduction

To better understand the problem of disease control in spinach production, it should borne in mind there are two different spinach products under consideration that require quite different production systems, and thus different methods for handling the disease problem. They are large leaf and baby leaf spinach. In earlier work in the CEA program, reported to NYSERDA previously, the spinach production system was modeled after that for lettuce. Large individual plants were produced with a transplant and one or two respacing steps. A growth chamber was used to produce seedlings prior to transplant. Beyond that, an NFT system was used for producing the crop. The products in that case were spinach plants with large leaves; in the market, the whole plant would be sold and it would be seen as primarily suitable for cooked use.

There is today considerable popular demand for bagged spinach ready for use in salads or cooking. Leaves range from very small to medium sized. All command a high price per pound. Only leaves are included in the product sold. We see the baby spinach product (taken to include a range of size categories and market values) as the one most likely to be economically profitable to grow in the greenhouse at the present time. Baby spinach is harvested when leaves are small and plants are young. Very high-density plantings are required and there is no need for transplant or respacing. After two days for germination, the crop must be placed in the greenhouse; there is no need for a seedling production facility and, indeed, the whole crop cycle is barely longer than the typical seedling production cycle for lettuce. Production of this crop is particularly well suited to a pond system in terms of logistics because a great deal of material must be moved about, but not as well suited in terms of disease control.

Extreme Hygiene Approaches to Disease Control

Disinfection of tanks and equipment between production cycles and use of clean solution for each production cycle, along with good hygiene during the production cycle, was shown to be feasible and effective means of avoiding PA problems during the experimental phase of this project. This "extreme-hygiene" approach has potential for use in any production method in which the volume of nutrient solution used is small enough that the solution can be discarded (or isolated and treated) between crop cycles. *Prima facie* it is unworkable in large-volume pond systems where the nutrient solution must be retained for use over many crop cycles because it would be too costly, or environmentally destructive, to discard. The nutrient solution is never isolated from roots in pond systems of this type and disease organisms can be expected to accumulate over time if left unchecked. Furthermore, large pond systems are so physically extensive and complex as to be virtually non-cleanable; once they are launched; they are likely to contain some level of PA inoculum forever.

The "extreme-hygiene" or "zero Pythium" approach has been adapted for use with a nutrient film technique in Japan, including an ingenious method of drawing down the nutrient content of the solution at the end of each crop cycle before disposal. It is a batch system rather than continuous production system. However, there is considerable labor time involved in cleaning operations between crop cycles, in dismantling and reassembling equipment and reconstituting nutrient solution, and there is a materials cost involved in throwaway plastic film liners and the water and nutrients discarded between cycles. The value of the spinach product in the United States (as compared to Japan) does not appear to justify the costs involved in using the "extreme-hygiene" approach in this country, nor is the practice of discarding nutrient solution on a frequent basis likely to be socially or legally acceptable in the future. (The nutrient solution could be retained between cycles, but then there would be the cost of storing and treating it and the cost of laboratory analyses to determine composition before and after treatment.)

Nutrient film and aeroponic techniques are inherently risky. They require constant human vigilance to guard against power breakdowns with resultant catastrophic crop failure, or else expensive automated detection and power backup systems must be installed. Despite their disadvantages, it is clear these systems can be used effectively for production of either baby or large leaf spinach in conjunction with a treatment process to eliminate zoospores during recirculation of nutrient solution. Both systems have the advantage of a degree of compartmentalization of plants. The economic feasibility of these systems depends on cost factors that remain to be determined.

Root Zone Temperature Control Approach for Large-Leaf Production

Katzman's research showed solution temperature could be used to diminish the effects of root invasion by PA in 9-day old transplants. It also showed the concentration of zoospores in the solution affected the severity of the disease. These findings provide a basis upon which to devise a production system for spinach using a pond that is not completely PA free, and one that can withstand outbreaks of PA infection. If the extent and rate of infection of transplants placed in the pond system can be limited by keeping pathogen concentration low through continuous treatment of the solution during recirculation, and the progress of disease in infected plants after they become infected can be slowed by use of a lowered solution temperature, it appears feasible to use a pond system for spinach production. It remains to be determined if it is profitable to do so. By implication, the end product is large leaf spinach. It is questionable whether the market price commanded by this product is sufficient to cover production costs.

To use the method of control outlined above to good effect, the plants need to be given a head start before transplant into the pond system. One needs to devise a seedling area separate from the main ponds in which seedlings are produced using extreme-hygiene methods of disease control. Some compartmentalization of the seedling production system is necessary to respond to any outbreak of disease and permit cleaning operations without a break in continuity of production. One boon is there is no problem with using copious amounts of newly constituted solution to produce seedlings because the used solution can be disposed of into the much larger pond system. Other details of the seedling production process are developed in the next section.

Several well-established methods for continuous treatment of large volumes of nutrient solutions are in commercial use (UV, sonication, heat treatment, and filtration). PA zoospores are fragile entities, and it is expected that a relatively inexpensive method of continuous treatment will be determined. However, such methods were not evaluated for cost and efficacy during this project. They are the subjects of a follow-on project. The cost of cooling of ponds below ambient air temperature is dependent on geographic and seasonal climatic factors. However, the desirable temperature is not exceptionally low and a great cost is not expected.

Katzman expresses the caveat that *Pythium dissotocum* was not identified in Cornell CEA nutrient solutions. It has been identified elsewhere as having a competitive advantage over *Pythium aphanidermatum* in attacking spinach at temperatures below 23°C. This raises questions such as how virulent is *P. dissotocum*, and what would be the best temperature for slowing spread of disease when both species of *Pythium* are present in the solution.

Using temperature of the nutrient solution to limit destructive effects of PA has great promise and may permit sustained production of spinach in ponds over multiple crop cycles. Before declaring the root disease problem solved, what happens in continuous production needs to be tested.

Limitations of Root Zone Temperature Manipulation for Baby Spinach Production

The foregoing has addressed disease control for producing large spinach plants. The spinach crop product we see as most likely to be economically profitable as a greenhouse crop is baby spinach, harvested when leaves are small and plants are young. Dealing with the question of PA infection in this crop is more problematical than in large leaf crops for reasons that will be apparent.

To achieve high productivity when harvesting very young plants, plant density needs to be in the vicinity of 1500 plants m^{-2} (see later sections) and no transplant or respacing step is needed or feasible. To achieve the same productivity as in large plants, the same area of greenhouse is required as for big plants, so in this production scheme, the greenhouse space is entirely filled by very small plants and the whole greenhouse in effect becomes the seedling production area. As a result, if a permanent pond system is desired, plants must be placed into nutrient solution from the very start, and it is likely the nutrient solution will be infected to some degree.

The question arises, could the combination of lowered temperature and continuous treatment of the nutrient solution work in this situation? Research on the effect of root zone temperature on spinach grown in this way (namely just the early part of the plant growth cycle) indicates there is a considerable penalty in productivity when the temperature is low enough to affect PA zoospore production. In an experiment conducted in the greenhouse at Cornell (Katzman and de Villiers, 2000), the cultivar Whitney showed an average increase in fresh shoot weight of 41% when grown in 26°C instead of 18°C ponds. The average was computed from the first three harvests, on days 20, 25, and 28. In this experiment the plants at 26°C went on to succumb to Pythium at day 41, but only then did the 18°C plants catch up in weight. In a more recent experiment within this project a substantial weight advantage was found to growing plants in 25°C compared to 20°C nutrient solution. Whole plant shoot fresh weight of the cultivar Eagle was increased 32% and economically useful "commercial cut" was increased 38.5% in two independent conditions. (See later sections). However, temperature effects do appear to be somewhat cultivar specific. In growth chamber studies, we found the Japanese cultivar "Alrite" performed similarly at 20 and 25°C in one

experiment and slightly better at 25°C in another (See following research reports). Katzman also found equal or better performance of Alrite at 18 versus 24°C in growth chambers, as reported earlier in this chapter.

Katzman demonstrated the devastating effect of PA infection on seedlings when exposed to a high concentration at day 1 at a solution temperature of ~24°C. Presumably, this is because very young seedlings have small root systems and a given number of zoospores are capable of disabling a greater proportion of root systems than those of older plants with larger root systems, including critical parts of the water transport system. At present, we have not ascertained what effects very low concentrations of zoss have on seedlings. One must expect that manipulation of solution temperature will not be as effective for seedlings as for older plants, and small dosages of pathogen will have larger effects than they do on older plants.

Treatment of nutrient solution is generally applied to a small volume of solution as it passes through the recirculation system. Treatment of this sort is incapable of making ponds completely pathogen free if the ponds are continuously filled with hosts for zoospores. Newly released zoospores need only cross to the nearest root in a veritable forest of roots; as a result, many are unlikely to circulate and thus are never exposed to the method of treatment.

Alternatives for Disease Control for the Baby Spinach Crop

For the baby spinach crop we would like to boost productivity by maintaining root temperature at a level optimum for spinach at this stage of growth, which is ~24°C. Unfortunately this temperature is also favorable for growth of PA. It may be the case that damage from Pythium infection will not amount to a significant problem regardless of temperature and even with exposure of very young plants if the zos population is kept very low and the crop cycle is very short. There is some hope for this being the case because it appears there will be little tendency to build-up PA due to post-infection release of zoss, because the time allotted to the crop in the pond (~14 days) is shorter than the zos post-infection reproductive cycle (~ 20 days). (This assumes that we have correctly identified the nature of such a cycle.) In this scenario, a pond system could be used for continuous production of baby spinach in conjunction with continuous treatment, even if the continuous treatment were not perfect. Temperature would be kept as low as possible without compromising productivity. We need to determine just how low the zoospore concentration needs to be maintained for such a system to work. We are currently investigating these questions.

If reducing zoss to a very low concentration in the pond is an insufficient remedy, what alternatives are there? Assuming we cannot prevent the occasional entry of PA spores into the system, we can at least try to prevent the spread of PA from plant root to plant root, or limit its rate of spread. This theoretically can be achieved using an aeroponic technique of nutrient solution delivery, coupled with clean nutrient solution.

The solution is expected to arrive at the roots as an aerosol then condense and fall down vertically. Zoss should have little opportunity to spread laterally. They also should have little opportunity to lodge on the roots before being carried away. As plants age and root systems tangle, more root contact between plants is expected, but only of adjacent plants. Thus, any spread of infection would be slow and unlikely to affect productivity in the short time available before harvest. Almost complete elimination of PA is required during recirculation of the nutrient solution, but given the much smaller volumes involved, may be feasible.

Restricting the spread of zoss can also theoretically be achieved by compartmentalizing deep flow systems. One form of compartmentalization would be to channelize ponds so that, should infection begin at a single point, only one channel will become infected. In this case it would still be possible float materials the full length of the channels. However, too high a degree of compartmentalization diminishes the special advantages of pond systems: safety, simplicity, low cost, and ease of materials handling.

A distinct directional flow of nutrient solution would prevent zoss from spreading against the direction of flow. If disease entered anywhere in the channel, it would only spread downstream; the source of infection would also move downstream as plants were harvested, and eventually no further source of infection would remain. Such a method of control would require a moderately rapid flow and thus would depend on it being possible to treat large volumes of solution quickly and effectively. PA zoss are fragile enough that this may be possible, but it is an empirical question also under current investigation. A fast flow would be compatible with compartmentalization into long narrow ponds.

What would be most desirable for growing very young plants in deep flow systems would be to attack *Pythium* zoss as they move from one root to another, when they are most vulnerable. There are several possible avenues of attack here: a fast flow rate or turbulence of the solution in the vicinity of the roots might be effective if it interfered with chemotaxis and hampered docking of zoss; sonication would be excellent if it did not also damage root cells. It might also be possible to deter PA from producing zoospores in the first place by altering the root environment.

CONCLUSIONS

The work of Katzman on disease control has led to a very promising protocol for producing large-leaf spinach in pond systems. As outlined above, this involves growing seedlings cleanly for 9 days before transplant and then transplanting them into a pond system in which the nutrient solution is cooled to 18°C, and which is also treated to keep ambient concentration of zoss to less than 250 ml^{-1}. Any shortening of the crop cycle possible is recommended as a safety precaution to avoid encountering large-scale release of zoss from infected roots, which might strain the treatment system.

Adoption of baby spinach as the end product adds to the difficulty of solving the problem of root disease in greenhouse spinach production because all plants are likely to be exposed to PA from the very start, when they are most vulnerable. At present, it is unknown what level of free zoss in the nutrient solution is tolerable for the baby spinach crop. We are presently working on this question.

Any solution to the problem of baby spinach production would appear to require two elements: 1) a guaranteed supply of *Pythium*-free solution (or near-free) as output from a continuous treatment process in the recirculating system, so that the recirculating system does not become the means of spreading disease, and 2) some degree of compartmentalization so that in the event of disease outbreak the damage is limited, and production can continue despite the outbreak. The one great advantage of baby spinach as a crop is the short crop cycle; secondary damage to the crop resulting from zoss released by infected plants may be discounted as a threat. Infected plants will be removed from the system before the zos reproductive cycle is complete. This also means it is unlikely there will ever be demands placed on the solution-treatment apparatus to cope with large concentrations of zoss.

We know of several ways that are currently available for producing disease free spinach. The question is which of these is economically feasible and will make it profitable to grow spinach hydroponically in greenhouses in competition with the outdoor crop. Our work in this study has pinpointed what information we need to determine which continuous treatment system is best, and whether a deep flow system is possible for baby spinach production or we need to turn to low volume hydroponic systems such as NFT and aeroponics.

References

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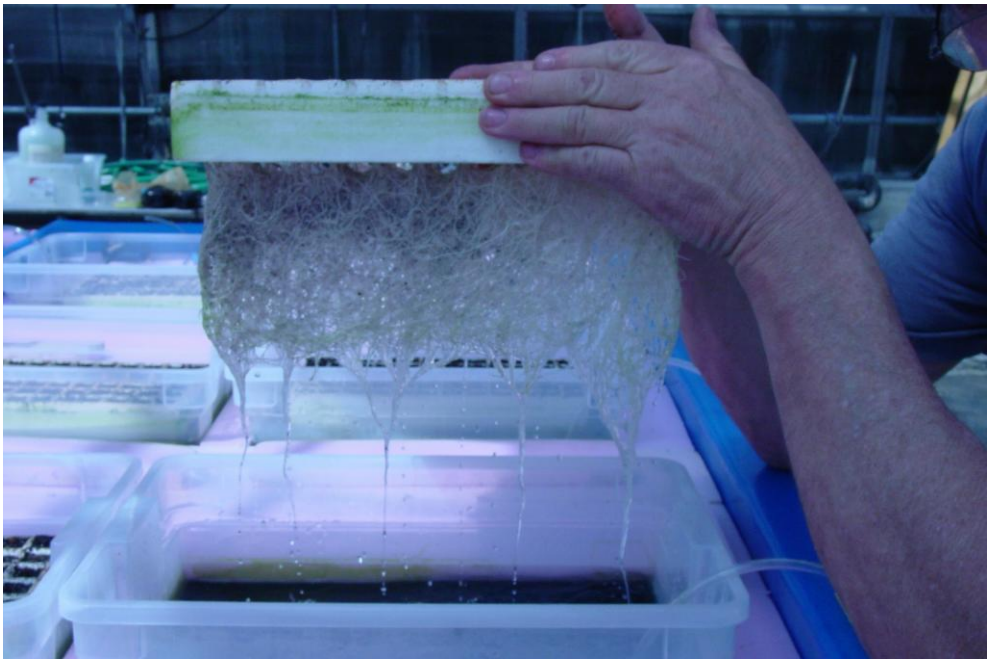
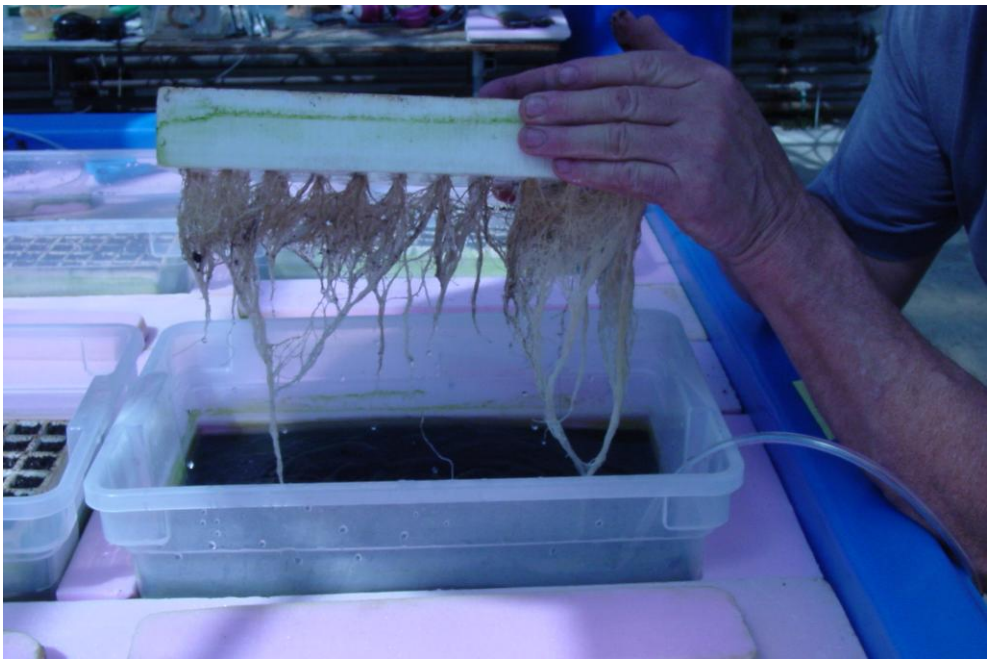
Katzman, L. and deVilliers, D.S. (2000). Unpublished data, Cornell University, Ithaca, NY.

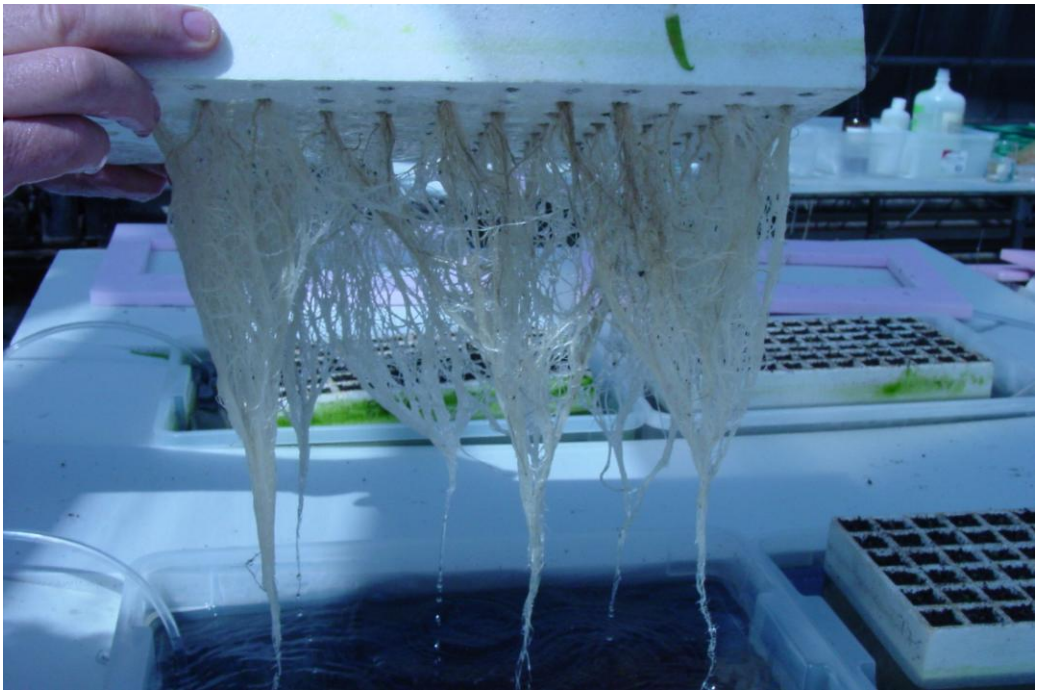


Appendix 1A-1. Healthy but soil-stained roots of 16 day old spinach plants



Appendix 1A-2. Badly diseased roots of 16 day old spinach plants

Appendix 1A-3. Clean healthy spinach roots of 16 day old plants



Appendix 1A-4. Partly diseased roots of 16 day old plants

CHAPTER 2. SEED GERMINATION AND SEEDLING PRODUCTION

Task 3: Seed quality, seed germination, and seedling production for large leaf and baby leaf products.

INTRODUCTION

Spinach germination has been historically problematical for seed scientists. Germination percentage is often poor and unpredictable and obtaining simultaneity of germination has proved elusive. As a result of our research under this grant, we have devised two seed germination methods that substantially overcome these difficulties, and we are now able to produce seedlings quickly, uniformly and with a high degree of predictability whether using traditional seed or newer de-hulled seed.

At the start of this project, we were aware of a seed pre-treatment process that makes spinach seed germination much easier, namely, removal of the thick outer shell called the pericarp. Our work on seed germination accordingly followed two lines of inquiry; to find a way to improve germination of traditional intact seed, and to develop a protocol for using the newer de-hulled seed. We considered it prudent to follow both lines of inquiry because the commercial future of de-hulled seed is uncertain. Although machinery to remove the pericarp has been invented, so far there has not been enough grower interest in obtaining de-hulled seed for it to have been made available commercially.

The two techniques we have developed are a priming technique that works independently of the particular medium employed, and a technique developed specifically for use with a peat-based medium, that relies on control of moisture content of the medium. The priming technique was developed using intact seed, and is most critically needed for problematic intact seed. The latter technique was developed using de-hulled seed in the production phase of this research; it lends itself to full automation using existing flat-filling and seeding technology. We foresee further improvements in seedling production techniques in the near future; work is in progress to dispense with medium entirely, but it is not yet at a stage where it is ready for commercial use.

Both germination procedures we have developed can be used with either intact or de-hulled seed. The moisture content method was developed for use with de-hulled seed, but if the quality of the seed supply is good, it is also effective for intact seed. In this case, it would be the preferred method because of its simplicity and ease of mechanization. On the other hand, in certain kinds of media or if problematical intact seed is being used, the priming method may be required to overcome the problems and then would be the preferred method.

RESEARCH APPROACH

This task fell into two parts; research on germination of intact seed, and research on seedling production using de-hulled seed. Both efforts resulted in development of effective new techniques.

Initially, a broad based approach to the problem of seed germination and seedling production was taken, including contacts with the seed industry and investigation of seed characteristics affecting quality and performance. We developed a laboratory-scale method for de-hulling seed and conducted basic research on effects of pH, EC, and temperature on germination. Investigation of the effect of medium moisture content on germination of intact seed led to development of the new priming technique mentioned above. In this very effective and widely applicable priming procedure, seed is first imbibed then dried down before being planted. Percentage germination and simultaneity of germination of normally intractable intact spinach seed are greatly enhanced. We concluded this phase of the work with detailed studies to optimize management of the first two stages of the priming procedure, imbibition and dry-down.

When the research focus shifted from germination to crop productivity, it was necessary to commit to an end product and a method of producing it; we decided to focus on baby leaf spinach as the commercial product and to use de-hulled seed, planted into plug trays filled with a peat moss-based medium as the means of producing it. To obtain satisfactory yields from baby plants, planting density must be very high, and seeding must be mechanized, which is possible with plug trays. We undertook research to determine the best way to produce seedlings within this scheme, which led eventually to the procedure of planting dry, un-imbibed, de-hulled seed into a medium with precisely controlled moisture content, then allowing a set time for imbibition and germination (incubation) before flotation. In this procedure, imbibition is controlled in such a way that it obviates the uncontrolled dry-down step that is a normal part of most germination methods.

Key findings of the seed germination and seedling production research are presented and discussed below.

BASIC RESEARCH ON SEED GERMINATION

Seed morphology and the order of events in seedling emergence are diagrammed and described in Appendix 2-A.

Provisional Method for Production of De-Hulled Seed

In the course of the germination research, pending access to de-hulled seed of northern adapted cultivars from Japan, we developed a laboratory-scale hand technique for de-hulling seed. Gently grinding the whole spinach seeds in liquid nitrogen with a mortar and pestle produced high levels of perfectly de-coated, viable spinach seeds. However, some damaged seed were mixed in with intact seed -- cracked de-coated seeds and de-coated seeds with damaged testae. A labor-intensive seed-selection operation was needed after seed de-coating.

Using this crush-and-select method, we were able to produce approximately 200 viable, de-coated seeds in 20 minutes. This method was usable for research-scale purposes, but would not be feasible for commercial scale production.

Research on pH, EC and Temperature as They Affect Germination of Intact Spinach Seed

Experiments on seed germination over a wide range of pH (4 – 8) and nutrient solution concentrations (EC 0 – 9000 $\mu S\ cm^{-1}$) showed some root reduction in pH 4 solutions and some reduction of germination in high salt conditions (EC's > 4000 $\mu S\ cm^{-1}$). This research indicated seed germination occurs well within a wide range of set points for these parameters. Normally we control levels of these parameters within a narrow range.

Historically, spinach germination temperature studies in the literature indicate that spinach, as a cool-temperature crop, has optimal germination in the range of 10 to 14 ^{o}C. At this temperature, germination is slow. The new seed priming and moisture content methods to be described allowed an increase in the optimal germination temperature for spinach seeds to warmer temperatures (25–30^{o}C), with corresponding increase in speed of germination.

Effect of Peatlite Moisture Content on Germination of Intact Spinach Seed

In this study, seed was placed on the surface of Cornell peatlite mix prepared to have a series of moisture contents (MC) ranging from 66% to 95% fresh weight basis, or 2.0 to 19.0 dry matter basis. (Peatlite is a one-to-one ratio by volume of finely sieved peat moss and vermiculite, with dolomitic limestone added). The seeds were sealed in with the peatlite in clear plastic containers, and germination over time was observed and recorded. The experiment was repeated three times.

The percentage of seeds planted that had germinated after 6 days may be seen in Figure 2-1 below. There was a good germination rate, a little over 80%, for moisture contents between 73.5% (2.8 MC) and 84.5% (5.45 MC). At higher moisture contents, percentage germination fell off very rapidly. At lower moisture contents, germination percentage was depressed, but as later research indicated, probably only because imbibition was slowed.

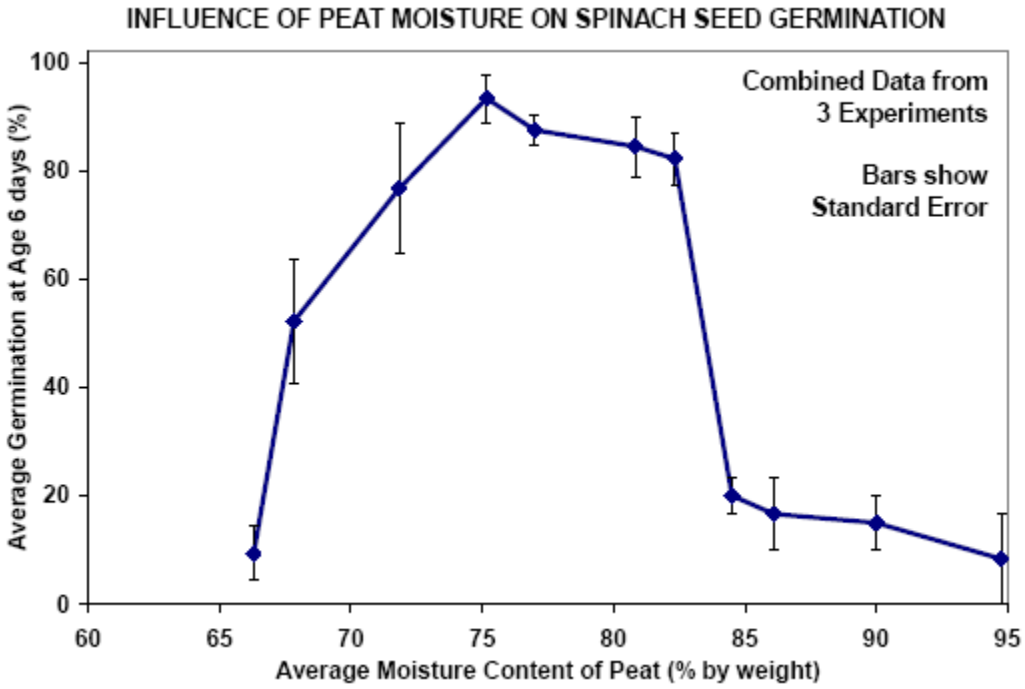


FIGURE 2-1: Influence of peat mix moisture concentration on cumulative germination of intact spinach seed 6 days after planting. Average MC of peat is calculated from actual measured (rather than desired) levels.

Effect of Vermiculite Moisture Content on Germination of Intact Spinach Seed

A similar experiment was conducted using vermiculite with moisture contents ranging from 12% to 65% fresh weight basis (0.14 to 1.86 MC). Seed was placed in centrifuge tubes and germination was observed after two days at 24°C.

The results are shown in Figure 2-2 below. Moisture content below 32% (0.47 MC) had low germination response, but moisture contents equal to or above 32% had excellent germination response by age 2 days. This response was quite different from that in peat mix described above. Levels of moisture content greater than 65% (1.9 MC) were not tested as the vermiculite was saturated beyond this point. (We may assume extremely low germination.)

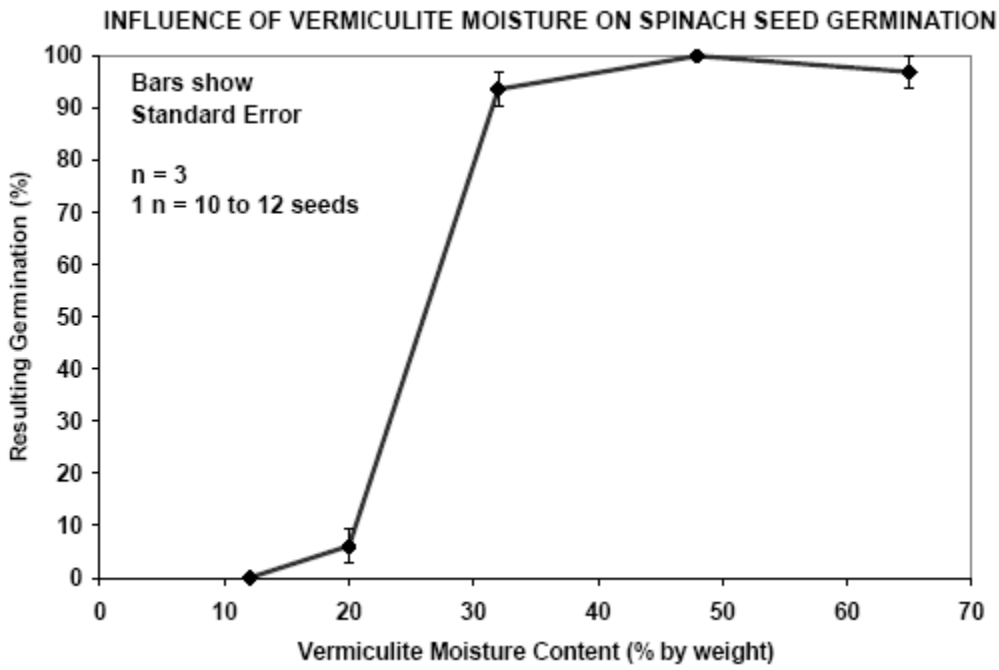


FIGURE 2-2: Influence of vermiculite moisture concentration on cumulative germination of intact spinach seed 2 days after planting

DISCOVERY OF NEW SEED PRIMING METHOD FOR GERMINATING SPINACH

The new priming method was discovered by chance. Lids of some germination boxes were accidentally not fully sealed, and as a result, seeds in these boxes slowly dried out after imbibition. We kept records identifying which germination boxes had inadvertently dried, and then re-wetted the contents. The result was that the spinach seeds in germination boxes that had dried down had significantly superior final percentage germination. This phenomenon was pursued and found to be useful both for increasing percentage germination and synchronicity of germination.

In subsequent work, we have developed a rationale for why this procedure works and why normal germination procedures often do not work well in spinach. Neither de-hulled nor intact spinach seed will germinate under water or in very wet media, although they will imbibe, and certain unseen biochemical events no doubt take place. (See research on moisture content.) Germination processes beyond the imbibition stage resume only when the seed or medium dries sufficiently. The course of events is left somewhat to chance in the normal way spinach seed is planted. If the period in which the seed is overly wet goes on too long, it impairs final germination percentage.

In this new priming procedure, the time allocated for the imbibition step is controlled and the rate of dry-down is controlled. Germination is restarted during the dry-down step with the seeds all at the same starting

point in terms of imbibition. Once germination progresses beyond a certain point during dry-down, the seed becomes more tolerant of wet conditions than at the start. At this point additional moisture may be safely applied to complete germination.

PROTOCOL FOR THE SEED PRIMING METHOD OF GERMINATION

The final optimized priming method was as follows:

a) **Preparation of paper media:** Place a 1 mm thick layer of paper in an airtight container. For blue germination blotter paper, one sheet is sufficient. For newspaper, germination paper, or paper towel, 4 to 6 sheets are sufficient. The layers of paper must be thick enough to hold a small reservoir of moisture. (The type of paper did not seem to matter in our studies. Newspaper performed just as well as germination paper and germination blotters for this purpose.)

b) **Moisten paper media:** Place an excess of water in the paper-holding container to moisten and rinse the layers of paper. Pour off most of the excess water. Allow gravity to drain off most of the water, but leave sufficient water such that the paper has a glistening appearance on the upper surface. This level of moisture should be maintained throughout the first imbibition. We accomplished this by using a continuous-feed felt material wicking system positioned below the paper, but simply adding a small excess of water also worked.

c) **First Imbibition:** Place spinach seed in a single layer on the moistened paper. All seeds should be in direct contact with the paper. Check that the surface of the paper still has a glistening appearance and add a small amount of water if needed to obtain the glistening appearance. Seal the container and allow the seeds to imbibe for 9 hours.

d) **Dry down period:** Remove the sealed covering/lid from the container, to allow air exchange and evaporation in a cool dry location. Place a moistened piece of paper on top of the seeds to reduce the rate of drying. The seeds should remain in contact with the paper during drying. Prevent any direct air currents that might result in rapid drying. Allow drying to continue for 24 hours. The paper may be dry to the touch or just slightly damp when the dry down period is complete. If blue blotter paper is used, the paper will have a speckled appearance (wet and dry spotting) when the dry down period is complete.

e) **Second imbibition:** Place the seeds into a moistened media or re-moisten the paper.

This method will provide uniform germination of most of the seeds within 24 hours of initiation of the second imbibition.

PERFECTING THE SEED PRIMING PROTOCOL

Changes in Seed Moisture Content During Initial Imbibition

Given the large effect the type of medium has on the relationship between germination and moisture content, we pursued events in the seed independently of the medium, in order to develop a procedure that was independent of the medium. The course of imbibition was tracked in the cultivar Bejo, with results shown in Figure 2-3 below.

After very rapid uptake of water over the first hour, there was a slow increase in seed moisture content for the next 6 hours.

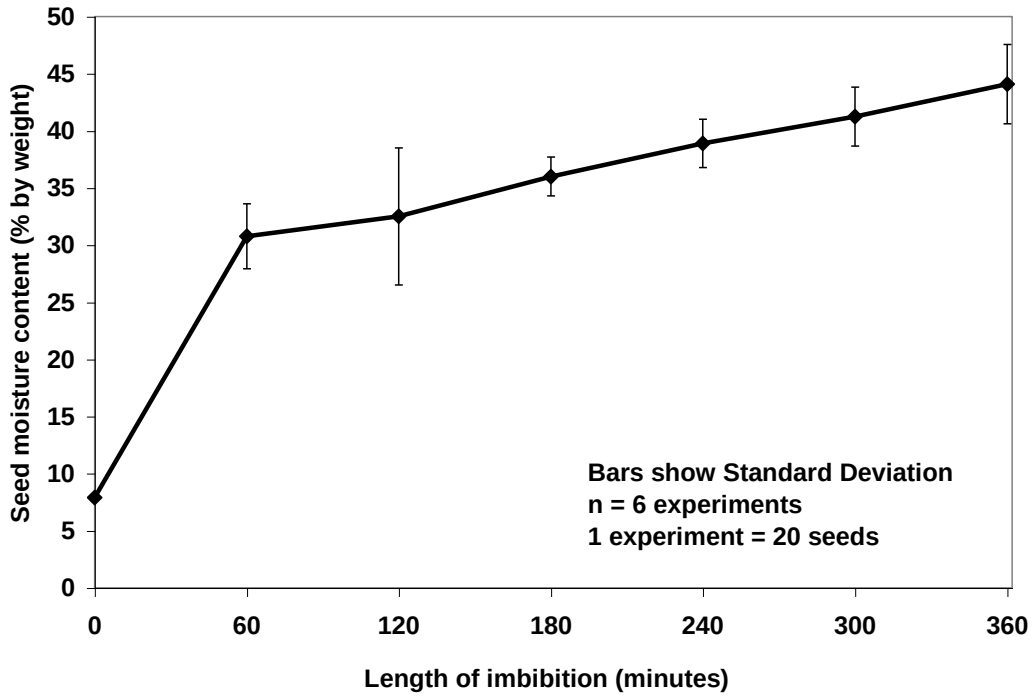


FIGURE 2-3: Increase in seed moisture content (% by weight) of intact spinach seed during the first six hours of imbibition

Effect of Duration of Imbibition on Germination of Intact Spinach Seed

The question arose: If the seed is fully imbibed after one hour, or even 6 hours, is this sufficient or is more time needed for seed biochemistry events not evident from weight measurements in the imbibed but quiescent seed?

After variable lengths of imbibition time, seeds were given a standard dry-down procedure lasting 24 hours then re-hydrated. Germination percentage was assessed after a further 24 hours with the results shown in Figure 2-4 below.

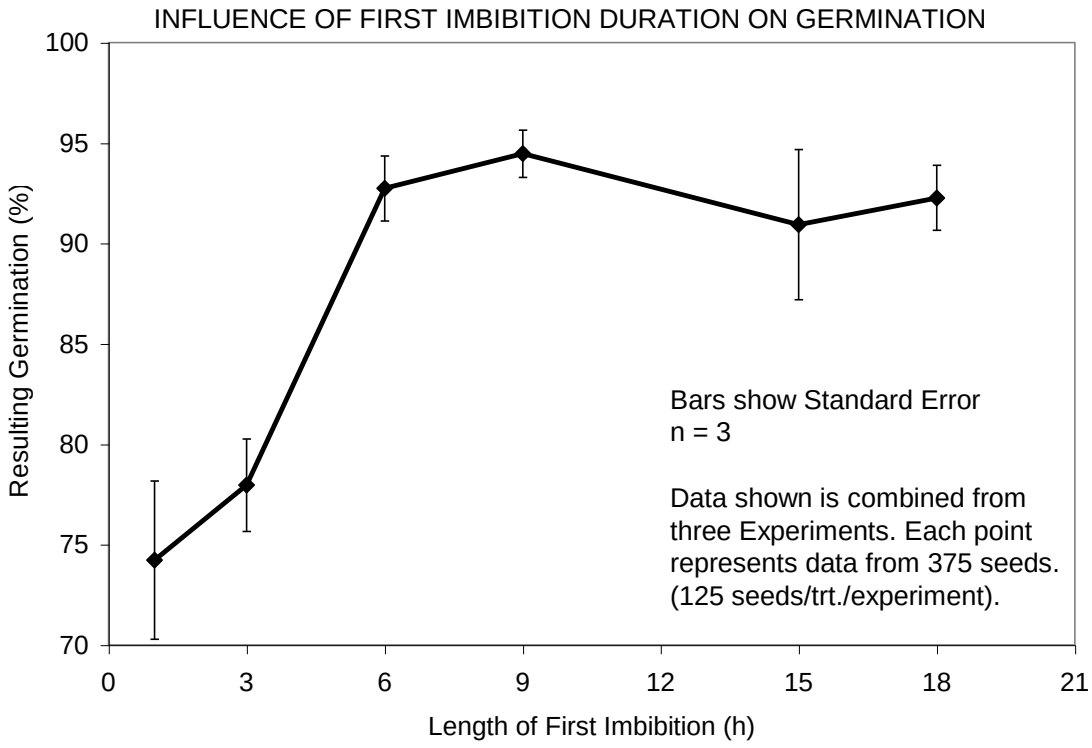


FIGURE 2-4: Influence of the duration of the first imbibition step of the priming protocol used to improve germination of intact spinach seed shows that an imbibition duration of 9 hours is sufficient and yields superior germination performance to imbibition durations less than 6 hours. (Note truncated ordinate)

An imbibition duration of 9 hours prior to the dry-down period was sufficient to obtain the maximum germination rate of circa 94%. A 6-hour imbibition duration yielded a similarly high germination percentage (92%). A First Imbibition duration of 15 to 18 hours also provided good average germination, but the results were more variable and less consistent than the 9-hour treatment. Thus, 9 hours is recommended as the duration for the first imbibition in the priming protocol.

Effect of Speed of Dry-Down on Germination of Intact Spinach Seed

In a series of experiments, changes in seed moisture content were tracked through first imbibition and then dry-down, using different methods of dry-down that changed the rate of dry-down. It became clear that a very slow a rate of dry-down failed to initiate the next stages in the germination process, and too fast a rate was detrimental. These outcomes are shown in the bar chart Figure 2-5 below.

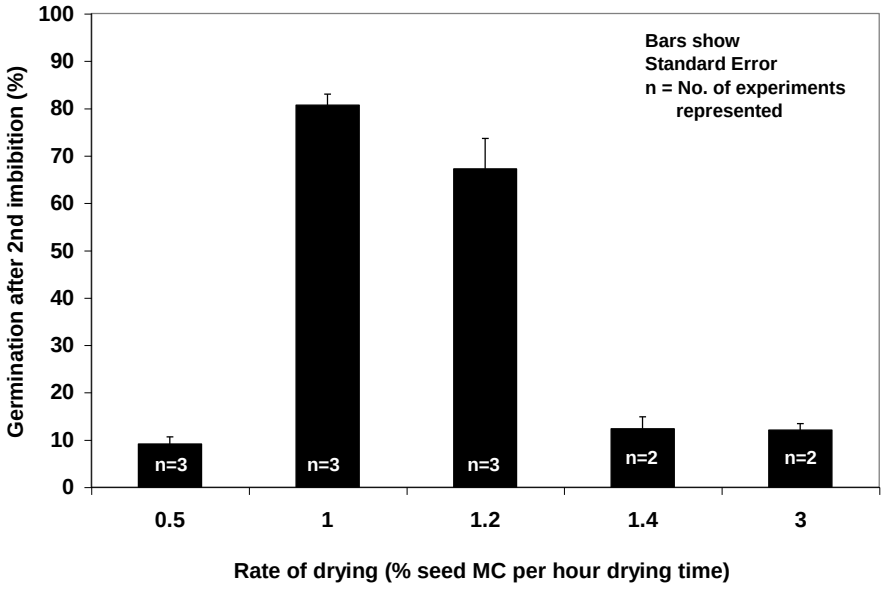


FIGURE 2-5: A 24-hr drying period at the rate of –1% per hour yielded the best germination rates after the 2nd imbibition. More rapid rates dried the seed too quickly. The slower rate tested (0.5% per hour) did not achieve the desired dried MC after 24 hrs.

Seed Priming Protocol – Unfinished Business

Optimal duration of the dry-down step in the priming protocol was not determined, although an estimate was suggested by the work on the effect of rate of dry-down on germination. Duration is rather closely tied to rate of dry-down, and initial moisture content of the seed, which in turn is determined by duration of imbibition.

The remaining step in the priming protocol is the second imbibition. In this case, it is known that the partially germinated seed is not highly particular about soil moisture content, but it is likely still that the transplant medium can be made too wet. Preferred moisture content of transplant medium and duration of exposure to this moisture condition during second imbibition prior to insertion into the final growing system remain to be determined.

SEEDLING PRODUCTION USING DE-HULLED SEED

Introduction

Work on seedling production falls between research on germination and research on biomass production. For production purposes, we would like plants to be as nearly the same size as possible throughout their growth, which means we are interested in synchronized emergence and continued uniform growth. We

would also like to automate flat filling and seeding as much as possible and simplify the conditions under which germination is brought about.

The initial task in the production phase of this project was to develop a protocol for seedling production using de-hulled seed. Prior to this project it was known that de-hulled seed is much easier to germinate than intact seed (specifically, from the work of Katzman, 1999), and it was our intention to evaluate use of de-hulled seed in commercial greenhouse production. Our initial research emphasis on germination of intact seed was because we might not be able to obtain de-hulled seed in the cultivars we wanted to use, either for current research or for the future, or that it might not be cost effective to use this type of seed. In fact, we were able to obtain a good supply of de-hulled seed from Takii Seed Company in Japan, holders of the patent on the de-hulling process, and we do believe it will be worthwhile to use de-hulled seed for commercial production of baby spinach; thus, it was necessary to perfect a protocol for seedling production using de-hulled seed. (The additional cost of using de-hulled seed is estimated in the chapter on cost of production.)

Indicators of Seed Quality and Seedling Performance

In this phase of the research, metrics were needed to evaluate seed and seedling performance. Although percentage of seeds that emerge above the soil is a very good indicator of seed quality, it does not tell the entire story. The speed with which emergence takes place, the synchronicity of emergence and the quality of the seedlings that emerge are equally important; ultimately, it is the percentage of seedlings that are "good", and same-sized that counts. No one measure encompasses all these desirable qualities. Each of the following indicators has some merit:

Germination/ Emergence Percentage. aka, Germination Rate, Germ, and Viability. This is by far the most common simple indicator of seed quality, but it tells little about seed vigor.

Germination/ Emergence Speed. A simple measure of speed is the hours from seeding (or start of imbibition) to 50% germination. This is known as T50. Other percentages such as Tzero and T90 are also of interest.

Synchronicity of emergence. 1. Time from Tzero to T50 or T90, or in other words, how long it takes for a given % of seeds to emerge from the time emergence begins, is a logical and easily read index of synchronicity of emergence.

Synchronicity of emergence. 2. The least time it takes for a given percentage of seedlings to emerge may be a better measure, although it is a little harder to read. This measure avoids undue influence of very early or late emerging seeds. Very early and very late seedlings are often of no use by harvest.

All the measures listed above can be determined from a graphical representation of the cumulative percentage of seeds planted that emerge, plotted against time since seeding, and a number of these graphs will be presented to visually summarize findings. The asymptote expresses the Emergence Percentage; the slope is an indicator of speed and synchronicity of emergence, etc. What is missing from such graphs is an indication of the quality of the seedlings. Typically, in this phase of research the seedlings were rated with regard to their deficiencies or problems, if any. Categories used included: "pop-ups"; stunted, deformed, very late, or trapped seedlings, and seedlings without a growing tip or missing other parts. Ultimately, these categories can be summed up to give a one-dimensional indicator, namely "poor seedlings" and "useful seedlings".

The operational definition of "emergence" used in this phase of research was any part of the seed being visible to the eye from above and visible without undue craning of the neck to get low angle views into crevices in the soil. (We repeatedly checked the reliability of implementation of this definition with different experimenters, and it proved quite reliable within and between experimenters.)

In thinking about emergence data as opposed to germination data, it should be borne in mind not all seeds that germinate are strong enough to emerge, or if they do emerge, to emerge quickly. Thus, emergence data will tend to give slightly lower percentages for viability than germination data. Time to emergence likewise can easily be 24 hours longer than time to germinate, if germination is taken to mean showing of any part of the radicle outside the testa. From a pragmatic standpoint, data on emergence are more valuable than data on germination, but even data on emergence convey a false picture if a large percentage of emerging seedlings have defects. For instance, under some conditions, a large percentage of seedlings that emerge do so without the root penetrating into the medium. These seedlings, which we call "pop-ups", later die and have no value.

Historical Methods

The starting point for producing spinach seedlings using de-hulled seed was the same method that has been used traditionally with many different crop species: dry seed is planted into moist soil, and is then watered in. After watering in, the soil medium is drained and/or dries down to a suitable level for germination to occur. In our case, we used slightly moistened peatlite (60 to 65% MC) as the medium to fill polystyrene plug trays in which to plant the seed, and then fully wetted the peatlite by floating the trays on water until the top surface glistened. (At 60% moisture content peatlite feels dry and is quite loose and friable, but is easily wetted further.) A period of dry-down of 48 hours in a warm place was found to be sufficient for germination to commence, after which it was safe to rewet or float the tray. This method was (and is) very successful with de-hulled spinach seed, and could have been used as described, but we wished to improve

upon it. The watering-in step is time-consuming and awkward logistically; furthermore we wanted to minimize the time required for the dry-down step.

Preview of Protocol for Controlled Moisture Content Method of Seedling Production

We reached the method we finally adopted for seedling production in the production phase of research, and recommend for commercial practice, by a roundabout route. Now that we have it, it appears to be extremely simple. What we do is plant dry seed into a peat-based medium (peatlite) of carefully controlled moisture content (~ 3:1 water to dry matter) and then wait for it to germinate under controlled temperature conditions (25°C). Germination is not actually visible since the seed is covered, but it is safe to fully wet the medium after 48 hours if the temperature is maintained at 25°C when de-hulled seed is used. (Intact seed requires 12 hours longer.) By this time germination has taken place, roots are emerging from the bottom of the flat, and some shoots have broken the surface. In our case, we float seeded flats in nutrient solution at this time. By 72 hours, all the shoots have emerged. We do not need to pre-imbibe or water-in the seed then dry it down. More details of the protocol will be described at the end of this chapter.

RESEARCH LEADING TO THE CONTROLLED MOISTURE METHOD OF SEEDLING PRODUCTION

Introduction

The order in which experiments were conducted, leading up to the protocol finally adopted for seedling production, was this: Initially we made an attempt to use the seed priming method developed for intact seed with de-hulled seed. A drawback to the priming method is that seeds are in a fragile state at the end of the dry-down period if their radicles have emerged. To avoid handling these vulnerable seedlings, primed de-hulled seed was planted into peatlite immediately after priming, with the intent of controlling rate of dry-down by the moisture content of the peatlite medium. Difficulties were encountered and the line of research was abandoned since there is no real need to prime de-hulled seed. Next, the focus was on comparing the emergence performance of seedlings in flats of different heights, in combination with different durations of dry-down after watering-in. The change in moisture content of flats over time in typical dry-down conditions was also examined without plants present. Finally, the effect of moisture content of the medium on seedling viability and synchronicity of emergence of de-hulled seed was examined. Seeds were planted into the medium without priming, watering in or dry-down, in a series of experiments that included intact as well as de-hulled seed. The range of moisture contents that worked well differed for the two types of seed. We determined which moisture content to use in the protocol for de-hulled seed by how easy it was to fill high-density seed flats using that moisture content; the MC adopted was that of the wettest medium that would still fill small cells without creating voids.

We undertook a preliminary experiment to determine whether it was necessary to acclimate seed coming out of cold storage, and if surface sterilization of seed was a good or bad thing. The procedure for this

experiment is presented in detail as an example of how many of the seedling production experiments were conducted, and to illustrate some of the points made above.

Experiment 1: Effect of Seed Sterilization Treatment and Acclimation After Long Term Storage on Emergence, Seedling Quality, and Early Biomass Production of De-Hulled Alrite Seed

Experiment Rationale. As a result of experiencing excessive and unexplained variation in seedling emergence performance of Alrite de-hulled seed, various hypotheses were advanced as to what might be causing the variability, touching on seed factors amongst other things. This experiment addressed two possible sources of variation in performance: whether seed had been acclimated or not coming out of cold storage, and whether a surface sterilization treatment had been applied to the seed. Since these effects could possibly be expressed as a weakening in vigor, the experiment was conducted as a seedling emergence experiment in a soil-like medium, rather than a germination experiment on absorbent paper, and early growth performance was examined as well as emergence performance.

This was a factorial experiment in which there were two levels of two factors. Either seed came directly out of cold dry long-term storage (un-acclimated condition), or it was allowed to acclimate to ambient room conditions for 10 days (acclimated condition). Either seed was treated with Clorox and ethanol following an established procedure (+ condition) or it was treated in similar fashion but with RO water (-condition) Each condition was represented in rectangular polystyrene flats of 72 cells, 1.75 inches high, plant density 1460 plnts m^{-2}, and there were two reps (one duplicate) for each condition.

This experiment provided 8 flats of potentially very similar plant material. It was decided to also use this material to answer questions concerning size of sampling unit appropriate for spinach. Using the individual plant as a sampling unit requires very large samples (to overcome the occurrence of failed seedlings and extreme divergence in plant size), and equally importantly, an excessive amount of time, labor, and materials in obtaining fresh and dry weights. It was thought a sampling unit comprised of a 3x3 matrix of plants might supply greater power of discrimination (as well as research efficiency), and this idea was tested empirically.

Hypotheses. The hypotheses were that a period of acclimation would be beneficial to seedling speed of emergence and subsequent growth, and that the seed sterilization treatment would be harmful particularly to germination percentage. It was anticipated that a sampling unit of 9 plants, giving an n of 8 in a flat of 72 seedlings (the typical "rep"), would compare favorably to a sampling unit of one plant giving an n of 72 per rep, as measured by p values in comparable t-tests for differences between treatments.

METHODS

Manipulated Variables. In the seed surface sterilization treatment, seed was wetted in RO water for a few seconds, drained, and then immersed and shaken in an 0.6% sodium hypochlorite solution (a dilution of 6% commercial bleach) for 20 seconds, then rinsed thoroughly in RO water, then immersed and shaken in a 70% solution of ethanol for 30 seconds before final rinsing. In the control, seed was wetted with RO water for an equivalent interval. Seed was then dried for 10 or more minutes on paper towels for ease of subsequent handling. (Method of Katzman, 2003).

During acclimation, seed was stored in a chamber where the temperature averaged 20°C, and the air was relatively humid, ~ 65%. Seed had been stored for many months at 1°C and a relative humidity of ~ 45%. Un-acclimated seed was used directly out of cold storage.

Seeding. An artificial soil medium, "peatlite", was prepared with measured water content of 1.8 MC (64% FW basis). At this water content, it was still friable and dry to the touch. Peatlite was prepared using a 1/8-inch hardware cloth sieve. It was considered important to reduce particle size to this extent for uniform filling of flats without air pockets, and to achieve uniform emergence of seedlings. Seeding was always done by one experimenter following a definite routine, which was as follows: Flats were filled by cascading/dribbling peatlite out of hand from a height of about 4 inches directly into each individual cell of the flats. Flats were all completely filled in this way, and then overfilled with up to 1/2 inch of medium. The excess material on top of the cells was then cleaned off with as little compression of the remaining medium in the cells as possible. A specially prepared "dibbler" was used to compact the medium in the filled cells 5/16 inches in the middle, 3/16 at the sides, with a flat in the center. The dibbler was keyed to the cell sidewalls, to give a uniform depth. (Flats were not dropped to settle the medium.)

Eight 72-seed lots of previously selected, treated seed were randomly allocated to conditions for seeding of flats. Tweezers were used to place seeds in the center of each cell, orientated with a flat side down (rather than on edge) in all cases. Seed was pressed into the soil using the dibbler, peatlite was dribbled over the flat to cover all cells to excess, and then the excess cleaned off. The final step was firming of the covering material, achieved by thumb pressure, with the thumb spanning the sidewalls of each cell to achieve a uniform result. (All steps will eventually be mechanized for commercial practice.)

Wetting In, Imbibition and Dry-Down. Immediately following seeding, flats were floated in RO water until water had wicked to the surface, indicated by darkening and glistening, a half-hour process. Flats were then set out in pairs on flat plastic trays on a bench in a growth chamber in which temperature was set to 25°C, and a 16-hour light cycle was employed. Flats were not covered. Trays were rotated in position morning and evening (approximately every 12 hours), without disturbing the individual flats. During this

period, some draining took place, and some evaporation, but the medium remained quite wet. Surface color did not change. Dry-down was continued for 48 hours from the time of imbibition.

Crop Flotation. After 48 hours dry-down, flats were floated in a common pond until harvest. The surface area of the pond was ~ 1.0 m^2, and its volume was 275 L. The nutrient solution was adjusted to an EC of c. 1200 micro Siemens cm^{-1} using equal parts by weight of Hydrosol and calcium nitrate. The nutrient solution was aerated with compressed air released through two airstones. Water use, pH, EC and DO were monitored daily and adjusted when necessary. Target pH was 5.8. DO was maintained at a level above 7.0 mg l^{-1}. The crop was rotated every day so that north and south and east and west positions were reversed, and all flats were exposed to a range of possible environmental conditions.

Dependent Measures and Harvest. The first part of the experiment concerned germination percentage, germination speed, and seedling quality, all of which variables were suitably measured. No stand adjustment was made, since germination was uniformly high. Half the flats (one rep) were harvested after 7 days had elapsed from seeding, half after 8 days. In the day-7 harvest, individual fresh weights of plant shoots were taken for half of the flat (36 plants from one rep in each condition) and dry weights were obtained for these plants individually. With a view to speeding harvest, for the other half of the flat, harvest was in four quadrants consisting of 9 cells in 3x3 matrices. Each 9-plant group was a sample unit. Seedlings within quadrants were counted and categorized for size, but plant material was bulked for both fresh and dry weights.

As far as data collection for shoot material, the day-8 harvest was treated in the same way as the day-7 harvest. Additionally, in one flat in the day-8 harvest complete matching root data were obtained by individual plant or quadrant, by extracting the root ball and washing away soil. In the remaining 7 flats, root data were only obtained as bulk dry weights for whole flats, and then only for roots outside the flats.

RESULTS AND DISCUSSION

Emergence performance. In Figure 2-6, it can be seen that emergence percentages were very high in all conditions (> 95%) and seedling emergence was essentially complete 78 hours after seeding. The big surge in emergence appears to have started 55 to 60 hours after seeding; approximately 90% of seedlings emerged during the 20-hour period following. (For lack of closely enough timed recordings of emergence, these indices are judged with some estimation based on the trend.)

The emergence performance was considered most satisfactory in all conditions, although there was some aberration early on in the un-acclimated-treated condition.

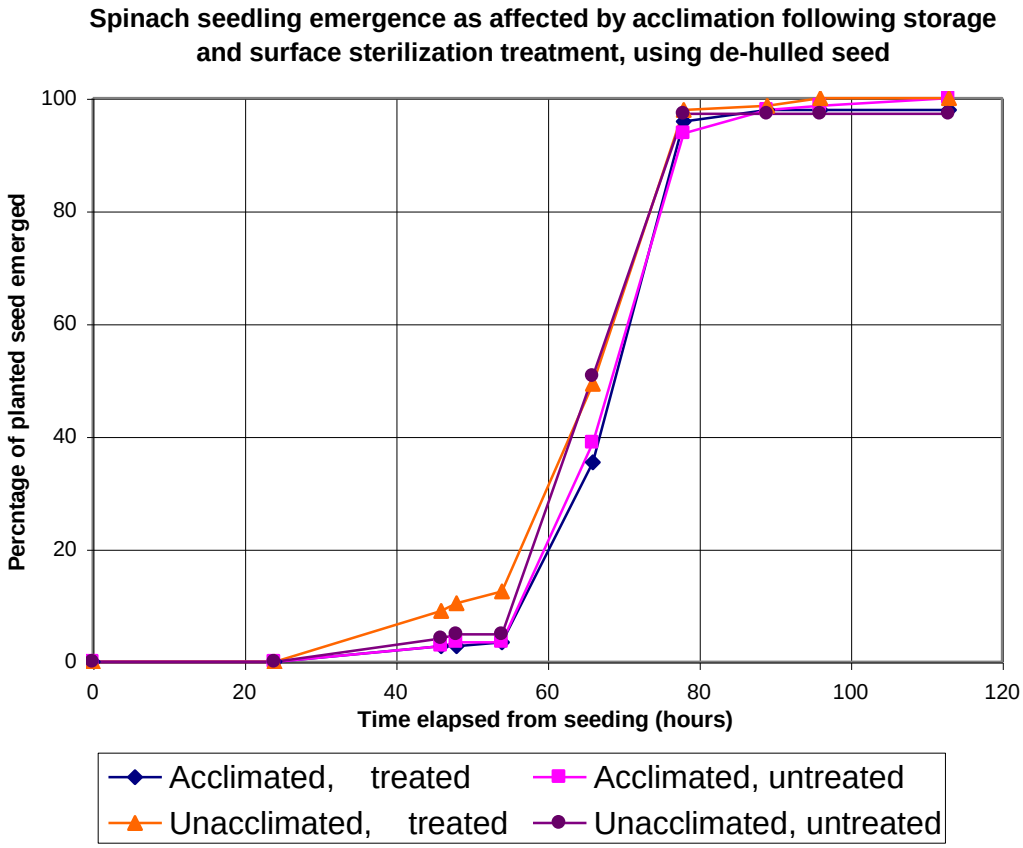


FIGURE 2-6: Spinach seedling emergence as affected by acclimation following storage, and surface sterilization treatment, using de-hulled seed

Seedling Quality. It is not uncommon to get nearly 100 percent emergence, as in this experiment, using de-hulled spinach seed. However, not all seedlings are of sufficient quality to contribute to yield later on. Table 2-1 below shows the kinds of defects that occur, and gives an estimate of percentage of seedlings that are expected to contribute to yield. It can be seen that in this case whereas on average more than 98% seedlings emerged, only 91% were expected to contribute to yield. This is an excellent percentage. It would not be uncommon to lose 25% of seedlings to defects in slightly lower grade seed of spinach.

Seedling Classification	Acclimated Seed						Un-acclimated Seed					
	Treated			Untreated			Treated			Untreated		
	rep 1	rep 2	**reps1+2**	rep 1	rep 2	**reps1+2**	rep 1	rep 2	**reps1+2**	rep 1	rep 2	**reps1+2**
Seedling visible	70	71	**141**	72	70	**142**	72	72	**144**	69	71	**140**
Pop-ups	2	3	**5**	2	0	**2**	2	3	**5**	2	0	**2**
Weak	2	0	**2**	0	2	**2**	3	3	**6**	0	0	**0**
Failed	0	1	**1**	0	1	**1**	1	0	**1**	1	4	**5**
Very late	2	2	**4**	0	7	**7**	0	3	**3**	0	2	**2**
Albino			**0**			**0**			**0**	1		**1**
blanks	2	1	**3**	0	2	**2**	0	0	**0**	3	1	**4**
Non-contributors	7	6	**13**	2	8.5	**10.5**	6	7.5	**13.5**	7	6	**13**
Useful plants*	65	66	**131**	70	63.5	**133.5**	66	64.5	**130.5**	65	66	**131**
% Emergence	97.2	98.6	**97.9**	100.0	97.2	**98.6**	100.0	100.0	**100.0**	95.8	98.6	**97.2**
% Useful plants*	90.3	91.7	**91.0**	97.2	88.2	**92.7**	91.7	89.6	**90.6**	90.3	91.7	**91.0**

*These figures included half of seedlings classified as very late

Table 2-1: Classification of seedling quality 96 hours after seeding

Biomass Harvests. Evaluation of seedlings 96 hours after seeding is still rather early to determine their fate. Emergence is only completed typically around 80 hours from seeding in the watering-in method of seeding that was used in this experiment.

Comparison of fresh and dry weights for seedlings harvested day 7 and day 8 showed very little difference between the treatments. Differences in biomass were not significant, nor did they appear to be different in trend. There was a significant difference in dry weight to fresh weight ratio, both days, with the ratio higher in the acclimated seed conditions than in the un-acclimated conditions. The difference in p values when performing t-tests using single plants as the sample unit versus blocks of 9 cells as the sample unit was small. It tended to favor the 9-cell sample unit.

CONCLUSIONS

There appeared to be no penalty incurred by sterilizing seed with chlorine and alcohol. Any gain in performance from acclimating de-hulled seed out of long-term storage was negligible. Collecting data for blocks of 9 cells instead of individual cells was in this case a satisfactory laborsaving alternative.

Experiment 2: Use of the Peatlite Medium for Dry-Down of Imbibed De-Hulled Seed

Methods. Concurrent with the previous experiment, three 72-cell flats were seeded into 1.8 MC medium using de-hulled Alrite seed that had been imbibed for 15 hours instead of dry seed. After seeding, one of the three flats was watered in followed by a 48-hour dry-down, the same as for the dry seeded flats, one was NOT watered in followed by a 48-hour dry-down (a priming procedure), and the last flat was floated directly with no dry-down period. Emergence was tracked as shown in Figure 2-7 below. A plot from the acclimated-untreated seed condition of the previous experiment described is included.

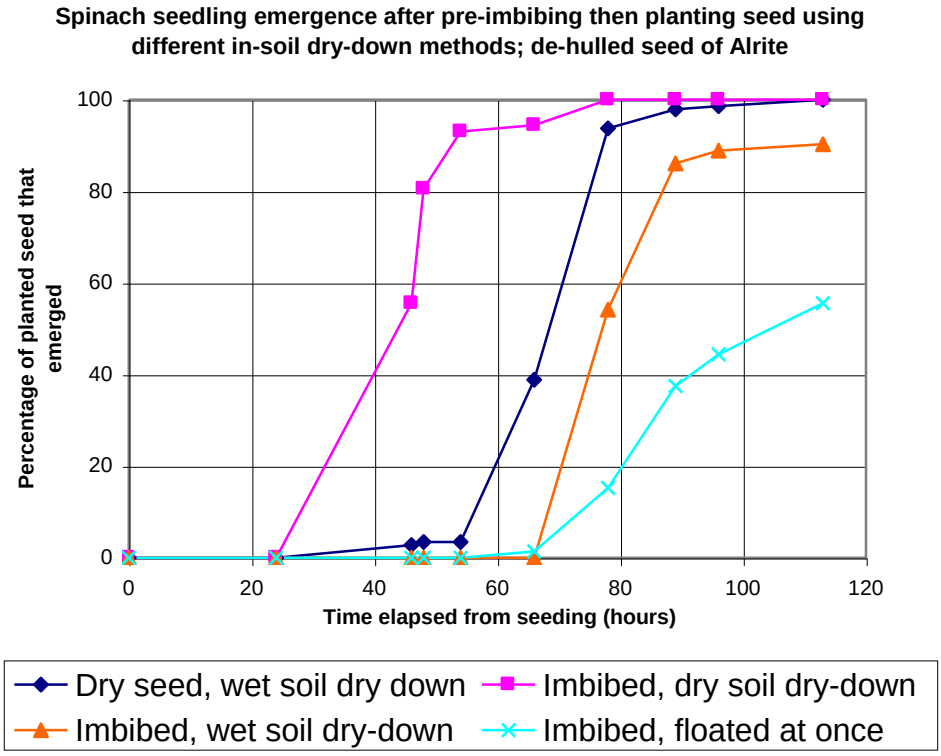


FIGURE 2-7: Fate of imbibed seed dried down in peatlite in various ways

Results and Discussion. These results are much as one would expect. The imbibed seed floated at once never had an opportunity to dry down, and this showed in much slowed emergence and reduced percentage emergence. The imbibed seed in the wet-soil dry-down suffered in the same way to a lesser degree. The dry-down of imbibed seed in dry soil (peatlite of 1.8 MC) was, superficially, a very effective method. Emergence was more than 20 hours ahead of the dry seed conditions of the previous experiment. However, there was a major drawback to this treatment: 51% of seedlings that emerged were "pop-ups" which later died. Since pop-ups are the first seedlings to emerge and they are abnormal emergences (because it is just the root emerging, not the shoot), the apparent speed of emergence in this condition was deceptive. It should also be kept in mind 15 hours had already been used to imbibe the seed. All in all, the time advantage for pre-imbibed over dry seeding was probably illusory.

Although we expect the right durations for imbibition and dry-down and appropriate MC of medium for dry-down in medium could be found to use with de-hulled seed, it appeared little advantage would be gained in this direction given the ease of germination of de-hulled seed in the first place, and, as much work would be entailed, this effort was abandoned.

Experiment 3: Experiment on Effect of Duration of Dry-Down Following Initial Watering In on Emergence of De-Hulled Seed of Cultivar Alrite in Flats of Two Heights

Introduction. This experiment represents an important step in the evolution of ideas leading to the seedling production procedure currently used. Previous experiments had shown that when 1.75-inch flats were floated directly after seeding, either no seeds germinated or germination was very poor. This was presumably because the medium was too wet for germination to proceed. If flats were taller (2.5 inches), it helped the situation, presumably because there was more air included in the medium in the vicinity of the seeds at the top of the soil column. It appeared the critical height for germination of de-hulled spinach seed in peatlite columns in continuous contact with a water table was somewhat more than 2.5 inches. The phenomenon described above is well illustrated in the zero dry-down conditions for the two flat heights shown in Figures 2-8 and 2-11 below.

Originally it appeared that watering in of flats after seeding is unavoidable, because initially the medium needs to be dry and loose enough to fill small cells in plug trays, and this MC is not wet enough for speedy imbibition. (This belief has since been proved wrong.) Our preferred method of watering in was by floating flats because the procedure is gentle, simple, and repeatable. However seed are watered in, it is then necessary to dry the medium down by allowing flats to sit in a well-ventilated location while drainage and evaporation take place.

In short soil columns it takes some time for saturated medium to dry sufficiently for germination to begin. The question of this experiment was how long after watering in do seeded flats need before they can be safely floated. Too long, and roots could emerge from the base of the flat, or have to cope with too dry an environment within the flat. Too short a time and the seed might not yet be committed to germination or have got beyond sensitivity to the wetness of the medium. Seed does not all germinate exactly on the same schedule, so whatever time is picked for the dry-down period could result in some seed receiving less than ideal treatment. Previous work had indicated that at 25°C de-hulled spinach seed offered sufficient water would extend the root out the bottom of a 2.5-inch flat in less than 72 hours. This information set the upper limit for dry-down in this experiment to 72 hours.

Hypotheses. It was observed in previous work that taller flats take longer to show signs of having dried down than shorter flats; as a result, it was expected the optimum dry-down period would differ in the two flats under consideration, being shorter in the 1.75-inch flat than the 2.5-inch flat. It was predicted 0 hours

of dry-down (also 24-hours of dry down for 1.75-inch flats) would adversely affect germination percentage in both flats, but more so in the 1.75-inch flat than the 2.5-inch flat. It was also expected that 72 hours of dry-down would be too long for the 1.75-inch flats, though not for the 2.5-inch flats, resulting in exposure of roots to a less-than-perfect environment, which would be reflected in reduced early growth. No strong predictions were made for performance after a 60-hour dry-down.

Method. Seeding was done in the normal way using a peatlite mix of approximately 1.6 MC (62% water content, fresh weight basis). Five 1.75-inch 72-cell polystyrene flats (plant density 1460 plnt m^{-2}) and three 2.5-inch 50-cell flats (plant density 1050 plnt m^{-2}) were seeded with de-hulled Alrite seed. The seeding procedure in use at this time involved filling the flats to excess by dribbling medium into cells vertically from above. After scraping off excess medium, the flats were dropped several times from a height of approximately 2 inches to settle the medium. Cells were then refilled, and the excess scraped off again, the medium was firmed, and 0.5 mm deep holes were opened to receive seed. Seed was settled in the bottom of the hole on its side, the sides of the hole were collapsed over the seed, and the surface was firmed again. The whole flat was planted in all cases, 72 cells in the 1.75-inch flats, and 50 in the 2.5-inch flats. Dry down periods of 0, 48, and 72 hours were applied to both flat types. Additional times of 24 hours and 60 hours were used for the 1.75-inch flats. There were no reps. The dry-down took place on a bench in a growth chamber where the temperature was maintained at 25°C day and night, and full light was applied for a 16-hour photoperiod during the day. Flats were not covered, but they were placed on an impervious surface and disturbed as little as possible prior to flotation. After flotation, flats were repositioned frequently to sample the possible variety of conditions present in the pond. However, light mapping showed that light, at least, was uniform throughout the growing area. No stand adjustment was made. Reflective barriers were not used in this experiment, in view of the early harvest. Progress of germination and seedling emergence was followed closely by counts and by qualitative evaluations. The experiment was terminated on day 12. Fresh weights were taken for individual plant shoots in all conditions.

RESULTS

Germination and seedling emergence. Results are presented graphically in a series of charts. Figure 2-8 dramatically shows the essence of the results for 1.75-inch flats, where few seedlings emerge if flotation is immediate, about 80% if a dry-down period of 24 hours is used, and 100% if a 48-hour dry down period is used. Figure 2-9 uses the same data to show that seeds which did emerge were slower to emerge in the shorter dry-down periods. Thus, the length of the dry down period affected both percentage and speed of emergence. Figure 2-10 shows all five dry-down durations used in 1.75-inch flats on the same graph. It can be seen that the 60-hour and 72-hour periods had much the same effect as the 48-hour period. Percentage germination was slightly off in the case of the 60-hour period, but that likely had nothing to do with the treatment. Occasionally some seeds in the sample simply are bad ones. The germination in the 72-hour period seems to have been slower than in the 60 and 48-hour periods. Since this divergence was

already present at 48 hours after seeding, before any of the three treatments had been floated, it seems likely it has to do with seeding technique. Probably the seeds were planted a little deeper or firmed more in this flat. Eventually, and quite quickly, the plants caught up in terms of percentage germination.

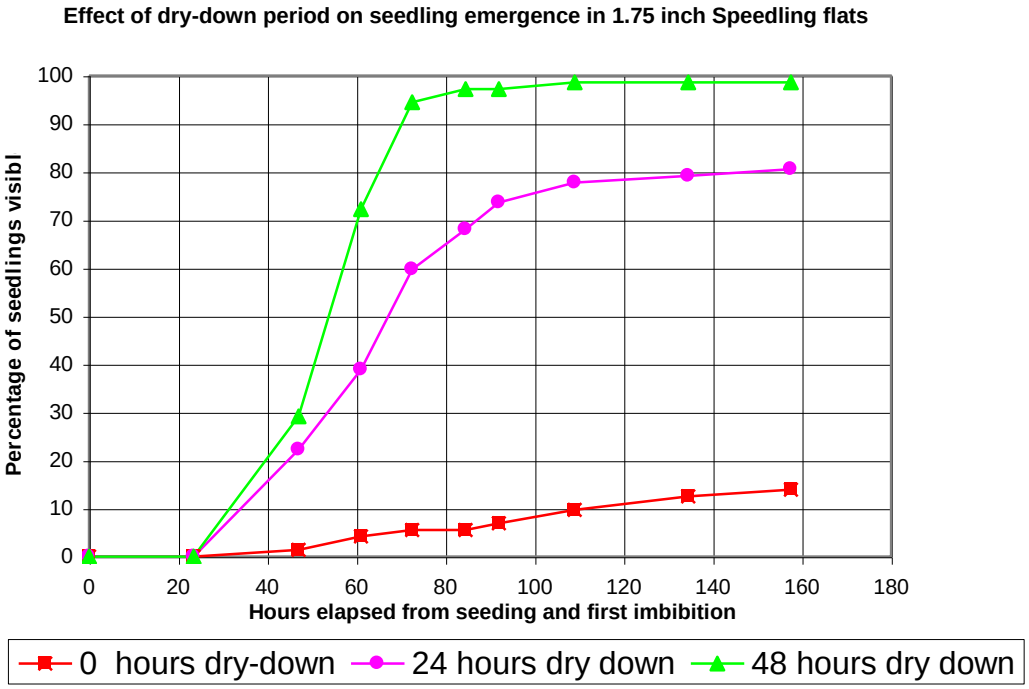


FIGURE 2-8: Effect of 3 dry-down periods between watering-in and flotation on emergence of de-hulled Alrite seed in 1.75-inch flats

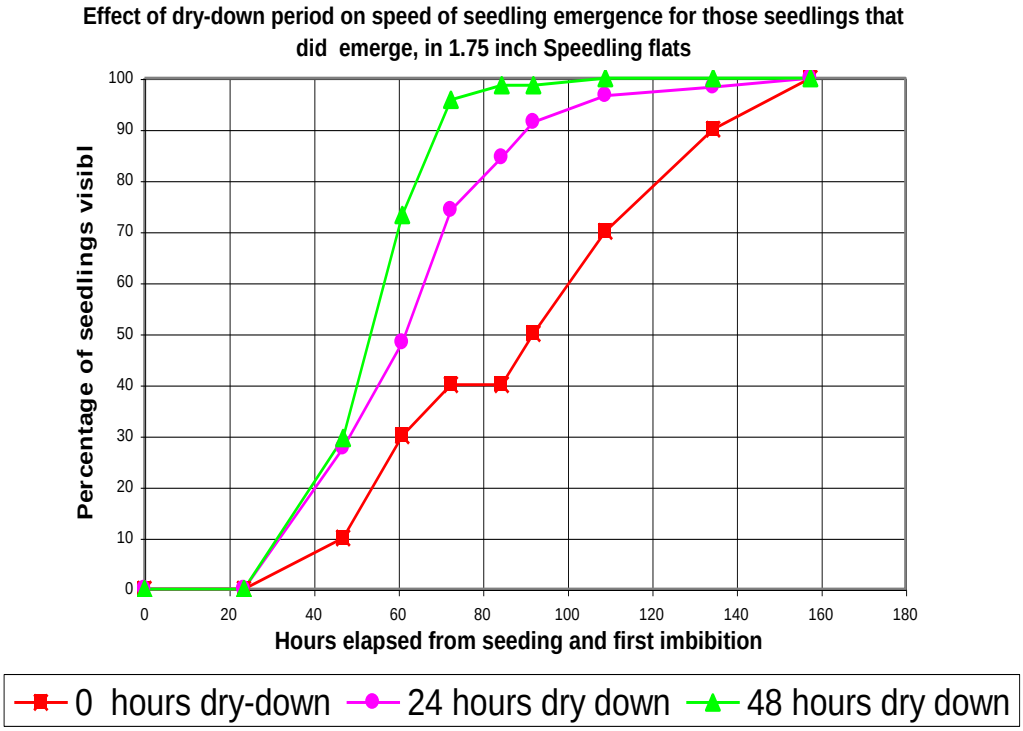


FIGURE 2-9: Speed of seedling emergence for those seedlings that did emerge, after 3 different durations of dry-down in 1.75-inch flats

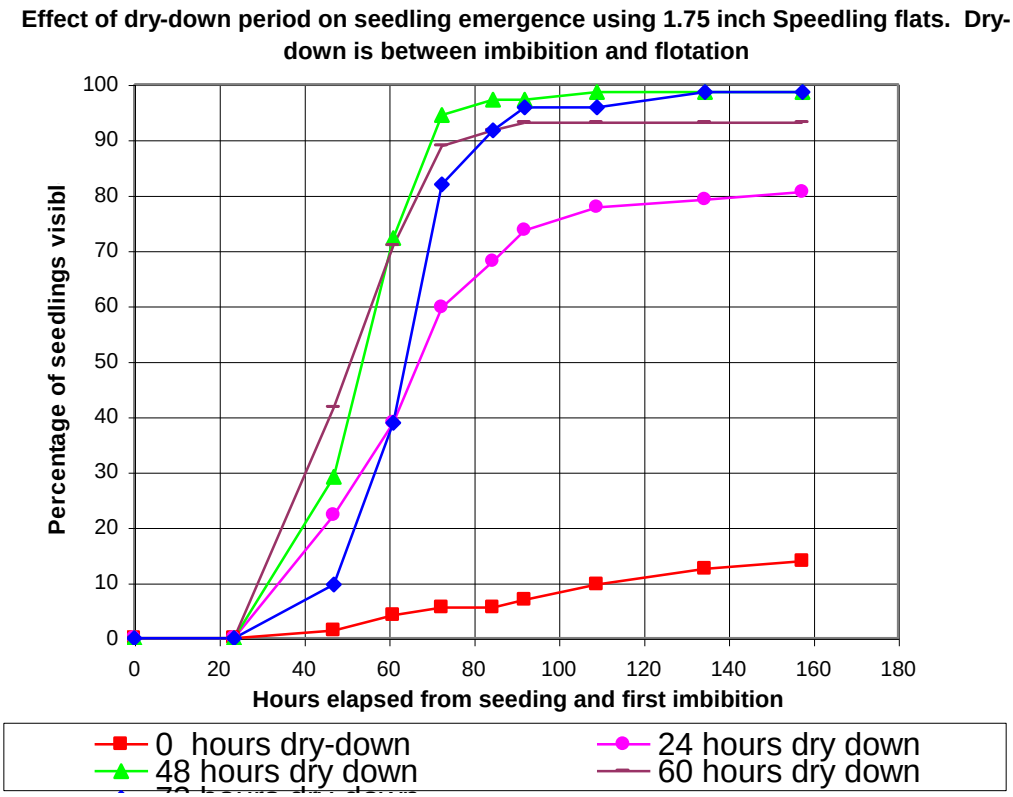


FIGURE 2-10: Effect of 5 dry-down periods between watering-in and flotation on emergence of de-hulled Alrite seed in 1.75-inch high flats.

Figure 2-11 below showing results for the 2.5-inch flats is analogous to Figure 2-8. Germination was 50% when flotation was immediate in 2.5-inch flats; it eventually went up to 92% with a 48-hour dry-down period, and 100% with a 72-hour dry-down. It is hard to say if the result at 48 hours was due to inhibition of germination after re-flotation or experimental error. It is certainly possible it is the equivalent of the effect found in a 24-hour dry down in 1.75-inch flats. In 2.5-inch flats, it may well take additional time for the medium to dry down enough for germination to proceed. This being the case, insufficient time may be left for critical events to take place if re-flotation is at 48 hours.

Figure 2-12 shows speed of germination in the 2.5-inch flats for those seed that did emerge. Speed of emergence was much less affected by the dry-down duration than percentage emergence.

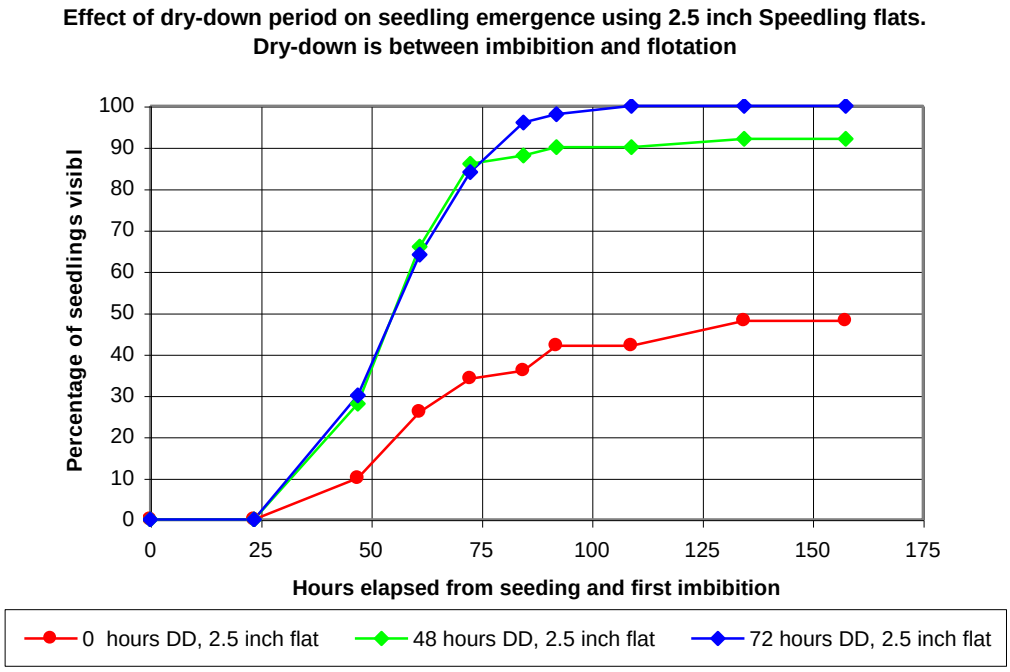


FIGURE 2-11: Effect of dry-down period between watering-in and flotation on percentage emergence of de-hulled Alrite seed in 2.5-inch high flats.

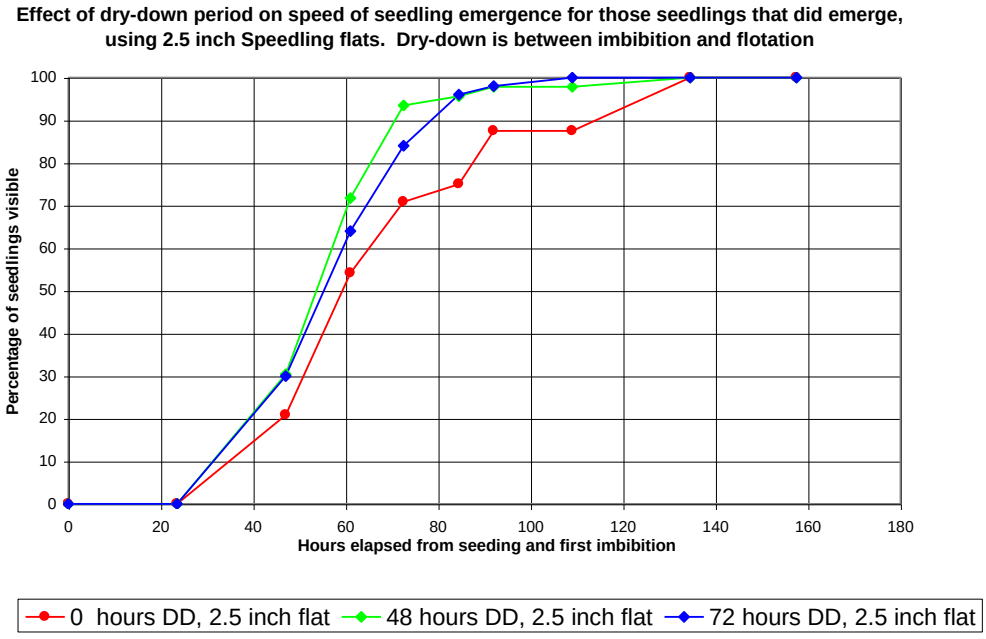


FIGURE 2-12: Effect of dry-down period between watering-in and flotation on speed of emergence for those seedlings that did emerge: de-hulled Alrite seed in 2.5-inch high flats.

Qualitative Data on Seedlings

Some attempt was made to come to grips with the fact that even if seedlings appear above ground, they are not always good ones that will produce vigorous plants. On day seven when the last germination counts were taken, all seedlings were classified in various ways, as shown below. In flats where dry-down duration was sufficient, (5 of 8 flats), germination/emergence percentage was 97%. Of those plants that did emerge, 82% were classified as "good". This percentage was remarkably constant flat to flat, ranging from 78 to 84%. The bottom line is that only approximately 80% of plants can be expected to be "good specimens", even when excellent seedling emergence is obtained, even if it is virtually 100%. Seedlings that were classified as "not good" were further broken down into 5 descriptive classes, namely:

1) Barely breaking ground - therefore very late to emerge,
2) Pop-ups,
3) Deformed - missing parts, broken off, and bound-up/trapped seedlings included here,
4) Runts - not growing well, and
5) Large deformed plants. The last category was a subcategory of deformed plants.

Deformed plants and runts comprised 80% of the bad plants, pop-ups 15%, and very late plants 5%. Incidence of the various defects did not appear to be related to the experimental treatments (duration of dry-down).

Root performance was evaluated at two times. It was in accord with speed of shoot growth or speed of emergence, as one might expect. Roots in the 48-hour dry-down condition for 1.75-inch flats were distinctly further along than other roots.

Biomass at Harvest

Just considering flats where seedling emergence was very high, one can say the 1.75-inch flats outperformed the 2.5-inch flats in total output - an average of 82 g FW per flat versus 66 g. This is explainable in terms of there being more plants per flat in the 1.75-inch flats (72 versus 50 plants). The average plant size for "good" plants was larger in the 2.5-inch flats, again as expected, because of less crowding. Figures were 1.70 versus 1.49 g plant $^{-1}$. There were no significant differences in biomass in flats with comparable emergence. It does not appear, for instance, that use of a 72-hour dry-down had any substantial detrimental effect.

CONCLUSIONS

This experiment determined that immediate flotation was detrimental to both percentage and speed of germination in both 1.75 and 2.5-inch flats, more so in the case of the 1.75-inch flats where germination rate was reduced to 12% than in the 2.5-inch flats where it was reduced to 48%. A 24-hour dry down was still not enough in the case of 1.75-inch flats (germination of 80%), but germination as good as could be was obtained with a 48-hour (or longer) dry-down. For the 2.5-inch flats it appeared that a 48-hour dry-down might be slightly too short, but this was not ascertained with certainty. Use of longer periods of dry-

down up to 72 hours had neither good nor bad effects in either flat type as far as could be determined. In this experiment, it was found that about 20% of seedlings that did emerge had defects of one sort or another, primarily in the category of deformities, breakage, or poor growth rate. Pop-ups were a relatively small percentage of the problem seedlings. None of the defects seemed to be related to the treatments.

Experiment 4: Changes in Moisture Content of the Medium in Flats During Dry-Down

Rationale. In Figure 2-1 of this Chapter, it was shown that if peatlite moisture content was above 5.0 MC (86%, wet basis), germination fell off precipitously in intact seeds. When flats are watered in, MC initially is raised to ~ 7.3 MC at start of dry-down. Even though de-hulled seed is less sensitive to moisture content than intact seed, this is probably too high for germination to continue past imbibition even in de-hulled seed. The results of the previous experiment can be understood in terms of the high initial moisture content of flats after watering-in, and differential dry-down rates of medium in flats of different heights. One can tell from inspection of the color of the surface of soil roughly how dry it is, and how MC is changing, but this is a very imprecise measure. This study was conducted to quantify what is happening to medium moisture content of high-density polystyrene plug trays during the dry-down period following watering in, under the protocol for seeding normally followed (given in detail for above experiments).

Hypotheses. Absolute amount of evaporation from the top surface of flats is expected to be proportional to the wet surface area exposed to stirred air. In general it is expected that evaporation from the soil surface will be at a constant rate while water in the soil is abundant, after which it will slow down as rate of replenishment of that which has evaporated becomes a limiting factor, or parts of the surface dry out. Thus, curves for change of MC over time will have an initial linear phase followed by deceleration. However, it is expected that the light cycle will influence rate of evaporation through a temperature effect, and this will be manifest in the slope of the curve as well.

Rate of change of moisture content (represented as the ratio of water content to dry matter content) is expected to differ from container to container in accordance with the surface area to volume ratio characteristic of the container, and the degree of compaction of the soil in the container. Specifically it is expected the moisture content of 1-inch flats will diminish faster than that of 1.75-inch flats, and that of 1.75-inch flats faster than that of 2.5-inch flats as a result of different surface-area to volume relationships of the cells. The degree of soil compaction (weight per volume) at any given MC would be expected to affect rate of change of MC by the same token, if it differed significantly by flat type. In this case it did not. The manner of bottom drainage and ease of evaporation of water from the bottom surface of flats is also expected to alter rate of change of MC, though in a less orderly manner. It is predicted that use of wicks under the flats will accelerate change in MC significantly during the early part of dry-down, when a direct capillary connection to the soil is briefly present, after which dry-down will become more similar to that in other flats without wicks. (The presence of the paper wick will still provide additional bottom ventilation.)

Method. In this study, the intention was to weigh saturated flats frequently as they dried down in a realistic setting, and compute how average moisture content of the medium contained in the cells of the flat changed over time. The two main candidate flat types in use were 1.75 and 2.5-inch high polystyrene Speedling plug trays (1460 and 1059 plnts m^{-2}); they have different sized cells, so the cell volume to surface area ratio is different. Three each of these flat types were prepared in the normal way, to provide replication. No seed was included. To determine the effect of using a wick to extract extra water from the bottom of the flat, two additional 1.75-inch flats were prepared, to be placed on a paper towel bed during the dry-down period. The paper towel bed extended several inches beyond the flat on all sides to provide an area for evaporation. In order to fill flats in a replicable way, custom tools (called dibblers) were made to compress the soil in the 1.75 and 2.5-inch flats. (The use of the tool also achieves a uniform depth of seeding. For this study, no seed was included.) Speedling flats of 3.0 and 1.0-inch heights are available, and examples were included for interest' sake. A 2.5-inch flat that had been cut down to 1.75-inches was also included. In the three less common flats compression of soil was done by judgment since no custom tools were available to perform this step. In all, eleven flats were prepared as though for producing seedlings, following the usual protocol. They were then fully wetted by bottom watering, and set to dry-down also in the normal way except each flat was placed on an individual plastic tray that could be transported and weighed without disturbance of the flat. To track moisture content, flats were weighed frequently through the typical 48-hour dry-down period and an extra day beyond.

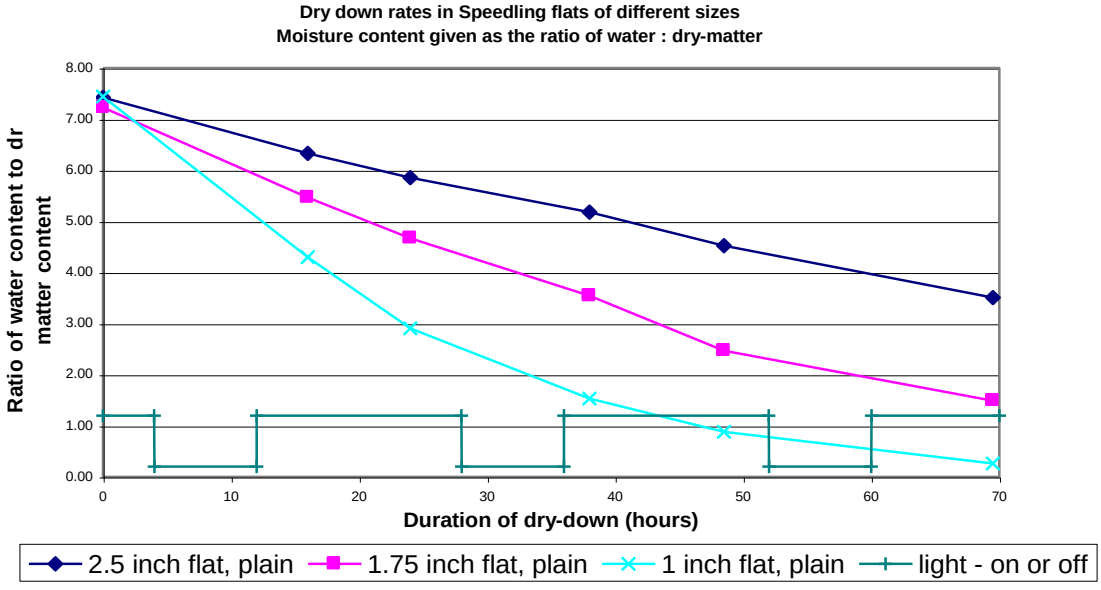


FIGURE 2-13: Course of dry-down of flats of different heights filled with saturated peatlite medium, at 25°C and with a 16-hour photoperiod.

Results. The results, which are presented graphically in Figures 2-13 above, and 2-14 and 2-15 below, broadly met expectations, and supported the hypotheses, but also demonstrated that many factors were influential at the same time, greatly complicating presentation and interpretation of the results.

A key characteristic of flats with respect to dry-down is the cell-surface-area to cell-volume ratio. Water contained in cells is closely correlated to cell volume, but water loss to evaporation is most closely correlated with surface area. Water loss during the first 16 hours was 0.41, 0.38 and 0.43 g cm^{-2} in the 2.5-inch, 1.75-inch, and 1.0-inch high flats respectively. For the 1.75-inch flats with a paper wick under them, it was 0.65 g cm^{-2}. Reduction of moisture content on the other hand, is fastest in cells with the highest surface-area-to-contained-water ratio. The surface-area-to-contained-water ratios of 2.5-inch, 1.75-inch, and 1.0-inch high flats were respectively 0.36, 0.64, and 0.97 cm^2 cm^{-3} H_2O. It can be seen in Figure 2-13 above, it took 12 hours for the medium in the 1-inch high flats to fall to 5.0 MC, 21 hours for the 1.75-inch high flats, and 40 hours for the 2.5-inch high flats. These findings support the explanatory mechanism proposed for the results in the dry-down experiment with respect to percentage and speed of emergence in flats of different heights.

The physical dimensions of cells and characteristics of the flats are given in Appendix 2-B.

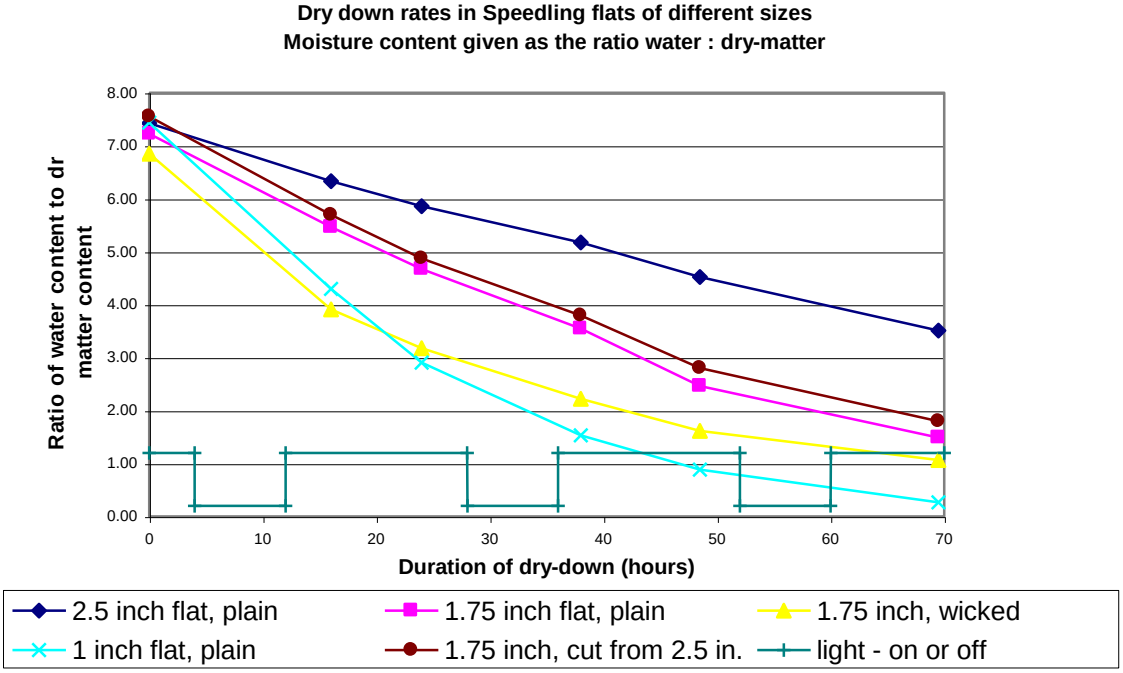


FIGURE 2-14: Course of dry-down of flats of different heights filled with saturated peatlite medium, at 25°C and with a 16-hour photoperiod.

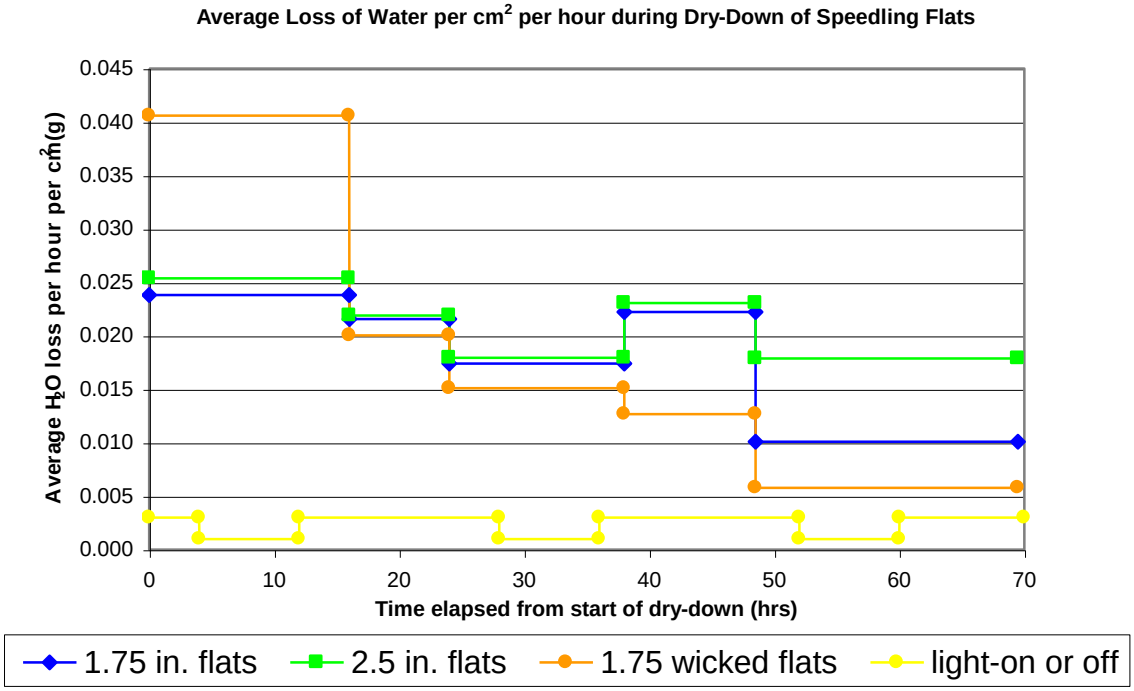


FIGURE 2-15: Average loss of water per unit surface area per hour, over the time period between measurements, for selected flats.

Experiment 5: Spinach Seedling Emergence at Different Peatlite Moisture Contents

Introduction. In studies on effect of flat height and duration of dry-down after watering-in, it became apparent watering in the flat after seeding was delaying the start of germination besides adding a labor step to the seedling production protocol. This led to the question: Is there a way to avoid watering-in the seed, and is there a medium friable enough to fill small cells in plug trays and at the same time with sufficient moisture content for seed to imbibe and germinate fast? And a second question: Would there be any penalty to seeding into a medium with controlled moisture content, and what would be the ideal moisture content? Two studies were conducted to make a preliminary survey of these questions. This experiment was designed to replicate and advance upon the two preliminary experiments investigating the effect of moisture content on seedling emergence using de-hulled seed. It was also an extension of the experiment on germination of intact seed at different moisture contents (MCs), reported earlier.

The series of moisture contents considered here included an additional low level, 0.50 MC, and an additional high level, 7.0 MC. The new MC levels were included to pinpoint the extremes at which seed germination will not take place. (MC here refers to the ratio of water weight to dry matter weight in the medium, standing for Moisture Content - Dry basis. This is also called the Gravimetric water content.) A procedure was also included by which to determine the air-filled pore space under each MC treatment level. The main thrust of this experiment was directed at determining a seedling production protocol for use with

de-hulled seed. Two containers of de-hulled Alrite seeds were prepared at each MC level to provide internal replication. However, it was decided to also include intact seed for a direct comparison of the two types of seed under the same experimental procedures. A series of single containers, each with 16 seeds, was prepared using intact seed of the cultivar Eagle. (Intact seed of Alrite from the same seed lot as the de-hulled seed was unfortunately not available at this time, nor de-hulled seed of the cultivar Eagle.)

Hypotheses. In the preliminary experiments using de-hulled seed mentioned, no seed emerged at 0.33 MC, but some did at MC of 0.67. It was expected no seed would emerge at the MC of 0.50 (33.3%, wet basis). At the wet end of the range, no seed emerged at a MC of ~ 6.0 in one experiment, but in another about 50% emerged. This was presumably because of differences in seeding technique this close to the wet limit for germination of de-hulled seed. In the current experiment it was predicted very little seed would emerge at 6.0 MC and none at 7.0 MC. It was expected the intact seed would start to emerge later than the de-hulled seed, and then take longer for the seed planted to complete emergence. It was also expected percentage emergence would in general be lower, and also be more sensitive to MC than would be the case with de-hulled seed (based on prior work in the CEA program).

Method. Two bushels of peatlite medium were prepared following a standard procedure, and the moisture content of the mix was estimated based on oven-dried samples. Portions of this bulk quantity of medium were used to prepare samples of peatlite at each of the 10 moisture content (MC) levels desired for the experiment. The target moisture contents for the experiment were 0.375, 0.50, 0.67, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0. The medium lots with different MC levels were prepared by adding calculated quantities of RO water. They were equilibrated for 24 hours before actual seeding was performed. Bulk density was determined immediately after mixing of samples, and at time of seeding 24-hours later. At time of seeding, a small aliquot of equilibrated medium from each of the treatment conditions was used to determine the density of the "solids" in the medium (solids and associated water) by Archimedes' displacement method. (A generous predetermined volume of water was poured onto a known weight of medium in a graduated cylinder, or conversely the medium was added to the water. The whole was agitated until all air was displaced, and the new volume recorded. By comparing final volume and starting volume of water, the volume of "solids" part of the medium added could be determined.)

Seeding followed the same procedures as those reported in detail elsewhere, using a standard mass-produced container as a germination box. Seed was carefully selected prior to the experiment by rejecting extremes of size and looking for defects. Seed so selected was divided into lots of 16 seeds, which were then randomly assigned to the treatment levels. In all, thirty groups of 16 seeds were prepared in this way. Germination boxes were filled to the brim with medium and then heaped a little more. The medium was settled by gentle shaking. The excess was cut off with a straight edge. A specially designed tamper was used to compress the surface exactly 3/8 inches, using the lip for a reference. 16 seeds were sown in a grid

using a template for accurate positioning. The 3/8th tamper was used to press the seeds into the soil surface. Visually it was checked that all seeds lay on their sides. More medium was added and again the excess cut off with a straight edge. Another tamper was used to firm the medium covering the seed - this time compression was to 1/4 inch below the rim - after which the medium was permitted to spring back to its natural level. Thirty germination boxes were prepared in this way, three for each moisture content level. Two boxes were assigned to de-hulled seed, and one to intact seed. The germination boxes were covered by a loosely fitting lid, and then placed inside larger containers also with lids, two at a time. Some water was included in the outer, larger container. The larger containers were themselves placed in two very large well-insulated containers in darkness and with free air circulation in a temperature controlled environment at 25°C. As a precaution, during the first week of the experiment the middle-sized containers were systematically rotated in position daily.

Seeding was accomplished in late afternoon. Observation of seedling emergence commenced a day and a half later. Two observations were made each day over the first three days of seedling emergence when germination activity was greatest, and thereafter at progressively longer intervals. When all seedlings had emerged in a particular box, the lid was removed to permit upright growth. No water was added. As part of the routine, all signs of seed life were observed and recorded, which is to say bumps in the soil surface as well as sighting of the seed or seedling itself. These were distinguished. Seedlings were also classified and counted as "normal" or "pop-ups", in which the root rather than penetrating the soil is blocked at the tip and grows above ground. When emergence was complete, seedlings were classified as to whether they had shed their seed coats or not. In those that retained their seed coats, three categories were recognized: Both cotyledons trapped at the tip by the seed coat (TC2), Seed coat attached to the tip of one cotyledon (TC1), and Seedling trapped around the middle by the seed coat (TCM). On the last day of the experiment, those seeds that had failed to germinate were excavated and inspected to see if any root emergence had started, etc. The germination boxes were all weighed again to see what water loss had occurred over the duration of the experiment. Medium from one of the three boxes in each moisture content level was then oven dried to determine the exact dry weight of medium that had originally been used, and thus permit precise determination of the moisture content that had been achieved, and see how closely it matched the intended moisture content.

Note on Air content determination. A given sample of peatlite at a specified MC has a fixed mass, but its volume depends on how compressed it is, how much water it has taken up etc.. The amount of air in the pore space also depends on how compressed the soil sample is. One could compress a sample to a certain degree, and then proceed to determine what the air content was for that particular volume. This could be done by slowly adding just enough water so that the whole was saturated and no air space was left, and measuring the amount of water added. However, in many cases, this is made difficult because of a tendency for the lighter parts of the medium to float; the medium has to be held down. And there also is a tendency

for some air bubbles to get trapped. A solution is to add water in excess and stir the medium-water mix to ensure all free air has left the mixture. The new volume of the soil-water mixture and the volume of water added need to be recorded. If more water is added than the increase in volume, then the difference equals the amount of air-filled pore space available for the water to fill before the volume of the mixture increased. Another approach is not to bother about compressing the mixture to any particular bulk density, but to instead concentrate on determining the density of the solids and fluids (particles and free water) comprising the mass, on the assumption the density of these components will not vary under the range of compressions used in practical horticulture. If we drop a known weight of medium (of a known MC) into a known volume of water, it displaces a volume of water equivalent to the volume of the non-air parts of the medium (solids and the film of water associated with the solids). Knowing this volume for the sample permits calculation of "solids" density, either as grams per cm^3, or cm^3 per gram. If now samples with this same MC are compressed various amounts and bulk densities are calculated, the part of the final volume occupied by solids (and integrated liquids) and the part occupied by air can easily be calculated from the bulk density coupled with the "solids" density. The air content so calculated is primarily that in the pore space that is easily removable by the displacement method.

Caveats. When preparing medium samples of different MCs, it is necessary to allow the water to be integrated into the peat, which can take several hours. Generally, 24-hours would seem a safe and sufficient time. The same is likely true for vermiculite. The volume of the fibrous peat material changes considerably as water is taken up into the fibers, and so too does density of “solids” until equilibrium is reached.

At a microscopic level, both ingredients of peatlite no doubt include air in the interstices of their fine internal structures. Vermiculite is expanded by the inclusion of air between the layers, much like puffed cereal. Peat reverts somewhat to its original plant form when wetted. In both cases, it can be expected water infiltrates the interior spaces to some extent, but this is probably quite a slow process. When volume of "solids" is determined by the displacement method described above, it is unlikely that the air inside the particles gets displaced to a significant degree in the short term, but nevertheless, it is advisable to conduct the procedure fairly quickly. By the same token, if the medium is compressed to a high degree, it is possible for the air (and water) that resides internally to be squeezed out. Under extreme compression, the "solids" density will change as a consequence. However, in horticultural seedbed preparation it is not envisaged that excessive compression will be used (and it certainly was not used in this experiment), and if the shapes of medium particles are momentarily distorted by compression, they are likely to spring back immediately.

Results. Results are presented graphically in charts plotting the time-course of seedling emergence for each MC level. Cumulative percentage emerged is plotted against time elapsed since seeding. Results for

the de-hulled seed are presented on separate charts from those for intact seed. To see differences better, the 10 MC levels are split into three groups, dry MC levels, wet MC levels, and optimal mid MC levels. In the case of de-hulled seed, results from the two reps were combined, inspection having indicated they were very similar to each other in respect to time-course of emergence. Thus, the behavior of 32 seeds is represented by the percentages in the graphs for de-hulled seed, but only 16 seeds in the case of intact seed. The medium for each MC level was made up assuming the MC of the bulk source was 0.375. It was later determined to be closer to 0.300. As a result, actual starting MC levels were systematically lower than the targets, as tabulated below. Since the deviations are reasonably small, for ease of presentation the nominal MC levels are used throughout the discussion.

Results for De-Hulled Seed, Alrite.

1. Optimal MC

Seedling emergence performance for de-hulled seed is best and almost identical under the MC levels 3.0, 4.0, and 5.0, as demonstrated in Figure 2-16. It commences at about 48 hours after seedling, and is 95% complete in 16 hours from when it commences for the 4.0 and 5.0 MCs, and 20 hours for the 3.0 MC.

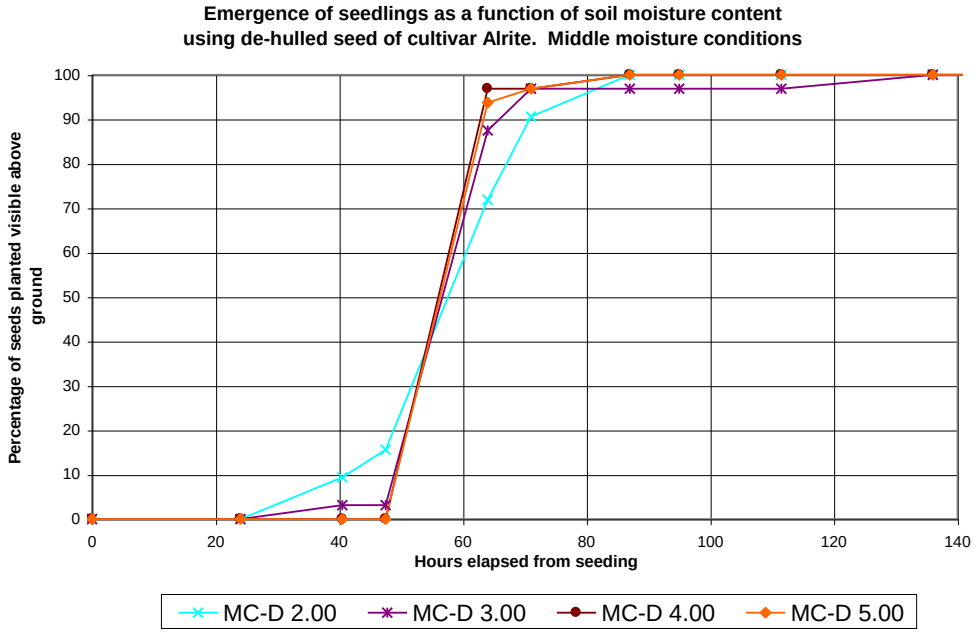


FIGURE 2-16: Middle moisture conditions: emergence of seedlings as a function of soil moisture content, using de-hulled seeds

2. Wetter conditions

In wetter conditions, represented in Figure 2-17, emergence appears to be delayed and slowed at 6.0 MC, then completely shutdown at 7.0 MC. At 6.0 MC, emergence appears to have begun 10 hours later than the less wet conditions (i.e., around 58 hours from seeding. Seedling emergence for this and

several other treatment levels began during the night, between observations. This figure was not observed, but rather is an extrapolation). It then took 30 hours to achieve 95% emergence, compared to 16 or 20 hours at MC 3.0, 4.0, and 5.0. Thus, it would appear that at 6.0 the soil is becoming too wet for optimal performance. Between 5.0 and 6.0 MC, volumetric air content of the peatlite dropped from 43% to 17% (see Table 2-1 below). From 6.0 to 7.0 it dropped further from 17% to 3%. It is possible that the reduction in air content was responsible for the slowed germination at 6.0 MC and the complete cessation of germination at 7.0 MC.

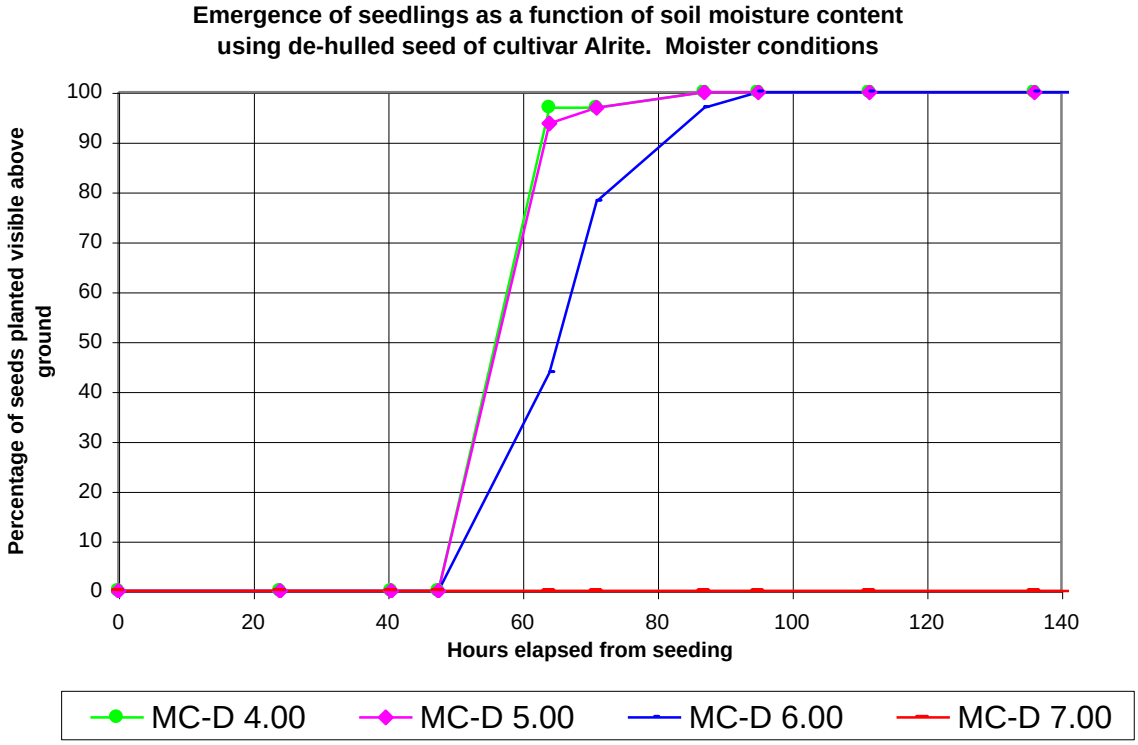


FIGURE 2-17: Wetter moisture conditions: emergence of seedlings as a function of soil moisture content, using de-hulled seeds

3. Drier conditions

At the dry extreme, there was no seedling emergence at 0.375 and 0.50 MCs, although at 0.50 MC some bumps were visible at the soil surface eventually. Excavation at the end of the experiment showed at 0.375 MC seed looked little different than dry seed. At 0.50 MC, most seeds had swelled but few radicles had managed to burst the seed coat, and if they had, they made no further progress. Figures 2-18 illustrates performance at 0.50, 0.67, 1.0 and 2.0 MCs. It can be seen as the soil became moister, emergence began progressively earlier and reached 100% sooner. However, the slope for the period of most rapid emergence did not change greatly suggesting once imbibition was complete the course of emergence went forward at close to the same rate.

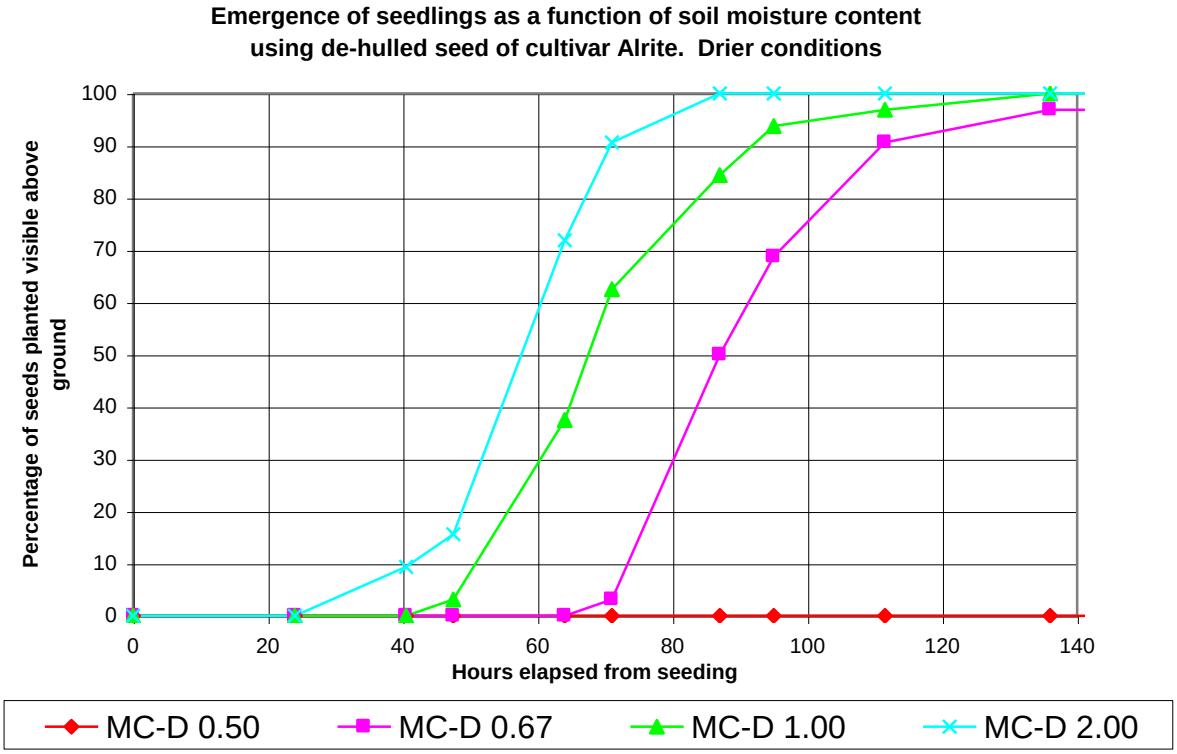


FIGURE 2-18: Drier moisture conditions: emergence of seedlings as a function of soil moisture content, using de-hulled seeds

What the graph does not show is that 17 out of 32 seedlings were pop-ups at MC of 0.67, 12 at MC I.0, and 5 at MC 2.0. There were no pop-ups at higher MCs and wetter conditions. Since pop-ups appear above ground prematurely because upward propulsion is from the root rather than shoot (hypocotyl), the time of onset of emergence is deceptive in these cases. By examining pop-up data, shifting percentages accordingly and using judgment to extrapolate, one may estimate that actual onset of emergence, had the seeds been held down better, would have been 85, 65, and 53 hours from seeding in MCs of 0.67, 1.0, and 2.0 respectively. 95% emergence was completed approximately 45, 35, and 25 hours from first emergence respectively. It will be recalled the equivalent values for optimal MCs of 3.0, 4.0 and 5.0 were 48 hours from seeding to onset of emergence and 16 to 20 hours from start of emergence to 95% emergence. Most of the figures given in the text above may be seen tabulated below.

Summary for Alrite De-Hulled Seed. All the curves may be seen at once in Figure 2-19. Some striking facts here are that germination and seedling emergence took place over a very wide range of MCs, from 0.67 to 6.00, and it was eventually 100% if any emergence occurred at all at that level. When performance for intact seed is considered, the uniformity of de-hulled seed performance will be more apparent by contrast.

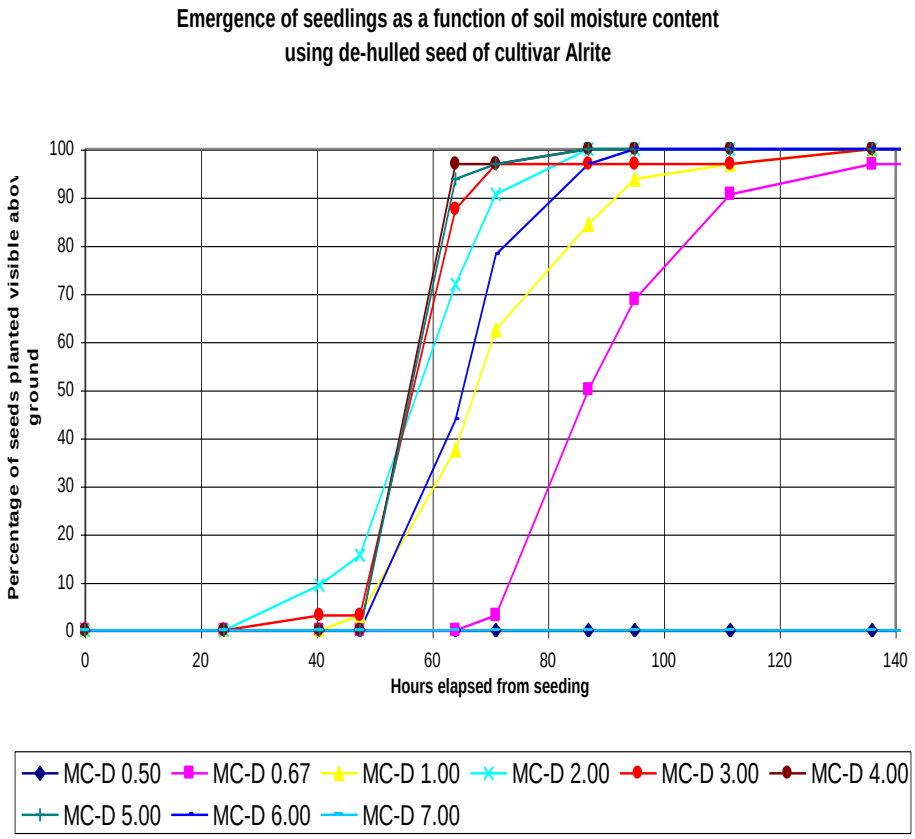


FIGURE 2-19: All moisture conditions: emergence of seedlings as a function of soil moisture content, using de-hulled seeds

The greatest synchronicity of emergence is clearly in the 3.0 to 5.0 MC range, and it is in this range we should germinate seed.

In practice, one is restricted in the MC range one can use for seedling production, despite the apparent wide choice. One would not want to use moisture contents lower than 3.0 because the likelihood of pop-ups occurring is substantial below this level. Pop-ups typically fail and die early on. On the other hand, it is difficult to work with moisture contents much above 3.0 because the medium becomes sticky and it is difficult to fill cells uniformly or avoid making large cavities. However, if a moisture content of about 3.0 is used for ease of flat filling, it should be quite safe to wet the medium further after seeding - for instance with some sort of misting system, as long as the ultimate moisture content is kept below 5.0. This represents a very large safety margin, so misting could well be incorporated into a practical seeding procedure.

Only at the very highest moisture content was the occurrence of seed coat retention reduced. Manipulation of moisture content thus does not appear to be a viable means for dealing with this problem. Reduced temperature is more promising.

Obtaining 100% emergence, and high quality seedlings as well, in this case depended on careful human seed selection. In de-hulled seed, even if almost all seeds are alive, very often, there are weaknesses in the seed coat caused by the de-hulling process, and if the cotyledons break through such a weak point, the root most often fails to emerge in a proper and timely fashion. The radicle generates the force to puncture or split the seed coat by internal pressure; if the seed coat is broken elsewhere, the pressure does not develop. In our view it was warranted to select seed carefully for this experiment because the purpose was understanding the effect of moisture content on seedling emergence; it was just as well not to confound the results with failures having more to do with seed condition than with moisture content. But in commercial production, it is doubtful it would be cost effective to visually inspect every seed for defects using human inspectors. It may be possible to use machine imaging or other seed technology to achieve close to the same result. In any case, an economic optimization study will need to be performed to see how much seed it may be cost effective to reject, and how much effort to put into seed selection.

Results for Intact Seed of the Cultivar Eagle

1. Optimal MC

The best performance with the intact seed was at MCs of 2.0, 3.0 and 4.0, as represented in Figure 2-20 below. The best germination rates for intact seed were 15, 14, and 14 seedlings out of 16 seeds planted, or around 90%, for MCs of 2.0, 3.0 and 4.0 respectively. For intact seed over one year old, this was an excellent germination rate; one could not expect more.

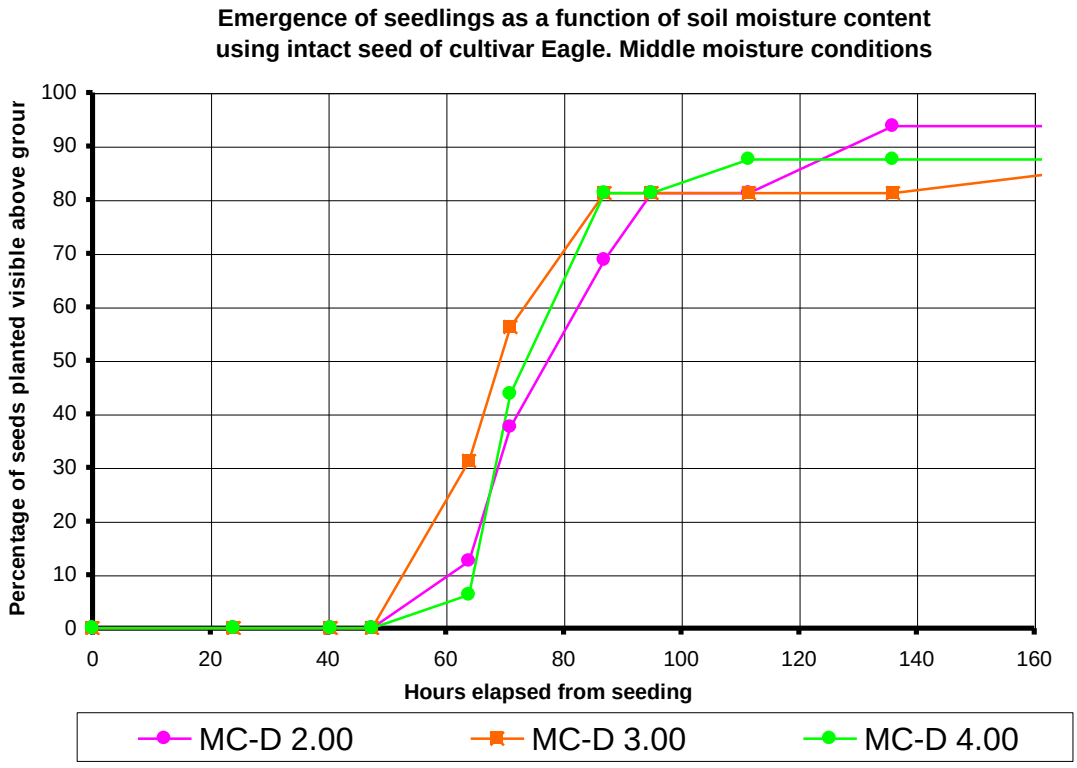


FIGURE 2-20: Optimum moisture conditions: Emergence of seedlings as a function of medium moisture content in intact seed of cultivar Eagle

Emergence began about 60 hours after seeding. If one extrapolates the nearest gradient, one might suggest onset times of 61, 58, and 63 hours for 2.0, 3.0 and 4.0 MCs respectively. It took an additional 27 or so hours for 90% of those seeds that were going to emerge to complete emergence. In comparison to de-hulled seed (Alrite) in the MC 3.0 condition, start of emergence was delayed approximately 10 hours, and speed of emergence was approximately 10 hours slower. It would appear the intact seed of this cultivar took about 20 hours longer to reach the same point as Alrite. But then 90% of seed planted had emerged for Alrite, but only 80% for Eagle on account of the difference in germination rates. (See also Table 2-1 below.) Two of the 14 seeds that emerged in the 2.0 MC condition were late-occurring pop-ups. No other pop-ups occurred in intact seed at any MC level.

2. Wetter conditions

Figure 2-21 below shows that emergence percentage fell from circa 90% at MC 4.0 to circa 60% at MC 5.0 and then 20% at MC 6.0. One can assume 0% for MC 7.0 based on the results for Alrite in this experiment and much previous research. It may be of significance that if emergence had not occurred within 4 days, it never occurred. Quite possibly seeds rotted or lost viability in other ways when maintained longer than this under very moist and warm conditions. Emergence appears to have commenced about 60 hours after

seeding for the 5.0 MC condition, and 70 hours or later for the 6.0 MC condition. After emergence started, it took 40 hours to achieve 90% emergence for those seeds that did finally emerge in the 5.0 MC condition. Although the percentage of seeds that emerged at these MCs differ, within the precision level of the sample sizes, speed of emergence for those seedlings that did emerge looks as though it was the same across the moister MC conditions, and fairly rapid. However, one can say MCs above 4.0 had a profound detrimental effect on percentage emergence of intact seed, in contrast to de-hulled seed, in which the detrimental effect on emergence began only above MC 6.0.

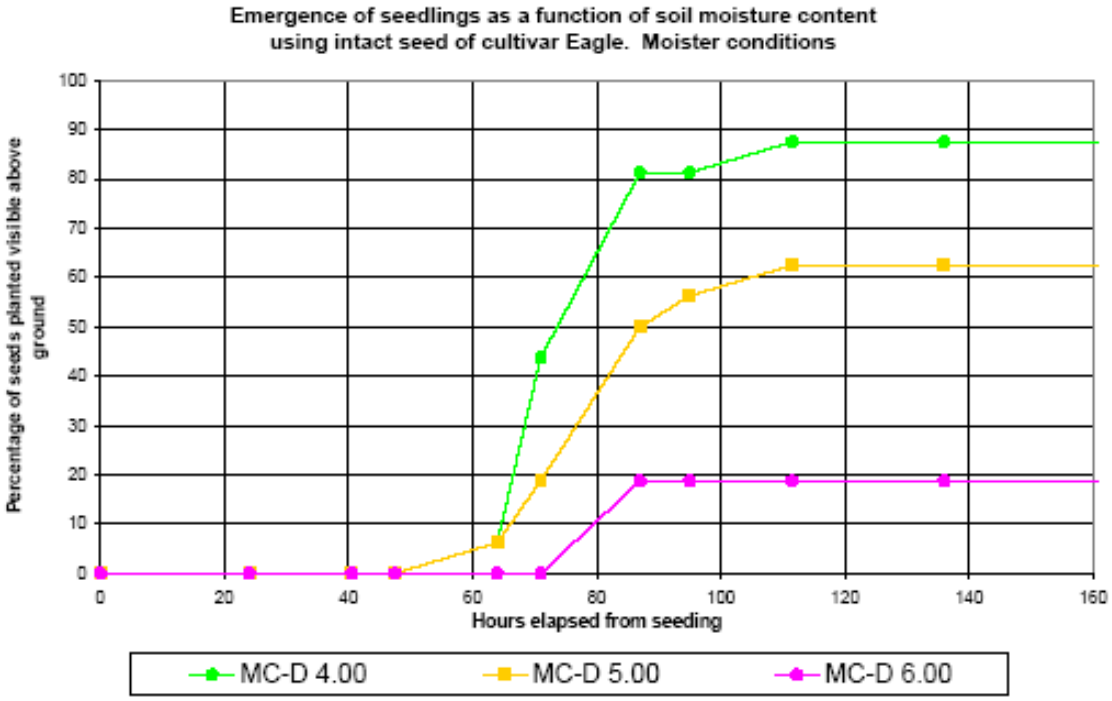


FIGURE 2-21: Wetter conditions: Emergence of seedlings as a function of medium moisture content in intact seed of cultivar Eagle

3. Drier conditions

The effect of restriction of available moisture by the drier MCs was to delay start of emergence progressively more and more and also to stretch out the time it took for emergence to be completed. At 2.0 MC, emergence started 60 hours after seeding; at 1.0 MC, it started 80 hours after seeding and at 0.67 MC at 120 hours after seeding. No emergence or even signs of impending emergence were found at MC 0.50 and MC 0.375 upon excavation. At MC 0.67 and 1.0, emergence continued as long as the experiment did (17 days), as can be seen in Figure 2-22. (Note change in time scale for this figure.) However, whereas in the 1.0 MC condition full germination for this seed lot (90%) was achieved in the 17-day period, in the 0.67 MC condition, only 55% was achieved. Remaining seed in the 0.67 condition showed no radicles or signs of impending germination, though the seed (pericarp) was opened. One cannot say for sure whether

additional emergence would have occurred given time. Presumably, it would have, so long as the medium was prevented from drying out and seed remained healthy.

With regard to speed of germination, speed was similar up to about 50% emergence (of those seeds that would emerge), in 0.67, 1.0 and 2.0 MCs, but after that emergence slowed considerably in the drier conditions. For 90% of the seeds to emerge that ultimately did emerge (in 17 days), it took 180 hours at MC 0.67, 240 hours at MC 1.0 and 57 hours at MC 2.0, measured from start of emergence.

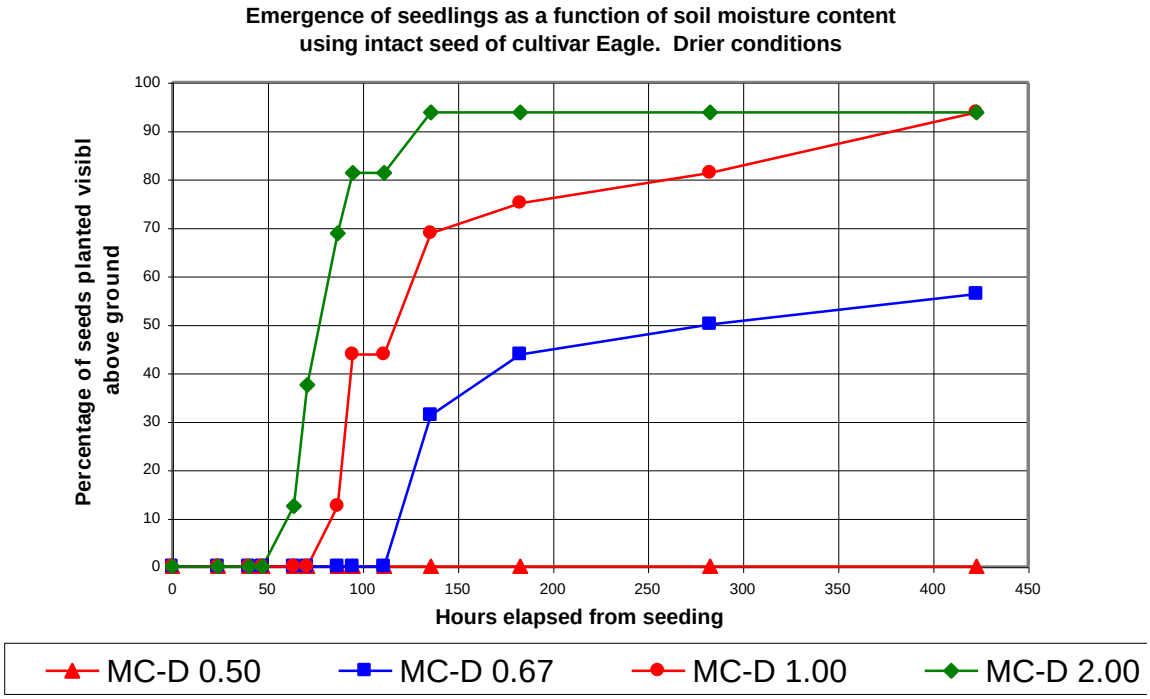


FIGURE 2-22: Drier conditions: Emergence of seedlings as a function of medium moisture content in intact seed of cultivar Eagle

Summary for Eagle Intact seed. The salient features of seedling emergence over the full range of MCs can be seen in Figure 2-23 below. In increasingly wet conditions, there was a substantial reduction in germination percentage, and we also see a small delay in start of emergence. Furthermore, emergence stopped abruptly after 4 days. In increasingly dry conditions, we see a substantial delay in start of emergence, and greatly slowed speed of emergence, with emergence continuing weeks beyond seeding. It is not clear if there was a reduction in germination percentage, even in the driest condition in which seed came up at all. In terms purely of speed of emergence it is notable that except for the extreme conditions, emergence commenced at nearly the same time, about 60 hours from seeding, and speed, for most of the emergence that was going to happen, was very similar.

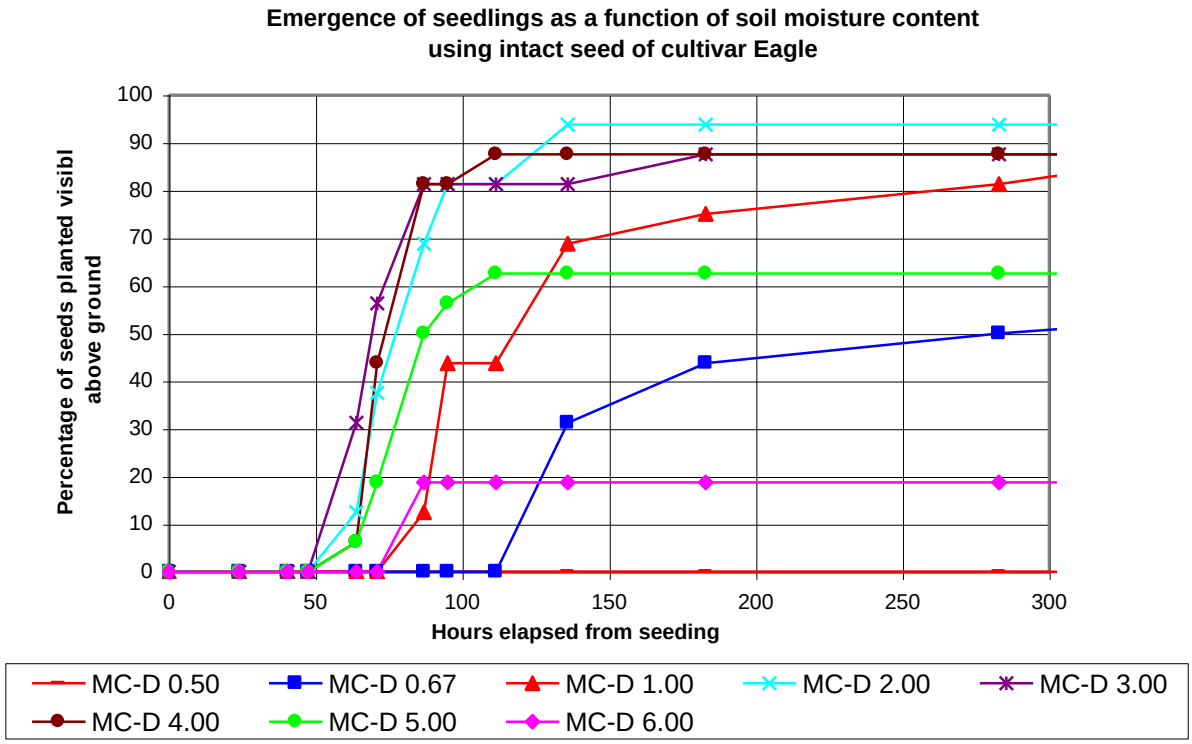


FIGURE 2-23: All conditions: Emergence of seedlings as a function of medium moisture content in intact seed of cultivar Eagle.

De-Hulled Versus Intact Seed; Comparison of Performance. Figure 2-24 presents emergence performance for both types of seed at MCs of 0.67, 3.0, and 6.0, representing the middle and the extremes of MC. These data are the same as already presented in separate charts, but have been brought together for direct visual comparison between intact and de-hulled seed. Note particularly the bunching of heavy solid-line curves for the de-hulled seed, as opposed to the wide separation of light dashed-line curves for intact seed.

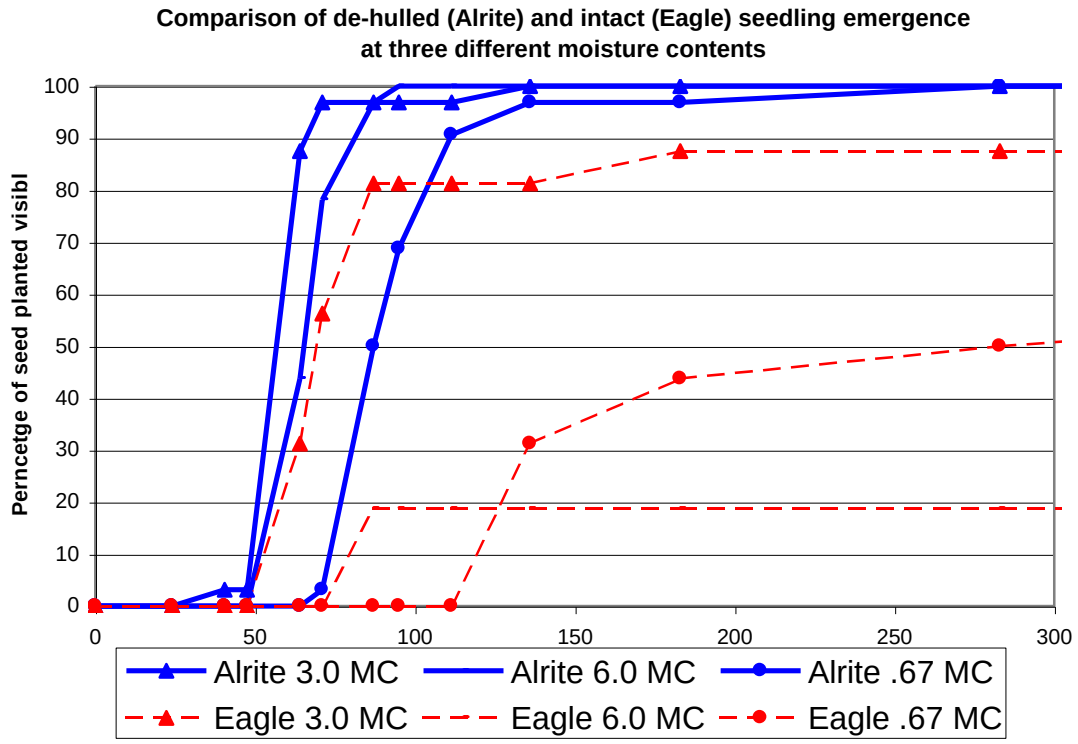


FIGURE 2-24: Comparison of de-hulled and intact seedling emergence performance at a range of medium moisture contents

Tabulated Data. Table 2-2 below provides data characterizing the medium (the independent variables), as well as summary statistics for seedling germination and emergence performance. Medium moisture level is given as intended and as actually achieved i.e. determined post hoc. Bulk density and density of the wet medium solids ands associated water films were determined empirically for each MC level, as previously described, which permitted calculation of volumetric contents of air and free water in the soil volume.

The seedling emergence data are presented in the lightly shaded right-hand section of the table. Leading off emergence data are two conventional indices used in germination work, Germination Rate and Germination Speed, though in this case we are dealing with emergence rather than germination. Germination speed is often indicated by time from imbibition to 50% germination, aka T50. Between de-hulled and intact seed, there is a large difference in speed of emergence after emergence begins. T50 does not indicate this very well, so T90 is also given, namely the time from imbibition to 90% emergence of seed planted.

Values for MC 3.0 are highlighted since this is the best peatlite moisture content for practical use without watering in.

Since, in production, seed trays or flats will be incubated in a special environment until emergence (in which the cost of space use is much lower than in the greenhouse), it is of interest how long it takes until emergence begins, when the need for light and greenhouse space also begins. Time to start of emergence is also given, which has been designated T-Zero. (Start of emergence inevitably falls sometime in the interval between when observations are made. Unfortunately, in many instances in this experiment the start of seedling emergence happened during a particularly long such interval, namely overnight. Considerable effort has gone into making plausible estimates of when T-Zero did occur in these instances, but it is still uncertain.)

For production purposes, we are particularly concerned about simultaneity of emergence. It probably does not matter how long it takes for seedling emergence to start (although we need to know), because storage of flats during this period can be made inexpensive. In fact, it may be helpful to time seedling emergence to fit nicely into daily work routines, in which case it may even have to be slowed down. Or if pop-ups or seed coat retention are affected by temperature, as appears to be the case, it might be desirable to reduce temperature and slow down emergence for the benefits it brings. But once emergence starts, we would like most of the plants to come up at the same time, so that large disparities in seedling size are avoided.

Several different measures of simultaneity of emergence are possible; here we have presented just one, the time it takes for 90% of the seedlings that are going to emerge to emerge, measured from the start of emergence. Because germination rate was 100% in Alrite, this figure is also the time it takes for 90% of seed planted to emerge, measured from start of emergence, a very informative figure. Because germination rate varied with MC condition in Eagle, the actual proportion of seed planted emerging in this time period is something less than 90%. It is found by factoring the germination rate by 90%.

Target MC Level dry basis	Measrd MC Level dry basis	Bulk Density of medium at start of expt.	Density of wet medium solids	Volumetric water content	Volumetric air content	Germination Percent		Germination Speed				Time to Start		Time for Emergence	
						Percentage emergence in 17 days		Hrs from seeding for 50% of seeds sown to emerge. T50		Hrs from seeding for 90% of seeds sown to emerge. T90		Hrs from seeding to start of emergence T-Zero		Hrs from start of emerg. for 90% of those that emerged to show	
						de-hulled	intact	de-hulled	intact	de-hulled	intact	de-hulled	intact	de-hulled	intact
MC-D	MC-D	g ml^{-1}	g ml^{-1}	ml ml^{-1}	ml ml^{-1}	Alrite	Eagle	Alrite	Eagle	Alrite	Eagle	Alrite	Eagle	Alrite	Eagle
0.375	0.29	0.17	0.45	0.04	0.64	0	0								
0.50	0.40	0.18	0.52	0.05	0.66	0	0								
0.67	0.57	0.20	0.53	0.07	0.62	100	56	87	285	111	(n/a. <90%)	85	120	26	180
1.0	0.89	0.23	0.58	0.11	0.61	100	94	67	120	92	380	65	80	27	240
2.0	1.8	0.32	0.81	0.20	0.61	100	94	58	80	71	130	53	60	18	58
3.0	2.8	0.38	0.91	0.28	0.59	100	88	57	70	65	150	48	58	17	27
4.0	3.7	0.47	0.98	0.37	0.53	100	88	57	75	62	150	48	63	14	21
5.0	4.7	0.59	1.04	0.49	0.43	100	62	57	85	62	(n/a. <90%)	48	56	14	39
6.0	5.6	0.90	1.09	0.76	0.17	100	20	65	(n/a. <50%)	72	(n/a. <90%)	58	78	14	(n/a. n = 2)
7.0	6.7	0.97	1.00	0.84	0.03	0	0								

Table 2-2. Characteristics of the medium (independent variable) and indices of germination and emergence

DISCUSSION

Characteristics of the Independent Variable. In this experiment, MC was systematically varied, but it is really more generally the condition of the medium that must account for the differences observed. Along with the different levels of MC went different bulk densities, different air contents and different volumetric water contents, not to mention different penetrability, different adhesive properties, different nesting of particles and other factors. None of these bears a one for one relationship to the others, because the physical properties of the medium components, peat moss and vermiculite, are affected by moisture content, and so as a result are the other measures. One also has to think in terms of the microenvironment of the seed. In the wettest conditions, say 5.0 upwards, it is possible there is some vertical gradation in moisture content. If enough water exists in a thick enough film on the outside of particles, and the films are connected through the soil column, water is drawn down by gravity so the true MC at the seed is a little lower than the average MC. In a very wet medium, there is also some concern that the particles do not pack together in the same way as in a friable medium. First large voids will tend to be formed, and then, when they collapse, particles will be arranged perhaps in a systematically different way than other conditions. There is also some concern that the degree of compression of the soil above and below the seed varies enough across MC conditions to affect seedling performance in breaking out of the soil and penetrating the soil. None of these are large concerns, as it is likely the effects are quite small if they exist at all, but it is advisable to do supplementary work to discount them as sources of error.

In this experiment, enough data was collected to calculate bulk density, volumetric water content, and air content, as has been described above. These indices have the advantage over moisture content as possible explanatory variables that they take into account the volume and degree of compression of the medium, whereas purely weight-based measures do not. Volumetric water content also has a systematic relationship to water potential that can be determined empirically for a particular medium. Volumetric water content can be measured directly with an existing electronic device, the theta probe. In large-scale commercial operations, it might be worth investing in this instrument if the moisture content of the medium is deemed critical.

If one wants a measure capable of accounting for the full range of responses of seed to the substrate condition, volumetric water content could perhaps serve, because it changes over the whole range of MCs. It is probably better than gravimetric water content (MC), because it does also take into account medium compaction, and thus to some extent soil aeration. However, mechanistically it seems likely oxygen supply is influential at high MCs, and this is most directly indicated by air content of the medium. If one accepts that different processes are at work in suppressing germination at high and low moisture contents, then in theory one would expect a measure of water availability to be best for low MCs and one for air availability for high MCs. It would be fortuitous if one variable accounted for both phenomena.

The four independent variables, volumetric air content, volumetric water content, bulk density, and moisture content itself (MC-D, or gravimetric water content) have been plotted against germination percentage of intact seed of the cultivar Eagle. For MCs from 4.0 to 7.0, it appears germination percentage has a strong linear relationship to both volumetric air and water content. These two independent variables tend to be highly inversely correlated. The relationship to bulk density and MC is not quite so good.

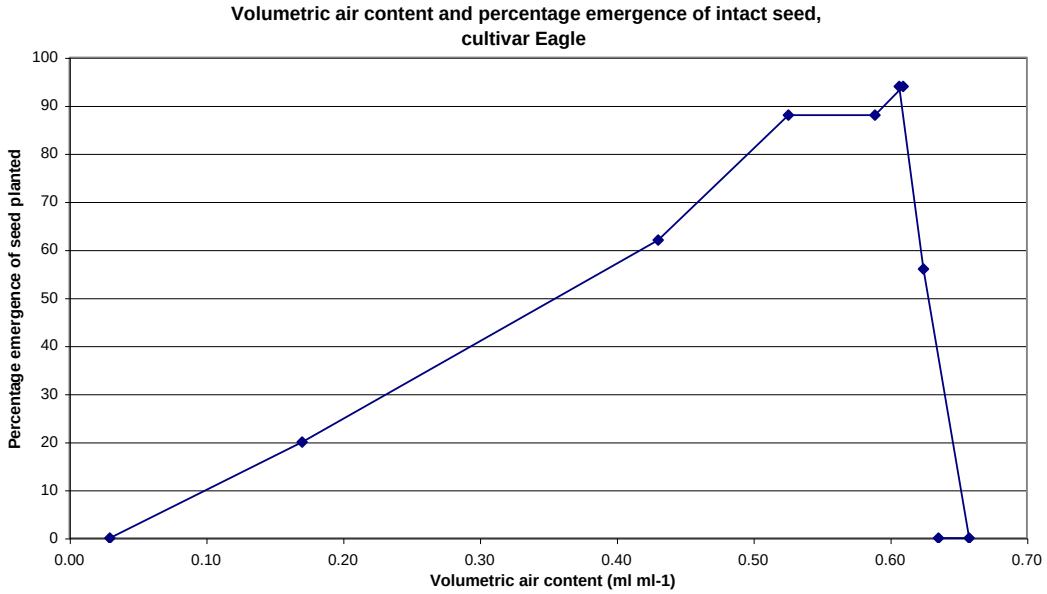


Figure 2-25: Volumetric air content and percentage emergence of intact seed, cultivar Eagle

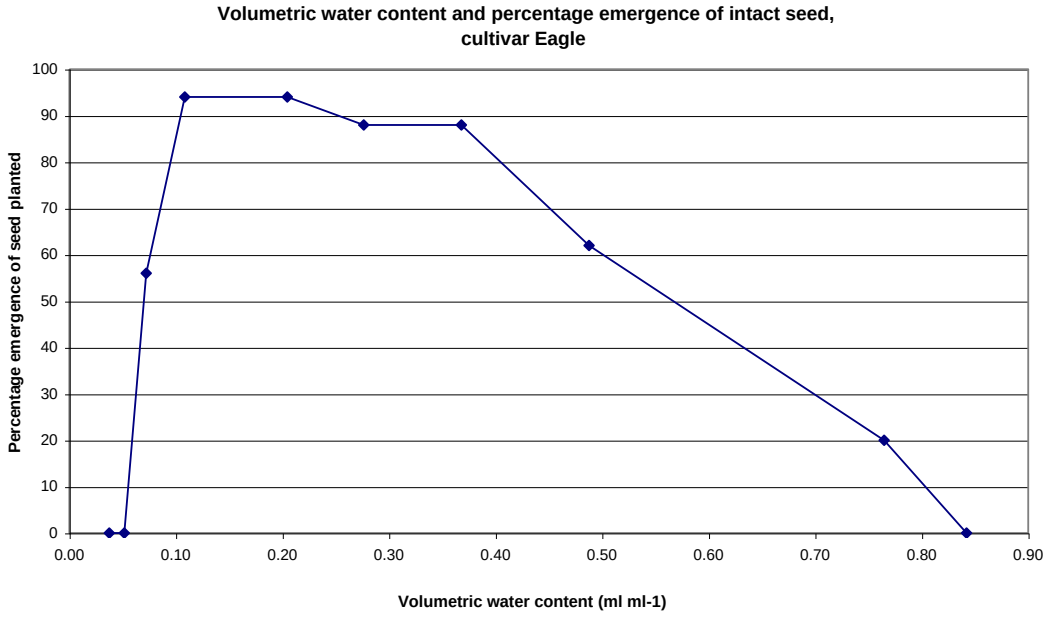


Figure 2-26: Volumetric water content and percentage emergence of intact seed, cultivar Eagle

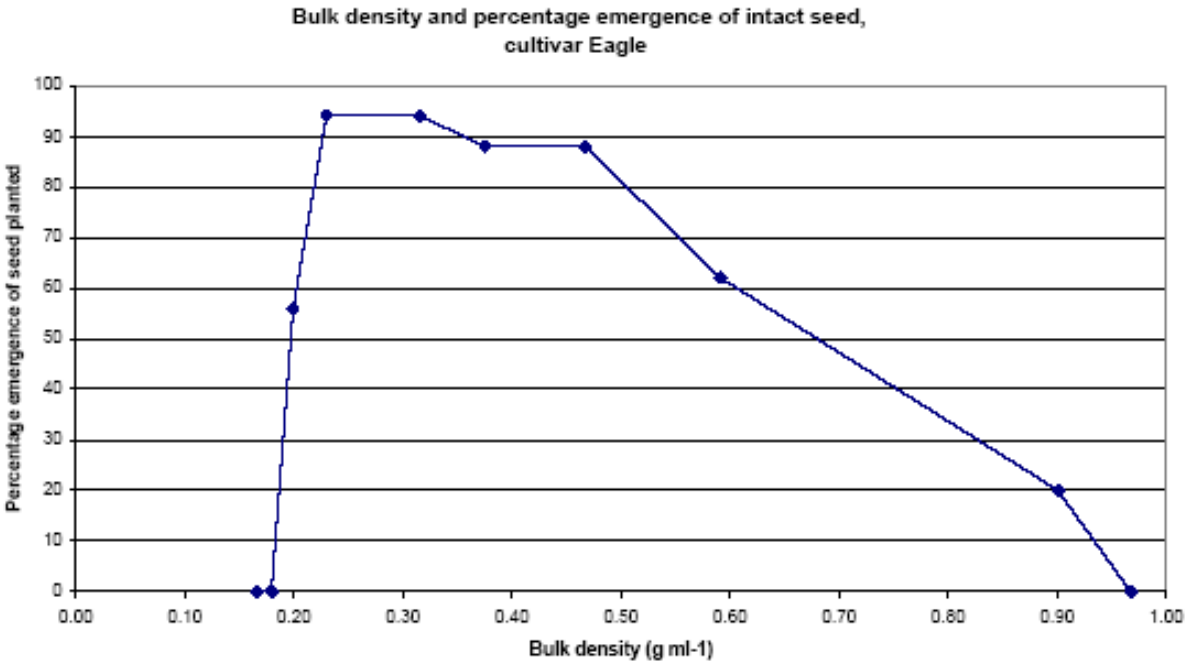
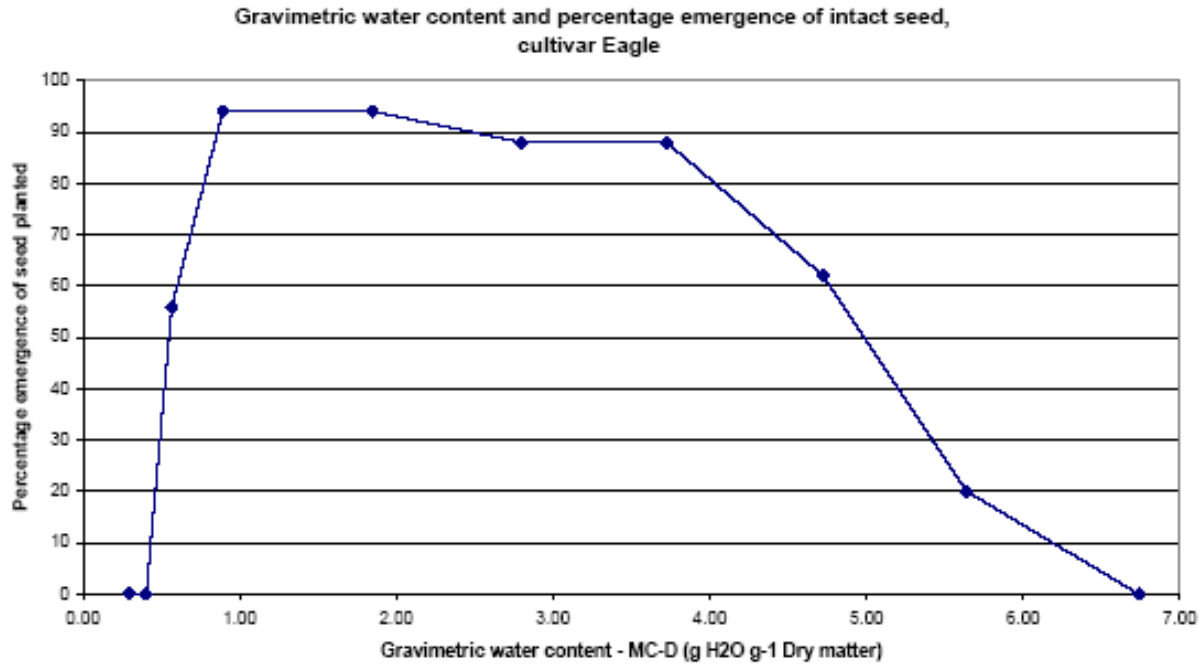


Figure 2-27: Bulk density and percentage emergence of intact seed, cultivar Eagle



Data from the high end range of MCs have been plotted and linear regression lines fitted as shown.

Figure 2-28: Bulk density and percentage emergence of intact seed, cultivar Eagle

Data from the high-end range of MCs have been plotted and linear regression lines fitted as shown.

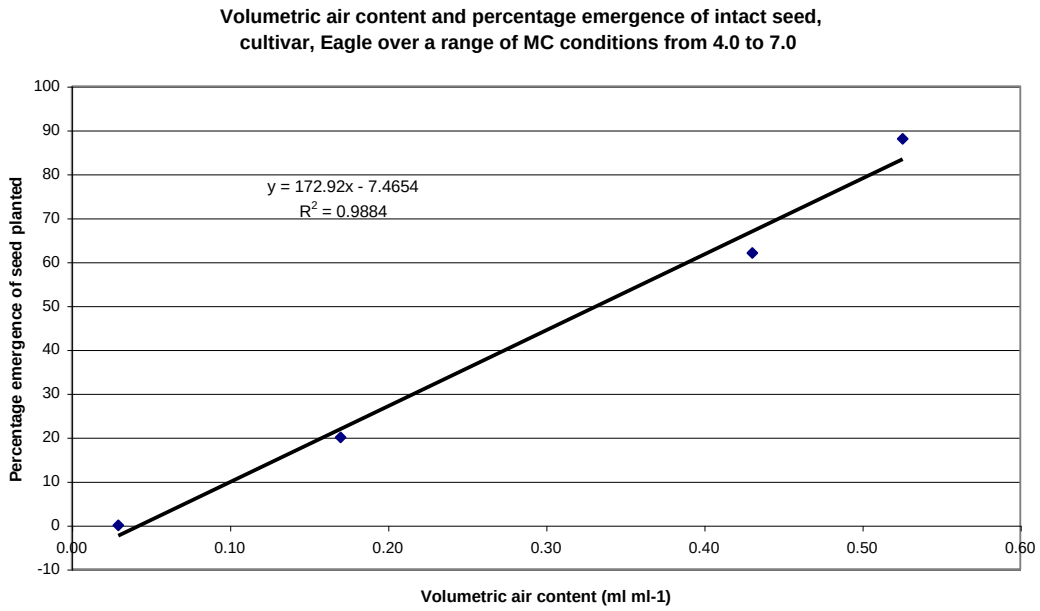


Figure 2-29: Volumetric air content and percentage emergence of intact seed, cultivar Eagle over a range of MC conditions from 4.0 to 7.0

Since emergence was all or nothing in de-hulled seed, it was not worth plotting these data for Alrite. The decline in emergence rate from 100% at 6.0 MC to zero at 7.0 MC in the case of de-hulled seed did correspond to a dramatic drop in air content from 17% to 3% in the medium, probably because de-hulled seed has a much lower threshold requirement for soil aeration than intact seed, between 17% and 3% (as opposed to a threshold around 50% for intact seed – see Figure 2-25 above). This range of air contents was not explored in this experiment. One could perhaps determine the threshold by manipulation of soil bulk density at the same MC, either with peatlite or some other medium. Direct manipulation of the partial pressure of oxygen around moist germinating seed would prove the point more easily.

Although emergence was 100% at MC 6.0, it appears to have been slowed in starting, and completion of emergence also took longer than at lower MCs. It would appear that lowered soil air content had some effect on germination in de-hulled seed even in the range of air contents between 43% and 17%, corresponding to MCs of 5.0 and 6.0.

Emergence rate in intact seed first declined between MCs of 4.0, and 5.0, suggesting if it is air content that is operative, the threshold is between 53% and 43% air content; if this is the case, it should be relatively easy to confirm even using medium, because these air contents are easily constituted by minor adjustments in bulk density without change in MC, or conversely, minor changes in MC without change in bulk density.

The data presented here are not incompatible with the hypothesis that gaseous oxygen content in the medium surrounding the seeds is determinative of seed germination and emergence performance in very wet media. But these data are limited to one cultivar, and may not be consistently found. In the literature, factors other than anoxia and hypoxia are considered as possibly affecting germination of intact seed. One is an inhibitory substance supposedly present in the pericarp, another is temperature-related seed dormancy, and a third is effects of mechanical constriction of the embryo by the pericarp. When increased moisture content affects germination of intact seed, two things happen. Start of emergence and speed of emergence are delayed and reduced, and germination percentage is reduced. If emergence is merely delayed, it is easy to envisage everything slowed down because of a reduced rate of production of the energy needed to fuel enzymatic reactions and growth, normally produced through oxidative respiration. It is less obvious why a large percentage of seeds never emerge no matter how long they are given. Unless seeds die, and it is that which cuts off germination, not a lack of oxygen, one has to envisage a process that not only slows germination speed but also completely halts it in many seeds, and is able to halt it over a large range of air and water contents. It looks as though there may be active suppression of germination. This could be through blockage of some key metabolic pathway, as a result of build up of waste products, or by inhibitory substances released from the pericarp.

Before speculating further it would be helpful to determine if it is water content or air content of the medium that is primarily holding back germination in intact seed close to the critical threshold, and how quickly and if in fact the seed is killed if it is held at 25°C for several days in a wet environment. The effects of dry soil conditions on germination have not been discussed, because the pattern revealed seems to be easily accounted for in terms of length of time required for imbibition to take place. It would be of interest to see if the timing of seedling emergence demonstrated in these data fits with theoretical modeling of imbibition.

Summary. The purpose of this study was not to determine the mechanisms underlying seedling emergence performance, nor was the design suited to doing so. The effect of peatlite moisture content on emergence of de-hulled and intact seed at 25°C was determined over a wide range of MCs. Measurements included emergence percentage, time to first emergence, time to 50% and 90% emergence, and speed of emergence once it had started or simultaneity of emergence. Some other features of seedling production were also observed, such as occurrence of pop-ups, and occurrence of seed-coat retention, neither of which are desirable outcomes.

It was found de-hulled seed has a very wide range of MCs in which 100% germination occurs. Of these 3.0, 4.0 and 5.0 would all be suitable for use in production if the medium was manageable, all leading to a high degree of synchronicity of emergence. The best option appears to be to use a peatlite medium made up with a MC of 3.0 or a little less for ease of handling, then to mist-in the seedbed to raise MC around the

seed a little higher. At 25°C, one would expect emergence to start after 48 hours and be 90% complete after 65 hours from seeding. Thus 90% of seedlings might be expected to emerge above soil in just a 17-hr time span. Quality of seedlings will depend on the degree of seed selection. With very careful selection as practiced in this experiment, almost all seedlings can be successful.

Intact seed had a narrower range of MCs that could be considered optimal; 3.0 and 4.0 were best. Germination percentage and speed fell off at MCs above 4.0. A high correlation and good linear relationship was found to exist between germination percentage and either volumetric air content or water content in the MC range 4.0 and above. When using intact seed directly, without priming or conditioning, the best result, based on this experiment, would be obtained by using a peatlite medium adjusted to MC 3.0. Misting-in could also be safely used, with care, and might speed up emergence slightly. In cultivars such as Eagle, with viability around 90%, emergence should begin after 58 hours and be 80% complete at 85 hours from seeding. In this experiment, 90% of the Eagle seed that was viable emerged in a 27-hour period when planted in 3.0 MC medium. Thus, the presence of the pericarp appears to both delay onset of seedling emergence and spread out the germination process in time.

SEEDING TECHNIQUE FOR SEEDLING PRODUCTION USING CONTROLLED MOISTURE CONTENT

Choice of Moisture Content

There is a fairly large range of moisture contents of peatlite (2.0 to 4.5, dry matter basis) over which seedling emergence is both good and fast for both intact and de-hulled seed. These data are very similar to those for seed germination. However, not all these moisture contents are feasible for automated flat filling. The preferred medium, peatlite, becomes sticky at the high end of the range, and leaves voids if used to fill small cells. The upper limit of moisture content that is feasible to use is approximately 3.25 parts water to 1 part dry matter, or a little over 75% moisture content on a wet basis. (The quality of the peat moss and the precise make-up of the medium in terms of ratio of peat moss to vermiculite affect the upper limit of friability. Poor quality peat moss gets sticky more quickly than high quality peat moss, and in general, peat moss can handle more water than vermiculite.) The moisture content adopted was the highest possible that would leave the medium still friable when using good quality peat moss in the mix (3.25 MC). The particular moisture content is perhaps not critical, especially for de-hulled seed, but using the highest practical content provides the greatest safety margin against accidental drying out.

When water is added to peat moss, it takes a number of hours to become absorbed and reach equilibrium distribution. During this time the peat volume increases, presumably as dried plant structures resume earlier shapes, much as dried sponges do. The peat moss becomes drier to the touch and more friable as the water is taken in. In preparing medium of known moisture content, it is also necessary to allow time for

equilibration of the medium. Overnight is an adequate length of time. Large batches of peatlite can be prepared ahead of time.

Flats and Flat Filling, and Seeding. Throughout the production phase of the project seedlings were produced using polystyrene plug trays (aka Speedling trays, although there are many manufacturers) with large numbers of cells. These come in different heights and with different numbers of cells. Typically the rectangular dimensions are standardized at 13.5 inches by 26.5 inches, and cell numbers are 11x22, 12x24 etc. The flat height we eventually settled upon was 2.5 inches, and cell number was 11x22 or more. Commercial equipment is available for filling and seeding this size of flat.

To fill flats of this type without voids, it helps to first break up any clumps and then to drop the medium down vertically into each cell. Both objectives can be achieved by passing the medium through a coarse sieve above the flat. After the flat is filled to excess, it needs to be vibrated to settle the medium in. Excess medium then needs to be scraped off.

To control depth of seeding precisely and firm the medium above the seed uniformly, two dibbling tools were made for each type of flat used. The first compressed the medium in the cell by 5/16 inches. After seeding and covering with additional medium, the medium in the cell was again compressed, this time by ¼ inch. The purpose of covering seed and firming the medium above it is to encourage roots to penetrate downwards rather than popping out of the soil. Compression of the medium above and below the seed was quite light, and depth of seeds not great, in both cases just enough to eliminate "pop-ups" as a serious problem, while not impeding root penetration or shoot emergence. Use of a standardized procedure and special tools ensured uniformity of conditions seed to seed, and repeatability across experiments. Questions as to ideal degree of soil compression above and below the seed were not systematically investigated experimentally, but were determined by trial and error and recourse to experience. Effect of depth of seeding was addressed in one study.

Germination. Flats were incubated in the dark in a humid place at 25°C for 48 hours, in the case of de-hulled seed, at which time they were floated. An additional 12 hours would be appropriate for intact seed. At this time, roots typically were just showing out the bottom of the tray and approximately 50% of seedlings had broken the surface. The speed of emergence can be controlled by manipulation of temperature. Use of 25°C is convenient because it permits flotation after exactly two days, which is easy to remember and fits into a work routine. Quality of seedling is also good at this temperature. Slightly faster germination would be possible with higher temperature (e.g., 30°C) but also at some risk of increased occurrence of pop-ups. The process of germination can also be slowed without apparent detrimental effects, by using a lower temperature.

References

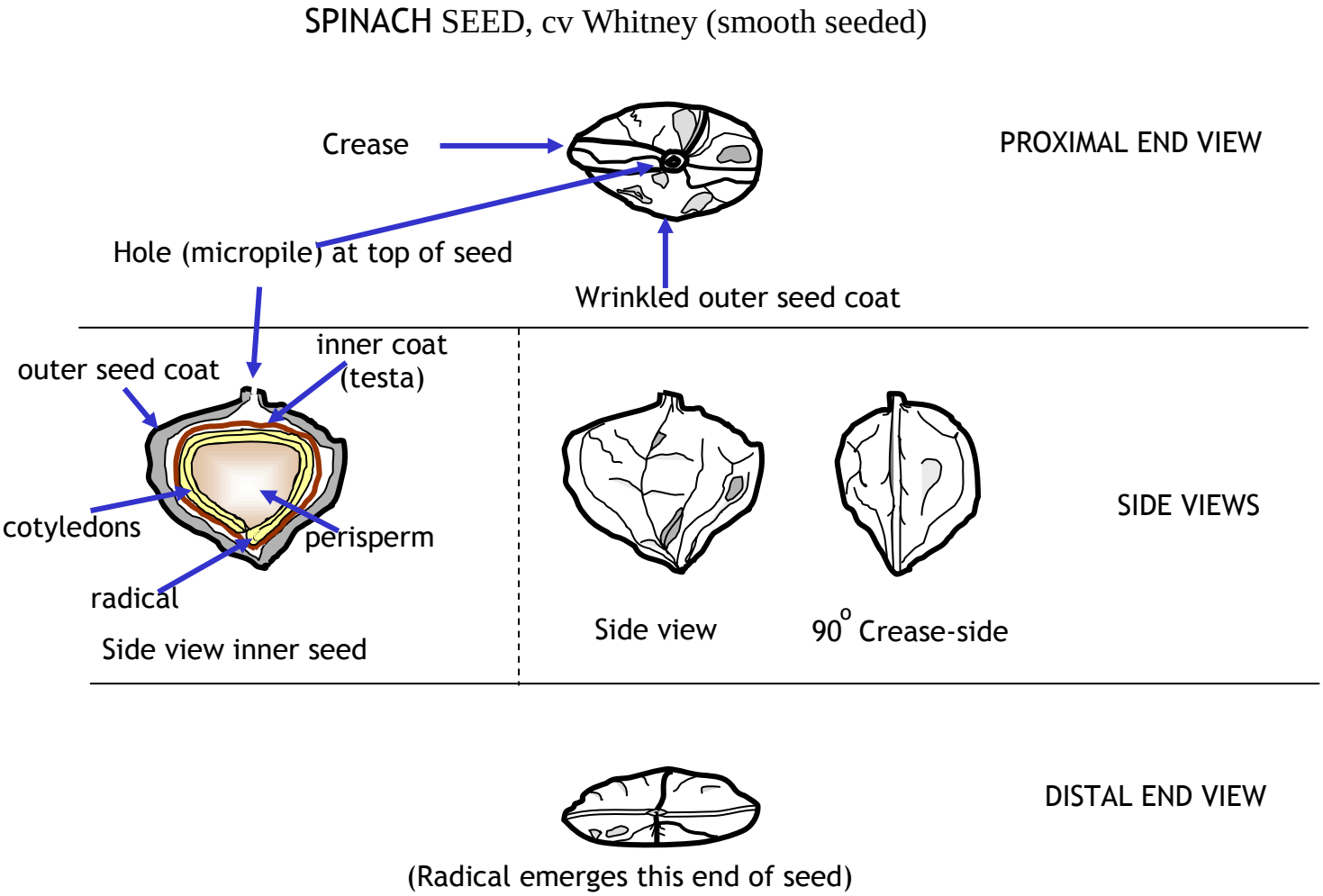
Katzman, L. (1999). Developing a system to germinate and grow hydroponics spinach, *Spinacia oleracea*. M.S thesis, Cornell University, Ithaca, NY.

Katzman, L. (2003). Influence of plant age, inoculum dosage, and nutrient solution temperature on the development of *Pythium aphanidermatum* in hydroponic spinach (*Spinacia oleracea* L.) production systems. Ph.D. dissertation, Cornell University, Ithaca, NY.

Appendix 2-A. Spinach seed morphology and seedling emergence diagrammed

SPINACH SEED MORPHOLOGY

Dry spinach seeds cv. Bejo, were placed under a dissecting scope and the following sketches were made of seed coat appearance.



C. Johnson

FIGURE 1. Sketches from views of spinach seed as seen under dissecting microscope (10 to 30 x magnification used).

Spinach is a dicot. Within the thick outer seed coat, the seed is covered with a thin membrane (testa). Two cotyledons (first leaves) are present within the seed. Below the testa, cotyledons are folded together, wrapped in an arch around the outside of the perisperm. The radical (root) is at the more pointed end of the seed. The perisperm is at the center of the seed and serves as a food-source during germination.

SPINACH SEEDLING EMERGENCE

During normal germination, the radical (root tip) is first to emerge from the seed coat (or testa in the case if de-coated seed). Radical emergence is followed by emergence of the cotyledons. The cotyledons emerge

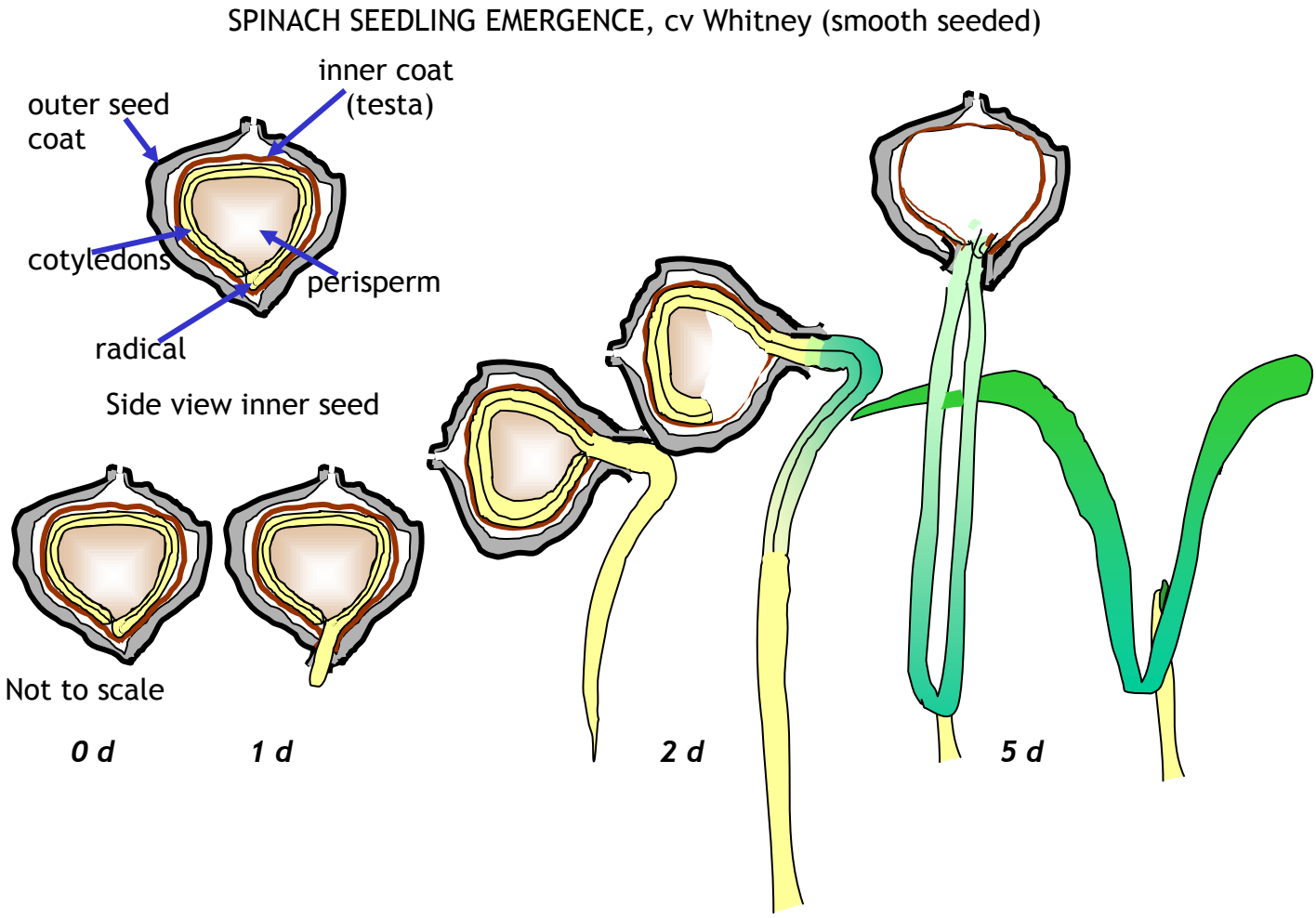


FIGURE 2. Sketches from views of spinach seed as they germinate from age 0 days (initiation of imbibition) to age 5 days.

pressed together and gradually separate from one another as the seed coat is released from the top of the cotyledons. In many instances, the seed coat release from the cotyledon tips was incomplete, and the tips of cotyledons were held together by the dried seed coat. This condition stunted seedling growth. Careful removal of the dried seed coat from cotyledons that were held together in this way was beneficial to seedling growth. Seedlings that were allowed to continue development with the two cotyledons stuck together by the seed coat often produced malformed true leaves. Occasionally, seeds with 3-cotyledons were observed. Seeds that produced three cotyledons also produced sets of three true leaves, (rather than the normal sets of two true leaves).

The first true leaves begin to emerge from between the two cotyledons approximately 4 days after germination. Additional true leaves follow in sets of two for the remainder of the growth period.

Appendix 2-B

Cell and Flat parameters	Flat height in Inches				
	1.75	2.5	1.75cd	1	3
Number of cells in flat	72	50	50	187	60
Whole Flat area (cm^2)	468	450	450	675	644
Flat height (cm)	4.6	6.4	4.55	2.7	7.55
Cell top: side of square (cm)	2.05	2.50	2.20	1.45	2.80
Cell bottom diameter (cm)	0.50	0.75	0.75	0.55	0.75
Cell volume (cm^3)	10.2	22.7	12.6	4.5	31.7
Cell surface area (cm^2)	4.20	6.25	4.84	2.10	7.84
Surface area to volume ratio (cm^{-1})	0.41	0.28	0.38	0.46	0.25
Cell border width (cm)	0.5	0.5	0.8	0.5	0.475
Border area per cell (cm^2)	2.3	2.6	4.2	1.5	2.9
Border area to cell area ratio	0.55	0.42	0.86	0.72	0.37
Cell area for whole flat (cm^2)	303	313	242	393	470

CHAPTER 3. PRODUCTION SYSTEM FOR SPINACH

Task 3: Develop Production System for Baby Spinach.

INTRODUCTION

Greenhouse production techniques for salad greens are most advanced in lettuce both in our program and worldwide. As a potential commercial greenhouse crop spinach differs from lettuce in significant ways. Tipburn is not a major issue for spinach, and thus one can use more of the available natural light throughout the year than is possible with lettuce. Since this energy source is free, and results directly in increased productivity at no increased cost, it can be a considerable advantage. (In deep winter, both crops need all the available light in temperate latitudes, and there is no advantage for spinach.) Spinach has a larger seed with greater initial reserves than lettuce and gets off to a faster start than lettuce, and thus it is possible to achieve the same crop productivity in a shorter crop cycle, an important consideration when a baby leaf product is under consideration. On the negative side, spinach is notorious for root disease problems when grown in hydroponics and germination is a greater problem. Plant to plant variation is much greater in spinach than in lettuce, and this also causes problems.

The cultural system we envisage for producing baby spinach has several features that differ from the system we devised for producing lettuce and several that are in common. Most of the differences result from adopting baby leaf spinach as the target product, which entails using high planting densities and requires automation in seeding and harvesting to cope with the increased number of plants involved. We continue to favor a deep-flow pond system over other hydroponic methods, for the safeguard it provides against catastrophic crop failure in the event of power outages, and logistical advantages in crop handling.

COMPARISON OF LETTUCE AND SPINACH PRODUCTION SYSTEMS

Differences Between the Baby Spinach Production System as Compared to the Head Lettuce System: Very high plant densities are employed. Plant density and timing of harvest are optimized for the particular size of baby spinach leaf product desired. For a standard baby-leaf product, plant density is circa 1500 plants per square meter.

The crop is seeded at final density, and there are no transplant and/or respacing steps as there are for head lettuce (or would be for large spinach plants if they were the desired product).[1]

[1] If technological developments permit automated respacing of seedlings from ultra high density to just very high density, without disturbance of the plants and checks to growth, respacing will be utilized.

Seeding is fully automated, using a custom designed seed tray that can be used many times over. (At present, we are using a commercially available polystyrene plug tray, in conjunction with a peat-based medium.) [2]

The crop cycle is very fast. First cutting is 12 to 16 days from seeding depending on the size of leaf required, the cultivar, and planting density. If repeated cuttings from the same crop stand are taken, second and third harvests follow at approximately one-week intervals.

Seed trays are incubated for 48 hours in a favorable environment outside the final growing system, while germination takes place. No light is required. Stacking is employed to economize on space. Temperature is controlled at 25°C, and high humidity is maintained.

After the 48-hour incubation period, seed trays are inserted directly into the greenhouse crop production system, where they remain untouched until harvest.

HACCP procedures are followed throughout the production cycle, from seeding to packaging. At no time are leaves wetted or touched by human hands. Consequently, post harvest disease (wet-rot in the bag) is avoided.

Harvest is fully automated, using a band saw-type cutter directly feeding leaves to packaging machinery. [3] The cut leaf is either packed in small, labeled bags for retail store sales, or bulked in larger bags for restaurant and institutional sales.

For purposes of root disease control the nutrient solution is continuously re-circulated and treated. To reduce the volume of solution needing treatment, a shallow pond is used (only a few centimeters deep below the float). To restrict spread of water-borne disease organisms when they do gain a foothold, the pond system is compartmentalized and a positive directional flow is maintained.

[2] A seedling tray that does not require medium will be substituted when it is perfected, or one designed to use a minimum quantity of medium.

[3] The design of the harvest machinery differs depending on whether a single terminal harvest is planned or repeated harvests are made from the same plants. If repeated harvests were made from the same planting, the cutting machine would preferably operate on the trays while they are still floating, so that the roots are not disturbed. If there is a single terminal harvest, the trays may be transported out of the greenhouse.

The objective of predictable, uniform, daily production is the same for the spinach crop as it is for lettuce, and the methods for environmental control are the same. Important commonalities of spinach and lettuce cultural systems are as follows:

Uniform daily growth year round is attained by use of supplementary light and/or CO_2 when needed, and shade control when too much light occurs naturally.

Management of daily growth is computer controlled by implementation of algorithms that monitor the crop environment and control application of supplementary light, CO_2, and shade.

Supplementary light is applied during off-peak hours as much as possible to minimize energy costs.

Photoperiod typically follows the outdoor photoperiod, but is extended up to 24-hours if/when supplementary light is needed to achieve uniform daily growth. Bolting-resistant cultivars are employed when necessary.

Deep-flow hydroponics is the preferred growing system. Seedling trays are entered at one end of long ponds, and harvested at the other end. Crop transportation is by flotation.[4]

Root zone temperature and air temperature are optimized for the cultivar and product desired – they are each between 20 and 25°C.

DESCRIPTION OF RESEARCH

In a crop harvested just barely beyond the seedling stage, seedling production is of utmost importance. A large part of the effort towards developing the cultural system for spinach was devoted to seedling production. This research has been described in detail in the previous section.

The research that will be described here includes a series of 4 studies on the effect of temperature on productivity and quality of product. Temperature has unexpected effects on spinach growth and plant characteristics. Effect of air temperature was separated from effect of root zone temperature in the final study in this series.

[4] Depending on how the question of root disease control is resolved, other possible growing systems are NFT and aeroponics. If a pond system is used, flats may be floated through a channel to and from the harvest machine, and enter the pond system at the start of the cycle in the same way.

Effect of plant density was examined both at the start and at the end of the project. If plants are grown at a high density, there is little latitude for error in timing of harvest if peak quality is to be obtained. Two large-scale studies on the effect of plant density, as interacting with type of cultivar, photoperiod, and timing of harvest, are described here.

It was important to determine whether supplemental lighting might be applied "at night" when the electricity rate is cheapest, and see what affect this had on plant quality and yield. The effect of photoperiod was examined in two studies. Fortunately, it appears there will be no difficulty in utilizing night rate electricity.

It was beyond the scope of this project to investigate all aspects of automation in the spinach production system we envisage. In any case, machinery already exists for most aspects of automation in which we are interested. However, a band saw-type harvesting machine was constructed, and tested on several crops. The practical possibility of automated harvest using this machine was demonstrated.

The final area investigated was repeated cropping (ratooning) of the same spinach plants. Preserving the "factory" between growth cycles can be expected to increase productivity; the issues are: when precisely should harvests be made: what is the effect on quality of the marketable product: and what are the potential gains. In the study presented, spinach growth was sampled and modeled through the initial growth cycle and then through re-growth after cutting at different heights. Under repeated harvesting, which extends the crop cycle and ultimate plant age, bolt resistant cultivars must be used.

Light integral effects were investigated previously in this program by Both et al. (1996). Research findings are presented in detail below.

Temperature Studies

Temperature was manipulated in four experiments of increasing sophistication.
First, germination of de-hulled seed was examined on germination paper where root activity could be observed directly.

Temperature Experiment 1: Germination of De-Hulled Seed of the Cultivar Alrite on Germination Paper Over a Range of Temperatures

Rationale. Historically, it has been considered necessary to germinate spinach at a rather low temperature relative to other species, in the range of 15 to 20°C, in order to obtain high percentage germination. Our work on intact seed (described earlier) showed that if an appropriate priming protocol was used to trigger germination, temperatures up to 30°C increased speed of germination and root growth. It was expected similar results would be obtained with de-hulled seed without use of a priming procedure.

It is worthwhile knowing precisely how temperature affects speed of germination and seedling emergence in de-hulled seed, which we are recommending for commercial greenhouse production to be able to manipulate the duration of the process to fit normal work schedules. However, speeding up germination by increasing temperature may have effects other than on rate of growth, some of them undesirable. In an experiment on seedling emergence at different soil moisture contents, two temperature conditions for germination were included. One set of flats was held at 16°C for 72 hours, after which flats were introduced to a 25°C chamber. The other set of flats was held at 25°C throughout. In the low temperature treatment, seedling emergence was delayed, as one would expect, but the number of pop-ups was reduced. Subsequent growth appeared normal. It seems likely that controlling rate of growth through temperature manipulation can be used to reduce the number of pop-ups, among other things. ("Pop ups" are where the root fails to penetrate the soil and instead the root and seed pop out of the soil. Subsequently the seedling typically fails.)

Rather than directly testing temperature effects on seedling emergence in soil, it seemed expedient first to survey response of de-hulled seed to temperature on germination paper, where rate of growth of the emerging radicle and relative growth of root and shoot could be observed and recorded directly, as was done in this experiment.

Methods. Five growth chambers were set to nominal temperatures of 16, 19, 22, 25, and 29°C to provide the range of temperatures desired. Subsequently, the actual temperatures in the proximity of the germination boxes were determined to be 17, 18, 21, 25 and 29°C, due to offsets in the growth chamber temperature sensors and local temperature gradients. Clear plastic germination boxes were fitted with a double layer of blue germination blotters that had been wetted in RO water, then drained for five seconds before being placed in the boxes. These blotters acted as a reservoir of water for the duration of the experiment. Twenty-five seeds of the cultivar Alrite were set out in a grid on the wet paper in each of the boxes, after which tight-fitting lids were put in place, and two germination boxes were randomly assigned to each temperature condition, giving 2 replicates per condition. The two boxes were themselves placed in a large closed clear plastic container in which free water was present at all times. (This was to slow down any tendency the germination boxes might have to dry out.) Rectangular, opaque, 1-inch thick, polystyrene sheets considerably larger than the containers with the seed boxes were then placed on each outer plastic container in order to exclude entry of long wave radiation directly from the light source in the chamber. Progress of germination was monitored by means of frequent overhead photographs, by counts of radicle emergence, recording of seed orientation, repeated measurements of root lengths after germination, and observations on root direction, root hair development, appearance of the hypocotyl hook, hypocotyl coloration, cotyledon emergence and separation, and other notable events such as raising of the seed and hypocotyl off the blotter surface. The above data were collected twice daily over the second, third and

fourth days following imbibition, and once on the fifth day. On day 6, a destructive harvest was made in which all germinated seedlings were measured as to root, hypocotyl and cotyledon lengths.

Results. Twenty-four hours after imbibition, just 5 seeds out of the 250 sown had germinated i.e., were showing any part of the radicle past the seed coat. By the next morning, (18 hours later) the minimum percentage germination was 56% in both reps of the 17°C condition, and all other conditions averaged 80% or more germination, as can be seen in Figure 3-1 below.

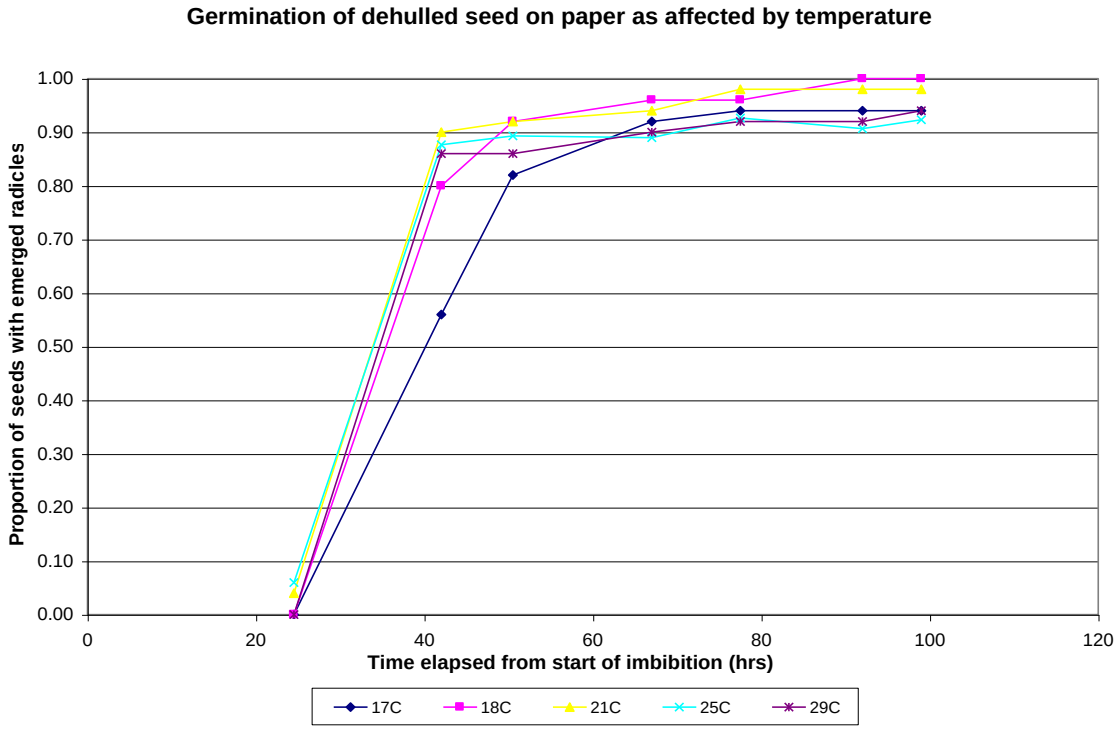


Figure 3-1: Germination of dehulled seed on paper as affected by temperature

By this time, 4 out of 5 treatments were indistinguishable in terms of germination percentage. The opportunity for observing differential effects of temperature on germination occurred overnight and was missed. These data underline the fact that speed of germination, defined as appearance of the radicle, is not a very practical indicator of performance in very fast-germinating species.

Germination percent was high in all treatments (greater than 90%) and consistent across reps. The small differences in viability were not significant, statistically or otherwise. Ultimately, the largest number of un-germinated seeds in any one box was 3 out of 25. No more than one seed in any box was considered dead. But in 7 out of 10 reps one or more seeds had problems with damaged testa (from the de-hulling process), which resulted in cotyledons bursting through the testa, which then slowed or prevented radicle emergence. If the radicle failed to emerge for this reason, by definition it failed to germinate. However, examination of

data taken on seed abnormalities (not shown) does not suggest a correlation between this kind of problem and temperature.

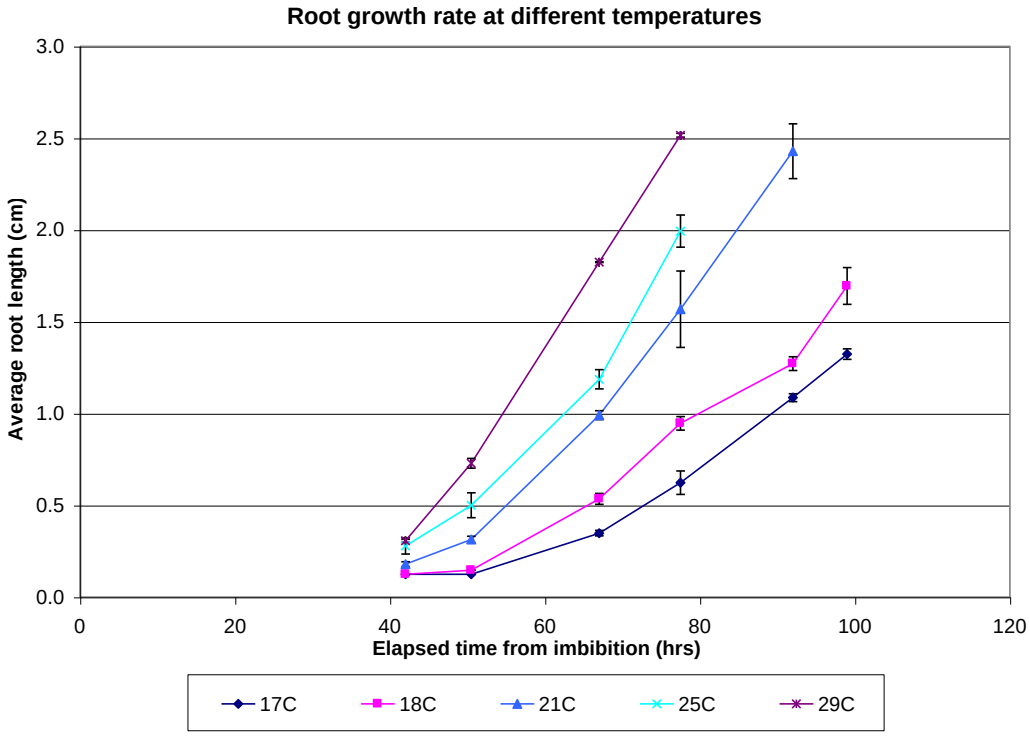


Figure 3-2: Root growth rate at different temperatures

Root length was estimated at frequent intervals during germination by assigning seeds to root length ranges. The categories used were > 4cm, >3cm, >2cm, >1.5cm, >1.0cm, >. 75cm, >. 50cm, >. 25cm, and > 0.0cm. Subsequently, frequency distributions by length classes and average and modal root lengths through time were determined. Average root length over time is shown graphically in Figure 3-2 : +/- 1 standard error is shown in the error bars. Since the temperature effect is prolonged in the matter of root growth, the effect of temperature is more clearly demonstrated than with simple germination. For instance, it can be seen that, by day 4, plants germinated and continued at 17°C were two days (48 hours) behind plants germinated at 29°C, in terms of root length, and there appeared to be an orderly progressive effect of temperature on rate of root elongation. The 25°C performance appears depressed, but there was a plausible reason for this, namely a deficiency of moisture in one of the reps.

Final observations were made after 6 elapsed days in a destructive harvest in which both root and shoot lengths were measured. Ratios between root, shoot, and hypocotyl were examined. (See Table 3-1)

Temperature (deg. C)		Root (cm)	Hypocotyl (cm)	Cotyledon (cm)	Ratio root:hypo	Ratio hypo: cotyl	Ratio root:shoot
17	Rep 1	4.45	5.80	6.82	0.76	0.85	0.35
	Rep 2	5.10	6.18	7.18	0.83	0.86	0.38
	Av.	**4.78**	**5.99**	**7.00**	**0.80**	**0.86**	**0.37**
18	Rep 1	5.92	7.33	8.43	0.81	0.87	0.38
	Rep 2	5.94	6.90	7.91	0.87	0.87	0.40
	Av.	**5.93**	**7.11**	**8.17**	**0.84**	**0.87**	**0.39**
21	Rep 1	8.15	9.89	11.27	0.82	0.88	0.38
	Rep 2	7.66	9.62	10.87	0.80	0.88	0.37
	Av.	**7.90**	**9.75**	**11.07**	**0.81**	**0.88**	**0.38**
25	Rep 1	8.59	9.81	11.55	0.88	0.85	0.40
	Rep 2	6.37	7.43	9.07	0.85	0.82	0.39
	Av.	**7.48**	**8.62**	**10.31**	**0.86**	**0.83**	**0.39**
29	Rep 1	7.27	8.18	9.30	0.88	0.88	0.42
	Rep 2	7.51	8.46	9.67	0.88	0.87	0.41
	Av.	**7.39**	**8.32**	**9.49**	**0.88**	**0.88**	**0.42**

Table 3-1. Seedling root and shoot lengths 6 days after start of imbibition

These data permitted calculation of root length averages for day 6 with which to extend the root growth curves of Figure 3-2, with the result shown below in Figure 3-3.

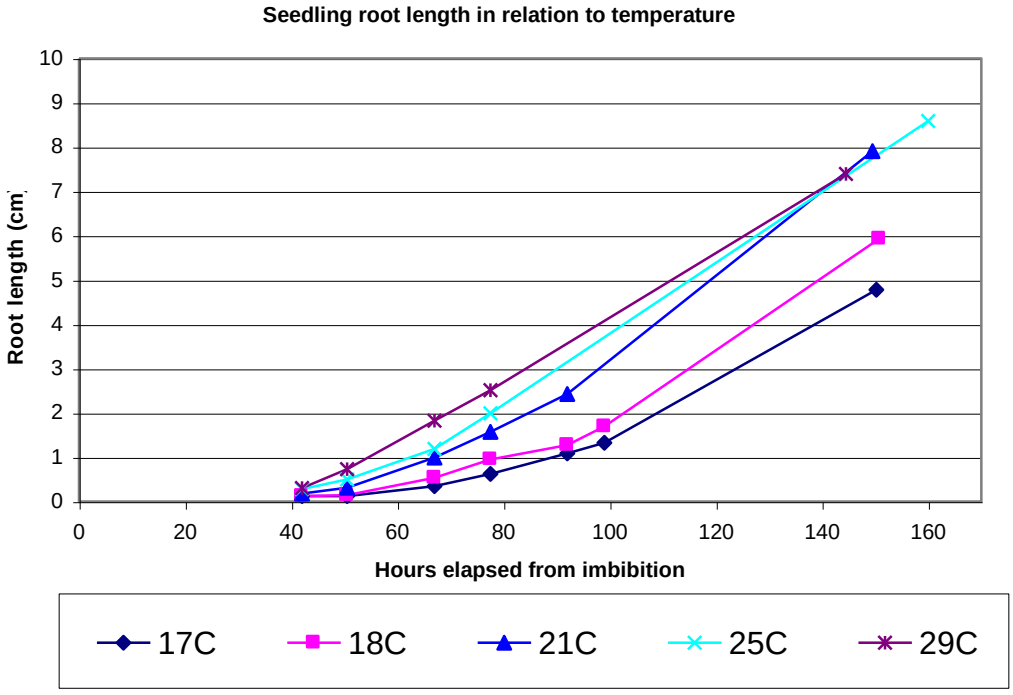


Figure 3-3: Seedling root length in relation to temperature

In these data, one would expect average lengths of roots to increase with temperature and continue to diverge from each other, based on the trends over the first 4 days. The average lengths did all increase substantially by day 6, and continued to diverge in the case of 17, 18, and 21°C conditions, but in the 25 and 29°C conditions root lengths converged so that very similar lengths were reached by day 6. We suspect this finding was an experimental artifact. It is possible by day 6 water stress was beginning to be experienced in the germination boxes of those treatments where plants had grown most rapidly. Water was required for Rep 2 in the 25°C condition on day 4, suggesting drought stress was already affecting performance in this germination box.

As data in Table 3-1 shows, there was excellent agreement among reps in all except the 25°C treatment. The divergence between reps started early in this case, and was correlated to a poor seal which led to drying out in this box.

It appears that the ratio between root and hypocotyl length and root and overall shoot length increased with temperature. The difference between the ratios at 17°C and 29°C was significant statistically (p values of .003 and .021 respectively).

Other distinctive seedling features were followed through time, such as development of root hairs, coloration of the hypocotyl, and emergence of the cotyledons (as indicated by the split between the two leaves showing). In all of these indicators, the same orderly delaying effect on growth with reduction in temperature was seen as in the case of root elongation.

Conclusions. This experiment provided useful parametric data on the effect of temperature on seedling germination, and particularly on root extension over time in the range of temperatures between 17 and 29°C. It supported the hypothesis that temperature level would affect root-shoot ratio, although the effect was small and in an unexpected direction. Differences in allocation of seed resources for root hair development were not evident, nor were any detrimental effects of temperature up to the level of 29°C demonstrated. The main effect of temperature observed was that of alteration in speed of growth. This was a powerful effect. As far as one can judge, it applied strongly to all plant parts.

Temperature Experiments 2 and 3: Introduction. Two temperature experiments were conducted in growth chambers to determine the effect of temperature on seedling emergence and early growth in a realistic seedling production protocol. Seeds were planted, as they would be for commercial purposes. In the first experiment, four different ambient air temperatures were maintained from the start to end of the experiment; in the second experiment, which was run concurrently, germination was accomplished at the same temperature in all conditions, and only then were the seedlings exposed to the four different temperatures. The focus of the first experiment was effect of temperature on seedling emergence, the

second on productivity under different temperature conditions. Plant morphology was examined in both experiments to a greater or lesser degree.

Temperature Experiment 2: Effect of Ambient Air Temperature on Seedling Emergence and Plant Quality and Morphology of Baby Leaf Spinach of the Cultivar Alrite

Rationale. An important objective of this experiment was to see if and how temperature may be used to mitigate some of the problems encountered in stand establishment of spinach, such as pop-ups and seed-coat retention or incidence of broken and deformed seedlings. The second main objective was to determine what effect temperature has on quality of the shoot, particularly qualities valuable commercially in a baby spinach crop such as shape of leaf, and durability under handling. Productivity, as always, was also a concern.

In the experiment just described it was shown that temperatures below 21°C substantially slowed germination and root extension of de-hulled seed early in the growth cycle when grown in germination boxes, while temperatures up to 29°C speeded germination. A disadvantage of the germination box method is that it does not address certain problems encountered in stand establishment in soil. For instance, for a seedling to emerge from a depth in a covering medium requires a degree of vigor which may not be revealed in surface-grown seed under no physical constraints; the problem of pop-ups does not occur; and although cotyledons are trapped by the seed coat in surface-grown seed, it probably happens in a different way than in soil. Obviously later phases of growth and shoot morphological characteristics cannot be observed in seedlings started in a germination box.

In this experiment the intent was to explore the effect of temperature in a more realistic setting – still in the growth chamber, but this time with seed planted as it might be commercially, i.e., in a peatlite medium - and exploring a range of temperatures likely to be encountered in production, either by choice or out of necessity (20, 25 and 30°C). The lowest temperature chosen was 20°C because previous studies in our program have shown this to be a lower limit beyond which substantial slowing of growth occurs. In the summer in a humid continental climate, it is not always possible to keep temperature below 30°C, even using shade cloth and evaporative cooling. A 35°C temperature condition was included because it may occasionally be encountered, and because an upper limit to the speeding effect of temperature was not determined for germination in the previous experiment. Although 35°C is only likely to be encountered briefly in greenhouse production when control systems fail, the effects could be catastrophic and it is valuable to know what the effects are when this happens.

Method. Eight 50-cell flats (cell density 1049 cell m^{-2}) filled with peatlite of gravimetric moisture content 3.0 (3 parts water to 1 part dry matter) were seeded with de-hulled seed of *Alrite*, and randomly assigned to four temperature conditions, two flats per condition. Seed was allowed to germinate for 48 hours in the

dark in chambers adjusted to provide 20, 25, 30, and 35°C air temperatures in the vicinity of the seed flats. Flats were floated in nutrient solution after 48 hours, and lighting was applied. Light level was carefully controlled to obtain the same integral and photoperiod in the four chambers, and checked and adjusted daily to achieve this goal. Seedling emergence was recorded frequently during the first 120 hours after seeding. When it was apparent seedling emergence was complete, seedlings were comprehensively evaluated and categorized, and failed/failing plants were removed in such a way as to equalize the stands among treatments, if possible. Destructive harvests to observe yield performance and plant quality characteristics were made on days 16, 17, and 20 and 21 from seeding.

Half of one flat (1 rep) was harvested day 16, the other half day 17. In the day 16 harvest, individual plant weight data were taken for all 25 cells in each of the temperature conditions. Fresh and dry weights were obtained, breaking down plants into leaf blade material, petiole and stem material. In the day 17 harvest, plants were separated into pairs of leaf blades, and leaf areas and weights were taken; petiole lengths and leaf blade dimensions were obtained for each of the primary (1st true) leaves. This kind of detail was obtained for all temperature conditions except 35°C, in which plants were doing poorly and the effort was not warranted. (For the 20°C condition, similar data was also obtained on Day 16.)

The day 20 harvests were also conducted in great detail. In this case, the sample unit adopted was a square area of 4 cells instead of the individual cell. Six sample units representing plants from 24 cells were harvested. The very detailed harvest was spread throughout the day, but rotated through conditions repeatedly to offset the effect of continued plant growth during the long time of harvest. The final harvest was on day 21; it was a fast harvest split between AM and PM. Just the distinction between leaf and stem was made. In this harvest the last of plants in the 35°C condition were harvested. Single plants were used as the sample unit.

Results. 1. Germination and Stand Establishment

The accompanying figure (Fig. 3-4) plotting cumulative percentage emergence of seed from peatlite at different temperatures, 20, 25, 30, and 35°C, showed that speed of emergence was greatest at 30°C and progressively slower at 25, 20 and 35°C. Although the standard-error error bars show extremely consistent findings across reps, the somewhat odd shape of the graph bears explanation. Seedling emergence in this experiment can be thought of as taking place in two phases, because there was a substantial change in temperature of the root zone when the flats were floated after 48 hours: for the observations before hour 50, the seeds truly were at nominal ambient air temperatures because they were sealed.

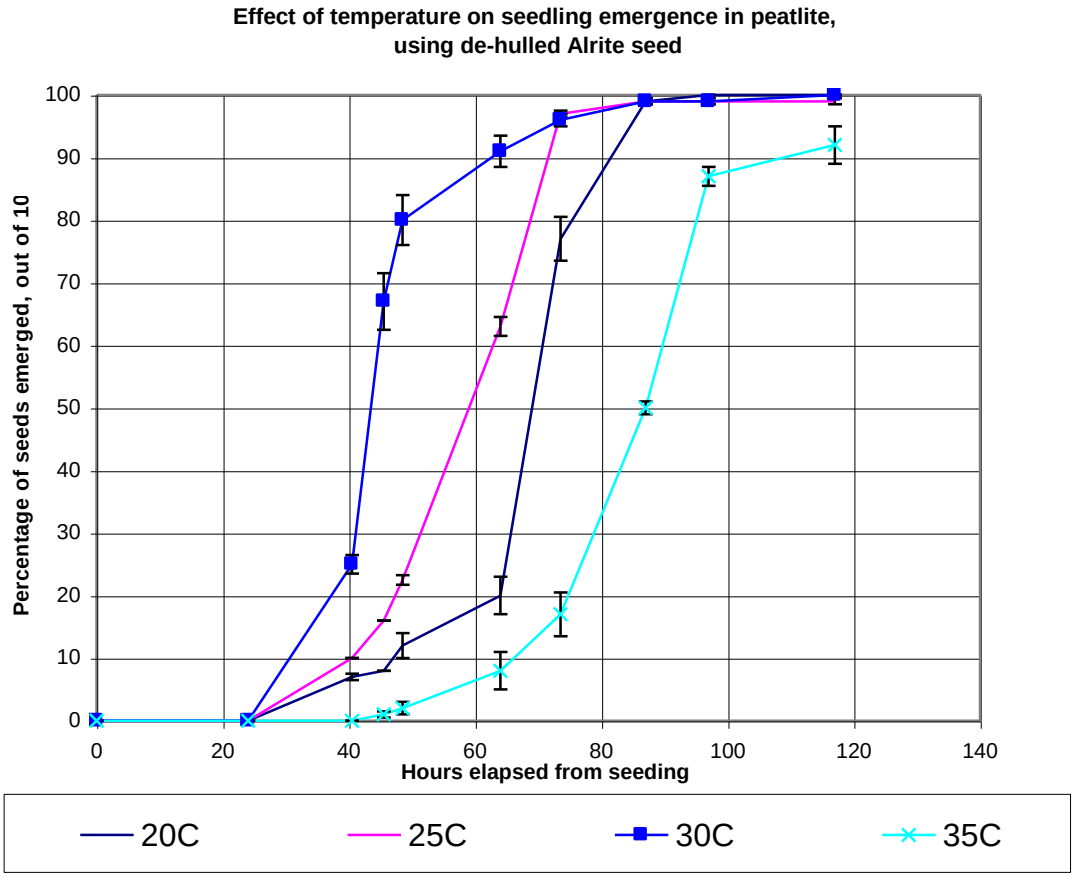


Figure 3-4: Effect of temperature on seedling emergence in peatlite, using de-hulled Alrite seed

After flotation, presumably due to evaporative cooling and convective heat exchange, the temperature of the nutrient solution fell considerably lower than the ambient air temperature (which remained the same) in three conditions. On day 4, the solution in the 20°C condition was c. 20°C, but it had fallen in the higher temperature conditions. In the 25°C condition, it was c. 22°C, in the 30°C condition it was c. 26°C, and in the 35°C condition c. 27.5°C. Temperature of the peatlite medium in the vicinity of the seed was not measured. Presumably, it fell between the air and nutrient solution temperatures. In interpreting the part of the emergence chart after 50 hours, one may speculate that seeds in the 35°C condition picked up in germination speed because the temperature was lowered and became more favorable, and seed in the 30°C condition slowed completion of emergence because temperature became cooler. However, in this case emergence was already nearly over.

Percentage emergence, defined as any part of the seed becoming visible from above the surface of the medium, was extremely high in this experiment, as it usually is with de-hulled seed. This does not mean that all the seedlings were destined to become worthwhile plants.

Day 5 Seedling Condition	Temperature Treatment							
	20C		25C		30C		35C	
	Flat 1	Flat 5	Flat 2	Flat 8	Flat 3	Flat 7	Flat 4	Flat 6
Emerged	50	50	49	50	50	50	49	43
Total Pop-Ups	1	3	2	3	4	3	>3	>3
% emergence	100	100	98	100	100	100	98	86
% rated "good"	0.92	0.90	0.92	0.90	0.76	0.86	0.42	0.62
Cots trapped top	5	7	7	7	21	16	2	0
Cots trapped lower	3	1	0	0	0	1	1	1
Single cot	0	0	1	0	1	7	1	0
Bad PU	1	2	2	3	3	3		
Small, very late	2	3	0	2	7	2	13	5
Deformed	1	0	1	0	2	2	16	7
Albino	0	0	0	0	0	1	0	1
No show	0	0	1	0	0	0	1	7
Good	46	45	46	45	38	43	21	31
Final stand after adjstmnt	44	44	44	44	39	41	22	22

Table 3-2. Seedling emergence and quality evaluation 5 days after seeding

On day 5 of the experiment, all the flats were evaluated carefully and seedlings categorized as to various problems they manifested. The results can be seen in the Table 3-2 above. Percentage emergence was close to 100% in all treatments, but whereas 90% of seedlings were rated as good in the 20 and 25°C conditions, only 80% were rated as good in the 30°C condition and 50% in the 35°C condition. (Note. Trapped cotyledons or single cotyledons did not disqualify seedlings from being rated “good”.)

One of the concerns in seedling emergence is whether the seed coat is shed. Retention of the seed coat does not appear to greatly impede later growth if it is at the tip of the cotyledons, but it is definitely not wanted in the final product or a salad where it is liable to be viewed as something dirty. The extreme rapidity of emergence at 30°C seems to have led to retention of the seed coat in about 37% of seedlings in this condition compared to 12% and 14% in the 20°C and 25°C conditions, which is definitely a negative feature of the 30°C condition. Less dramatically, there seemed to be a small increase in number of bad “pop-ups” with each increase in temperature. Since these seedlings will die and in effect are worthless, they subtract from the number of “good” plants. (Trapped cotyledons are considered a cosmetic problem, and do not affect this figure.). The 30°C temperature seems to have also encouraged a small increased percentage of late plants, or in other words spread out the emergence time. Late and deformed plants were epidemic in the 35°C treatment.

Some small adjustments were made to the plant stand to equalize plant density where possible. Final number of plants closely followed number of plants rated as "good", as can be seen in the last two rows of the table.

Results 2. Terminal Harvests

Summary. The two main harvests were made 4 days apart, on days 16-17 and 20-21, and each time they were split between fast harvests -- basically for biomass, but with blade material separated from stem material -- and very detailed harvests used to characterize plant morphology. The simpler biomass harvests gave an indication of what proportion of the plant might be saleable, and the detailed harvests indicated what the harvest would look like in terms of leaf shape, size, number and composition.

Summary results for both days of the first harvest are presented in the Tables 3-3 and 3-4 below.

Day 16 Summary All weights are in grams	**Temperature Condition**							
	20C		25C		30C		35C	
	Average	SD	Average	SD	Average	SD	Average	SD
Av. Fresh Weight of Whole shoot	**2.38**	0.507	**2.48**	0.763	**2.53**	0.837	**1.13**	0.284
Av. FW of Blade only	**1.97**	0.424	**1.90**	0.616	**1.89**	0.672	**0.92**	0.229
Av. FW of Petioles and Stem	**0.38**	0.094	**0.53**	0.162	**0.58**	0.163	**0.17**	0.053
Ratio: Blade FW to Whole Shoot	**0.83**	0.019	**0.76**	0.036	**0.74**	0.023	**0.82**	0.029
Av. Dry Weight of Whole shoot	**0.138**	0.037	**0.148**	0.057	**0.154**	0.057	**0.09**	0.026
Av. DW of Blade only	**0.122**	0.034	**0.126**	0.050	**0.129**	0.049	**0.08**	0.022
Av. DW of Petioles and Stem	**0.016**	0.004	**0.022**	0.007	**0.025**	0.008	**0.01**	0.004
Ratio: Blade DW to Whole Shoot	**0.88**	0.018	**0.85**	0.025	**0.84**	0.016	**0.87**	0.025
DW/FW ratio for Whole Shoot	**0.058**	0.008	**0.058**	0.007	**0.060**	0.006	**0.084**	0.009
DW/FW ratio for Blade only	**0.061**	0.008	**0.065**	0.008	**0.068**	0.007	**0.090**	0.010
DW/FW ratio for Pets. and Stem	**0.043**	0.009	**0.041**	0.005	**0.042**	0.004	**0.070**	0.011
n	**22**		**22**		**20**		**15**	

Table 3-3. Fresh and dry biomass by main plant parts in the Day 16 harvest

Day 17 Summary All weights are in	Temperature Condition					
	Averag	SD	Averag	SD	Averag	SD
Av. Fresh Weight of Whole	**3.22**	0.79	**2.90**	0.84	**3.25**	1.23
Av. FW of Blade	**2.57**	0.65	**2.18**	0.66	**2.37**	0.93
Av. FW of Petioles and	**0.62**	0.15	**0.69**	0.21	**0.09**	0.30
Ratio: Blade FW to Whole	**0.80**	0.02	**0.75**	0.03	**0.73**	0.02
Av. Dry Weight of Whole	**0.196**	0.067	**0.173**	0.061	**0.196**	0.080
Av. DW of Blade	**0.170**	0.059	**0.144**	0.053	**0.160**	0.068
Av. DW of Petioles and	**0.026**	0.009	**0.029**	0.009	**0.036**	0.013
Ratio: Blade DW to Whole	**0.87**	0.018	**0.83**	0.022	**0.82**	0.025
DW/FW ratio for Whole	**0.060**	0.008	**0.059**	0.005	**0.060**	0.005
DW/FW ratio for Blade	**0.065**	0.009	**0.065**	0.006	**0.067**	0.006
DW/FW ratio for Pets. and	**0.041**	0.006	**0.042**	0.004	**0.043**	0.004
n	22		22		21	

Table 3-4. Fresh and dry biomass by main plant parts in the Day 17 harvest

It can be seen there was very little difference in average shoot fresh weight between the three viable temperatures of 20, 25 and 30°C on either day. The air temperature of 35°C resulted in an average shoot weight less than half the others showing that not only in germination but in shoot growth as well, 35°C is well beyond the optimum temperature range for growth. The proportion of the plant consisting of leaf blade (as opposed to stem and petiole) and was significantly higher in the 20°C condition than the 25°C and 30°C conditions on both days and on both a fresh and dry weight basis (e.g., 83% vs. 76% and 74% on a FW basis for day 16, with p values for a two tailed test of 5xE-9, and 9xE-16), a result recommending use of the lower temperature. However, the total for leaf blade material includes the cotyledons, and there is some question as to whether they should be considered saleable.

Dry matter content of plants (DW/FW ratio) was nearly the same in 20°C, 25°C and 30°C conditions, and consistent across the two harvests. It was 6% overall. Dry matter content increased slightly in the blade material with increasing temperature. Dry matter content was very much higher in the 35°C condition than the other temperatures.

Detailed Day 17 Harvest. The day-17 harvest was devoted to a very close look at plant make-up in the three viable temperature conditions of 20, 25, and 30°C. The most salient plant characteristics were quantified by counts and measurements as far as possible. Data are presented in Table 3-5 below. (Plants were badly stunted at 35°C. These plants were not characterized in detail.)

Day 17 Summary All weights are in grams	Temperature Condition					
	20C		25C		30C	
	Average	SD	Average	SD	Average	SD
Average FWs of leaf-blade pairs						
Cotyledons	**0.62**	0.11	**0.47**	0.08	**0.39**	0.14
Primary leaves	**1.54**	0.42	**1.10**	0.32	**0.92**	0.33
2nd pair of true lvs	**0.36**	0.16	**0.52**	0.25	**0.77**	0.46
3rd pair of true lvs	**0.04**	0.03	**0.10**	0.10	**0.23**	0.20
Total for All Leaf-blade Material	**2.57**	0.65	**2.18**	0.66	**2.32**	1.07
Leaf areas (cm2)						
Cotyledons	**8.8**	1.48	**6.3**	1.47	**4.6**	1.77
Primary leaves	**42.1**	9.39	**27.3**	6.77	**19.8**	5.80
2nd pair of true lvs	**13.9**	5.36	**17.4**	7.38	**22.3**	11.05
3rd pair of true lvs	**1.1**	1.00	**3.2**	3.49	**7.7**	6.70
Total for All Leaf-blade Material	**65.8**	15.48	**54.3**	16.16	**54.3**	23.32
n	**22**		**22**		**12**	
Primary leaves' characteristics						
Longest petiole (cm)	**4.9**	0.56	**5.3**	1.00	**5.3**	0.53
Second longest petiole	**4.6**	0.56	**4.8**	0.98	**4.8**	0.47
Largest leaf-blade:						
Length	**8.0**	0.90	**7.5**	0.98	**6.8**	1.07
Max. width	**4.0**	0.53	**3.4**	0.44	**2.7**	0.45
Ratio of length to width	**2.0**	0.19	**2.2**	0.28	**2.5**	0.26
Second leaf blade:						
Length	**7.6**	0.95	**7.0**	0.93	**6.1**	1.12
Max. width	**4.0**	0.51	**3.3**	0.46	**2.5**	0.48
Ratio of length to width	**1.9**	0.19	**2.2**	0.19	**2.5**	0.34
n	**22**		**10**		**12**	

Table 3-5. Detailed leaf characteristics in the Day 17 harvest

Spinach produces leaves in pairs of about the same size, starting with the cotyledons, then the first true leaves, etc. By day 17, these plants typically had three pairs of measurable true leaves in addition to the cotyledons. Plants were dismembered and divided into pairs of leaves, which were then separated into blade and petiole. The fresh weight and area of each leaf-blade pair was obtained individuallywith the results shown in the top half of Table 3-5 above. (The fresh weight of the petioles and stem was obtained collectively. Dry weights were obtained for all leaf blade material collectively, and all petiole and stem material collectively, as presented in Table 3-4 above.)

The largest leaves in all treatments were the first true leaves, also known as primary leaves; they could be expected to dominate any display of baby leaf spinach in the market place, and also would be the first leaves to get too big to qualify as “baby”. The physical characteristics of the primary leaves (petiole length and overall leaf length and width) were obtained for each of the primary leaves on every plant, in addition to leaf area and fresh weight. These data are presented in the lower half of Table 3-5.

Although differences in average whole plant fresh and dry weight at day 17 were insignificant, examination of plant the data tabulated shows distinctly different morphological and developmental characteristics of the plants in the three temperature conditions. Seedlings grown at 20°C were least advanced in terms of number of leaves produced or size of most recently showing/expanded leaves. For the most part, at 20°C the third pair of true leaves barely showed at the end of day 21, and averaged just a fraction of a square centimeter in area, as compared to plants at higher temperatures where the fourth pair of true leaves was also sometimes showing, and the area of the 3rd pair of leaves was considerable (see leaf area data for the 3rd pair of true leaves in Table 3-5). Conversely, the cotyledons and first true leaf pair were dramatically larger in the 20°C condition than in other conditions; the leaf area of the primary leaves in the 20°C condition was more than twice that of primary leaves in the 30°C treatment. Distribution of leaf area between leaf pairs within each temperature condition was also radically different. In the 20°C condition the 1st pair of true leaves was three times the area of the 2nd pair of true leaves, whereas in the 30°C condition the 1st and 2nd pairs of true leaves were equal in area. It appears that at higher temperatures new plant organs were instigated faster than at lower temperatures, and, evidently, these became competitors for photosynthate at the expense of the first-produced organs such as cotyledons and primary leaves, which then received a lower allocation. One must suppose in order to generate the same amount of dry weight (as appears to have been the case), the same amount of photosynthate was produced in all temperature conditions and the light catching apparatus or leaf area would need to be similar across conditions in order to do so unless photosynthetic efficiency differed widely.

The differences in plant morphology and of weight distribution between plant parts may be more clearly seen if the data are normalized as has been done in Table 3-6 below. Here, the different leaf pairs have been expressed as proportions of either the whole plant weight or just the leaf-blade part of the plant. It is interesting that despite the fact that more of the plant weight is in leaf in the 20°C condition than higher temperature conditions, the proportion of the plant that is most desirable for sale, the true leaves, is exactly the same in all temperatures, at 60%. This is because the cotyledons became progressively smaller as temperature increased, and a greater proportion of total leaf weight (and area) belonged to true leaves.

Although there was no particular advantage in terms of saleable biomass to one temperature over another in these harvests, certain quality factors favored the 20°C condition. Generally, plant appearance was superior in plants grown at 20°C, for a number of reasons that will be detailed.

Ratios of FWs of different leaf-blade pairs	Temperature Condition								
	20C			**25C**			**30C**		
	Average Ratio to all leaf	SD	Average Ratio to all shoot	Average Ratio to all leaf	SD	Average Ratio to all shoot	Average Ratio to all leaf	SD	Average Ratio to all shoot
Cotyledons	0.25	0.048	0.20	0.23	0.064	0.17	0.18	0.049	0.13
1st true leaves	0.60	0.037	0.48	0.51	0.053	0.38	0.42	0.087	0.31
2nd true leaves	0.13	0.040	0.11	0.23	0.062	0.17	0.31	0.071	0.23
3rd true leaves	0.01	0.010	0.01	0.04	0.035	0.03	0.09	0.053	0.06
All true leaves	0.75	0.048	**0.60**	0.77	0.064	**0.59**	0.82	0.049	**0.60**
All leaf blade	1.00		0.80	1.00		0.76	1.00		0.73
Whole shoot			1.00			1.00			1.00

Table 3-6. Ratios of fresh weights of leaf types to total leaf weight and shoot weight

On day 17, the prototypical leaf in the 20°C temperature condition was of an elliptical shape with major and minor axes of 8 and 4 cm (3 and 1.75 inches). This is an ideal size for a baby leaf spinach product. Leaves were flatter and more upright than at higher temperatures, and cotyledons were bigger and straighter. As shown in the Table 3-5 above, primary leaves were rounder than at higher temperatures, in which the ratio of length to width progressively increased from 1.9 to 2..5.

At progressively higher temperatures (30 and 35°C), there was progressively more tendency towards two types of leaf curvature in true leaves -- edge rolling and mid-rib curvature. At 25°C, the edges of the first true leaves (in particular) tended to be rolled under on both sides, reducing projected leaf area by 25% to 50% (though functionally this probably mattered little in a dense stand as far as light capture was concerned). There was also some epinastic curvature along the long axis of the leaf (mid-rib curvature); that is, the midrib formed a convex arch viewed from above/the side. This was particularly found in the 2nd true leaves. (If the leaf was turned over and placed in the palm of the hand, it would act as a cup for water. For taking area, this epinastic curve was inverted, which helped to straighten out the edge -rolling, if it was not too exaggerated. The leaf was also split if necessary, particularly at the base and tip, to assist in flattening out the leaf for taking areas.)

Additional types of leaf deformation were found just in the cotyledons. The ends of cotyledons, which in spinach are much longer than wide, were often rolled up into spirals in higher temperature plants (25 and 30°C). The tip was curled under the leaf blade, with curvature tightest at the tip. Alternatively, in many cases cotyledons took a lateral corkscrew twist towards the end of the leaf in 30°C and 35°C plants. Only in the 20°C condition were cotyledons typically straight and untwisted. In the two highest temperatures, there was some incidence of disease and necrosis of the cotyledons.

With increase in temperature from 20 to 25°C, petioles tended to become longer in both first and second true leaves, but there was little difference in petiole length between the 25°C and 30°C conditions. Petioles were, if anything, shorter in the 35°C condition than other conditions, because of overall stunting of the plant.

The shape of the first true leaf looked quite different depending on temperature, apart from the elongation mentioned above. As temperature increased above 20°C, there was more frequent occurrence of a type of leaf where the start of the leaf blade was ill defined on the petiole, and tapered up to maximum width only gradually. (This type of leaf is Romaine-like in appearance in this respect, but it is not folded about the midrib as Romaine is. If it exhibits epinasty, the curvature is opposite to that of Romaine.) These leaves tended to be small and leathery, often with edge rolling, and thus not particularly attractive from the consumer viewpoint.

One cannot determine from this experiment where temperature is acting to bring about these effects. Probably both air and root temperatures contribute to both the developmental and morphological changes to at least some degree. It is also likely that cultivars exist that behave rather differently from Alrite in respect to temperature, and for which temperature is less critical. Temperature does seem to be very important in controlling product quality, and also the ease with which the crop can be cut and harvested without disfiguring leaves or including too much petiole.

Day 20 Summary	Temperature Condition					
Weights are for 4-cell Sample Units	20C		25C		30C	
(g)	Average	SD	Average	SD	Average	SD
Av. FW of Whole Shoot per SU	**22.73**	2.89	**22.52**	6.34	**18.74**	4.63
Av. FW of Blade alone per SU	**16.74**	2.32	**15.79**	4.32	**12.92**	3.06
Av. FW of Pet. and Stem per SU	**5.72**	0.82	**6.42**	1.96	**5.46**	1.42
Ratio: Blade FW to Whole Shoot	**0.74**	0.02	**0.71**	0.02	**0.70**	0.02
Av. DW of Whole Shoot per SU	**1.27**	0.21	**1.32**	0.48	**1.09**	0.33
Av. DW of Blade alone per SU	**1.06**	0.19	**1.07**	0.39	**0.87**	0.26
Av. DW of Pet. and Stem per SU	**0.22**	0.03	**0.25**	0.09	**0.22**	0.07
Ratio: Blade DW to Whole Shoot	**0.83**	0.02	**0.81**	0.02	**0.80**	0.01
DW/FW ratio for Whole Shoot	**0.057**	0.01	**0.059**	0.01	**0.059**	0.00
DW/FW ratio for Blade only	**0.063**	0.01	**0.067**	0.01	**0.067**	0.004
DW/FW ratio for Pets. and Stems	**0.038**	0.00	**0.039**	0.00	**0.041**	0.00
n (no. of sample units)	**6**		**6**		**6**	
Av. no. of plnts per SU	**3.67**		**3.17**		**3.17**	
Av FW of single plants	**6.20**		**7.11**		**5.92**	

Table 3-7. Fresh and dry biomass by main plant parts in the Day 20 harvest using a 4-cell sample unit.

The second harvests (days 20 and 21) were done with the detailed harvest first and the fast harvest second. As with the first harvests, summaries are presented first for whole plants with a simple breakdown into blade and stem materials. See Table 3-7 above and Table 3-8 below.

For the detailed harvest, the sample unit was a square area of 4 cells with whatever number of plants fell within that area (which varied from 2 to 4 plants).

Day 21 Summary Weights are single plant (g)	Temperature Condition					
	20C		25C		30C	
	Average	SD	Average	SD	Average	SD
Av. FW of Whole	**6.11**	3.28	**5.69**	2.53	**7.14**	2.35
Av. FW of Blades	**4.41**	2.41	**3.85**	1.75	**4.89**	1.67
Av. FW of Pet. and	**1.66**	0.87	**1.82**	0.81	**2.20**	0.69
Ratio: Blade FW to Whole	**0.72**	0.02	**0.68**	0.03	**0.68**	0.02
Av. DW of Whole	**0.34**	0.22	**0.30**	0.18	**0.36**	0.13
Av. DW of Blades	**0.27**	0.18	**0.24**	0.15	**0.31**	0.11
Av. DW of Pet. and	**0.07**	0.04	**0.07**	0.04	**0.09**	0.03
Ratio: Blade DW to Whole	**0.79**	0.03	**0.77**	0.02	**0.78**	0.80
DW/FW ratio for Whole	**0.051**	0.01	**0.051**	0.01	**0.054**	0.00
DW/FW ratio for Blade	**0.057**	0.01	**0.059**	0.01	**0.062**	0.00
DW/FW ratio for Pets. and	**0.038**	0.00	**0.036**	0.00	**0.038**	0.00
n	**9**		**11**		**11**	

Table 3-8. Fresh and dry biomass by main plant parts in the Day 21 harvest

Although overall shoot weights were somewhat variable, it is clear there still was no significant difference between the temperature conditions in terms of overall biomass. In the four days between harvests, the proportion of the whole plant weight as leaf blade decreased considerably (from 83% to 72% in the case of the 20°C condition), and the three temperature conditions converged towards the same proportion. Dry matter content fell steadily as well. A flat of plants in the 35°C condition was saved for harvest on day 21. Fresh and dry weights were obtained for 22 plants. Average fresh weight was 3.53g, still half that of the other treatments, and dry matter content remained high at 6.9%.

It can be seen in Tables 3-9 and 3-10 following that in this detailed harvest, as the last, the distribution of weight (fresh and dry) and leaf area was greatly different in the different temperature conditions, with more of the area and weight in the older leaves in the lower temperature condition than the higher temperature conditions.

In the day 20 harvests, dry weights were obtained separately for different leaf types permitting examination of dry matter content for each age of leaf. A consistent pattern in all temperature conditions was for dry matter content to be progressively higher in the younger leaves. This effect was strongest in the 20°C

condition. At the same time the specific leaf area, or amount of light-catching surface projected per unit weight of leaf, was also progressively higher in younger leaves.

Day 20 Leaf Wt Details	Temperature Condition					
Averages are for 6	20C		25C		30C	
4-cell sample units. (g)	Average	SD	Average	SD	Average	SD
Whole Plant FW - Av. SU (g)	22.73	2.89	22.52	6.34	18.74	4.63
Av. FWs for leaf-blade pairs (g)						
Cotyledons	2.40	0.37	1.57	0.40	1.36	0.18
1st true lvs	8.97	1.05	6.00	1.76	3.85	0.77
2nd true lvs	4.04	1.00	4.93	1.47	4.62	1.00
3rd true lvs	1.17	0.39	2.76	0.82	2.45	1.06
4th true + lvs	0.16	0.06	0.54	0.17	0.64	0.51
All True Leaves	14.34	2.08	14.22	3.99	11.56	2.89
All Leaf-blade material	16.74	2.32	15.79	4.32	12.92	3.06
Av. DWs for leaf-blade pairs (g)						
Cotyledons	0.11	0.029	0.07	0.021	0.07	0.011
1st true lvs	0.54	0.063	0.37	0.135	0.22	0.046
2nd true lvs	0.29	0.078	0.35	0.144	0.32	0.084
3rd true lvs +	0.12	0.045	0.28	0.099	0.26	0.140
All True Leaves	0.95	0.172	1.00	0.366	0.80	0.247
All Leaf-blade material	1.06	0.187	1.07	0.386	0.87	0.256
Av. DW/FW ratios						
Cotyledons	0.046	0.010	0.045	0.008	0.053	0.002
1st true lvs	0.060	0.006	0.062	0.006	0.058	0.003
2nd true lvs	0.071	0.006	0.069	0.008	0.068	0.004
3rd true lvs +	0.087	0.008	0.083	0.007	0.083	0.004
All True Leaves	0.066	0.006	0.069	0.007	0.068	0.004
All Leaf-blade material	0.063	0.007	0.067	0.007	0.067	0.004
n	6		6		6	
Average no. of plants per SU	3.67		3.17		3.17	

Table 3-9. Detailed biomass characteristics of different leaf types in the Day 20 harvest

Day 20 Leaf Area Details	Temperature Condition					
Averages are for 6	20C		25C		30C	
4-cell sample units. (g)	Average	SD	Average	SD	Average	SD
Areas (cm2)						
Cotyledons	32	5	19	5	15	1
1st true lvs	211	29	129	41	73	15
2nd true lvs	130	28	133	42	122	32
3rd true lvs	46	17	95	29	79	26
4th true + lvs	4	2	19	7	32	25
All True Leaves	**391**	60	**375**	113	**290**	77
All Leaf-blade material	**423**	63	**394**	118	**305**	78
Specific leaf area (cm2 g-1) FW basis						
Cotyledons	13.5	0.8	12.1	1.4	10.9	1.0
1st true lvs	23.5	1.4	21.3	1.3	19.0	0.9
2nd true lvs	32.6	3.1	26.8	2.1	26.6	5.9
3rd true lvs	39.0	2.2	34.4	2.8	32.9	3.2
4th true + lvs	25.2	2.0	34.1	2.2	34.3	2.6
All True Leaves	**27.3**	1.4	**26.2**	1.6	**25.1**	2.9
All Leaf-blade material	**18.6**	0.5	**17.4**	0.9	**16.3**	1.9
Specific leaf area (cm2 g-1) DW basis						
Cotyledons	306	82	272	40	206	19
1st true lvs	392	50	348	35	328	20
2nd true lvs	461	53	392	44	392	92
3rd true lvs +	429	47	416	41	370	29
All True Leaves	**417**	47	**382**	38	**367**	43
All Leaf-blade material	**405**	48	**374**	38	**353**	40
n	6		6		6	

Table 3-10. Detailed leaf area characteristics of different leaf types in the Day 20 harvest

Day 21 Details	Temperature Condition								
Ratios of FWs of different leaf-blade pairs	20C			25C			30C		
	Average Ratio to all leaf	SD	Average Ratio to all shoot	Average Ratio to all leaf	SD	Average Ratio to all shoot	Average Ratio to all leaf	SD	Average Ratio to all shoot
Cotyledons	0.14	0.018	0.11	0.10	0.013	0.07	0.11	0.011	0.08
1st true leaves	0.54	0.041	0.40	0.38	0.034	0.27	0.30	0.043	0.21
2nd true leaves	0.24	0.036	0.18	0.31	0.028	0.22	0.36	0.019	0.25
3rd true leaves	0.07	0.018	0.05	0.18	0.027	0.13	0.19	0.040	0.13
4th true leaves	0.01	0.003	0.01	0.03	0.006	0.02	0.05	0.025	0.03
All true leaves	0.86	0.018	**0.64**	0.90	0.013	**0.64**	0.89	0.011	**0.63**
All leaf blade	1.00	0.000	0.74	1.00	0.000	0.71	1.00	0.000	0.70
Whole shoot			1.00			1.00			1.00

Table 3-11

In Table 3-11 above, showing ratios of different plant parts to the whole shoot in the day 21 harvest, again it was found that a consistent proportion of the plants across the three viable temperature conditions, this time 63 to 64%, was in the form of leaf-blades of true leaves, the part that is most saleable. (The same percentage was found in the day 20 harvest, which may be calculated from data in Table 3-9.) Whether all of this 63 to 64% could be recovered when cutting in a single plane remained to be determined.

One cannot determine from this experiment where temperature is acting to bring about these effects. Probably both air and root temperatures contribute to both the developmental and morphological changes to at least some degree. It is also likely that cultivars exist that behave rather differently from Alrite in respect to temperature, and for which temperature is less critical. Temperature does seem to be very important in controlling product quality, and also the ease with which the crop can be cut and harvested without disfiguring leaves or including too much petiole.

Temperature Experiment. 3: Temperature Effects on Early Growth Subsequent to Germination at the Same Temperature

Rationale. This concurrent second experiment was intended to complement the experiment just described above. The same four different temperatures spanning the full range likely to be encountered in production were used. In that experiment the timing of seedling emergence, degree of stand establishment, and subsequent plant growth and morphological characteristics were shown to be strongly affected by temperature, as expected, but it was difficult to make precise evaluations of biomass production because the

length of time over which plants were harvested was protracted in order to collect detailed morphological plant data.

In this experiment, the major change was that germination and seedling emergence were accomplished under the same near-optimal temperature conditions for all flats, and only then were the different temperature conditions applied. This was so effects on crop productivity due to differences in timing of seedling emergence could be separated from effects due to subsequent differences in developmental growth rate and/or photosynthesis and respiration associated with temperature differences. In production, seedling preparation will be separate from crop grow-out. Each of these phases can be optimized for temperature separately.

After seedling emergence was complete, flats were placed under the same temperature conditions as in the previous experiment and harvested when 19 days old. Harvest was fast. Fresh and dry weights were obtained and also leaf blade to total weight ratios, with a view to evaluating productivity.

Hypotheses. It was not expected that the different temperatures used for germination in the first experiment would have long-term effects on subsequent growth other than in starting the growth cycle slightly sooner or later. The prediction was the same plant morphological effects of temperature would be found in this experiment as in the previous one once seedlings emerged.

It was thought best biomass performance in terms of shoot fresh weight would be at 25°C; it was expected 20°C would produce better than 30°C, and performance at 35°C would be worst. It was thought DW to FW ratio (dry matter content) would decrease with temperature increase, and as a consequence, there would be less difference in dry weight than in fresh weight over the temperature conditions.

Method. Eight 1.75-inch high polystyrene Speedling flats of 72 cells (cell density 1464 cell m^{-2}) in a 6x12 matrix were filled with peatlite mix, which had been adjusted to 3:1 water to dry matter content, and seeded with carefully selected de-hulled seed of the Japanese cultivar Alrite. The technique of seeding flats followed standard procedures that have been described elsewhere.

Flats were placed in plastic bags that were loosely closed then placed in closed Rubbermaid tubs in the presence of free water. Tubs were then set in growth chambers for germination. Seeds were germinated for two days at 25°C after which flats were removed from the plastic bags, but remained in the closed tubs. Germination and seedling emergence were continued for two days at 20°C, with daily rotation and repositioning of the tubs. Emergence performance was closely examined.

At the end of the germination period (4 days), all flats showed a minimum of 68 out of 72 seedlings visible, but not all could be considered of good quality. Stands were adjusted so that seedlings were equal in number and quality. Weak and late seedlings were removed. Plant stands were eventually adjusted down to 59 seedlings. Three matched pairs of flats were made up and randomly assigned to the temperature conditions 20°C, 25°C and 30°C. Each flat was considered to be an internal replicate. Two flats were also assigned to the fourth temperature condition, 35°C, but only one of these was comparable in plant stand to the other six flats. The second flat was short in number of seedlings, having suffered an excessive occurrence of "pop-ups". (Note. The best reason for the excessive occurrence of pop-ups in the one flat we were able to suggest was that the medium was drier or more loosely packed in this flat on the evidence that the filled flat was the lightest after seeding.) Flats were floated in small ponds (large polyethylene Rubbermaid tubs), one pond containing two flats per temperature condition. The nutrient solution used was drawn from a common reservoir of nutrient solution prepared as described previously, and adjusted for EC and pH. This reservoir was used throughout the experiment to maintain the solution level in the tubs and make up for losses to evaporation and plant uptake. Solution parameters were monitored, but no adjustments made except to volume during the 15 days the flats were in flotation. Two air stones supplied with compressed air were inserted into each of the tubs, to provide aeration and water circulation. The ponds were rotated every few days to balance for any position effects. Light intensity was monitored daily and altered as necessary to provide the same daily integral in each chamber within a 16-hour photoperiod. Air and water temperature were also periodically monitored.

Harvest was on December 24, 2002. One set of flats (F2 flats) was harvested between 3.10 PM and 4.15 PM. Just FW and DW of sample units (SUs) of 9-cell areas were obtained as well as number of plants included in the sample unit. Order of harvest was 20°C thru 35°C. The second set of flats was harvested between 4.15 and 6.15 PM of the same day, in the reverse order. In this case, blades were separated from stem and petioles in taking weights and putting material to dry. Accessible root material was bulked by flat.

Results. The final harvest in this experiment (day 19) was one day later than the final harvest in the previous experiment described (day 21), and the same growth chambers were shared for each of the conditions (but not the same ponds) in both experiments. Consequently, it was possible to make direct visual comparisons between the stands of plants under the same temperature condition, looking back and forth between the crop stands. Apart from the slight difference in age, there was no difference in appearance of the morphological characteristics detailed in the report from the previous experiment, between plants under the same temperature condition of either experiment.

The result for fresh weight of crop is shown for the combined replicates in Figure 3-5 below.

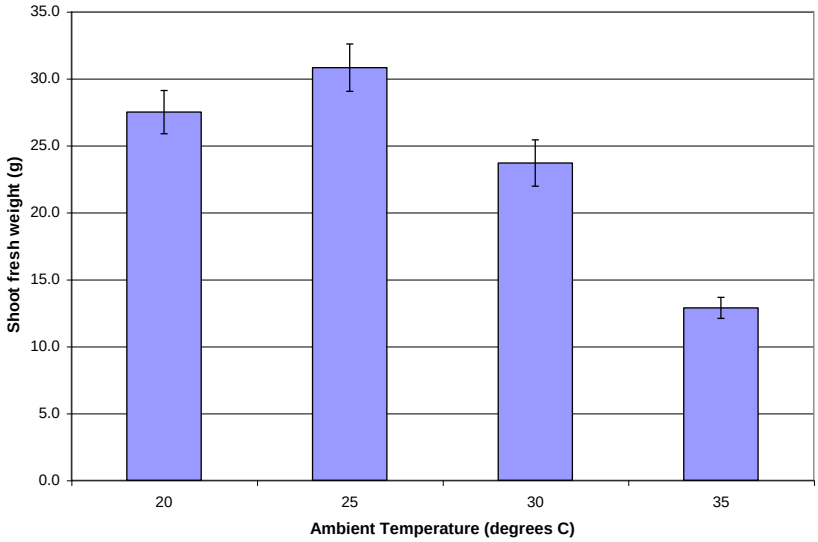


Figure 3-5: Mean shoot fresh weight as affected by ambient temperature.

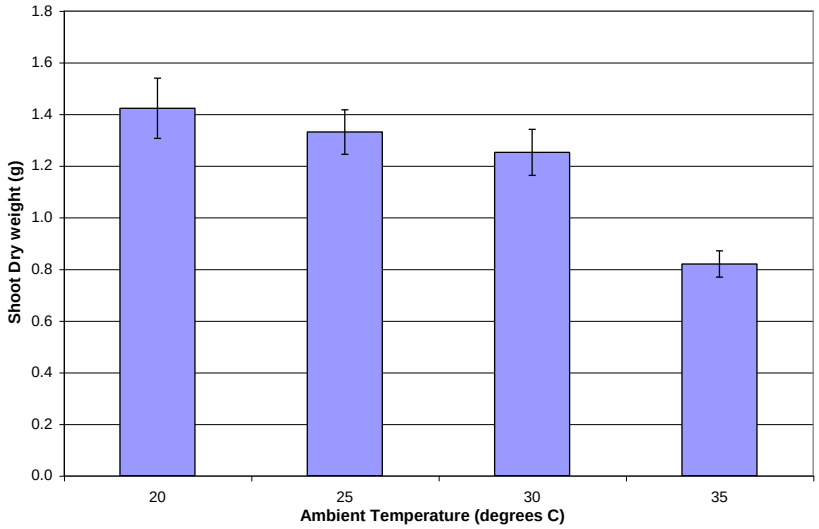


Figure 3-6: Mean shoot dry weight as affected by temperature

The results for fresh weight followed the hypotheses: 25°C gave the best result, 35°C the worst, and 20°C was better than 30°C. The results for dry weight (Fig. 3-6) were considerably different. There was no statistical difference in dry weight between the 20, 25 and 30°C conditions, although there was a small trend downwards with each rise in temperature. The 35°C condition was sharply down.

The different results for dry and fresh weights appear to result from a difference in water content, as reflected in dry-weight-to-fresh-weight ratio (DW/FW). This is a U shaped function as shown in Figure 3-7 below.

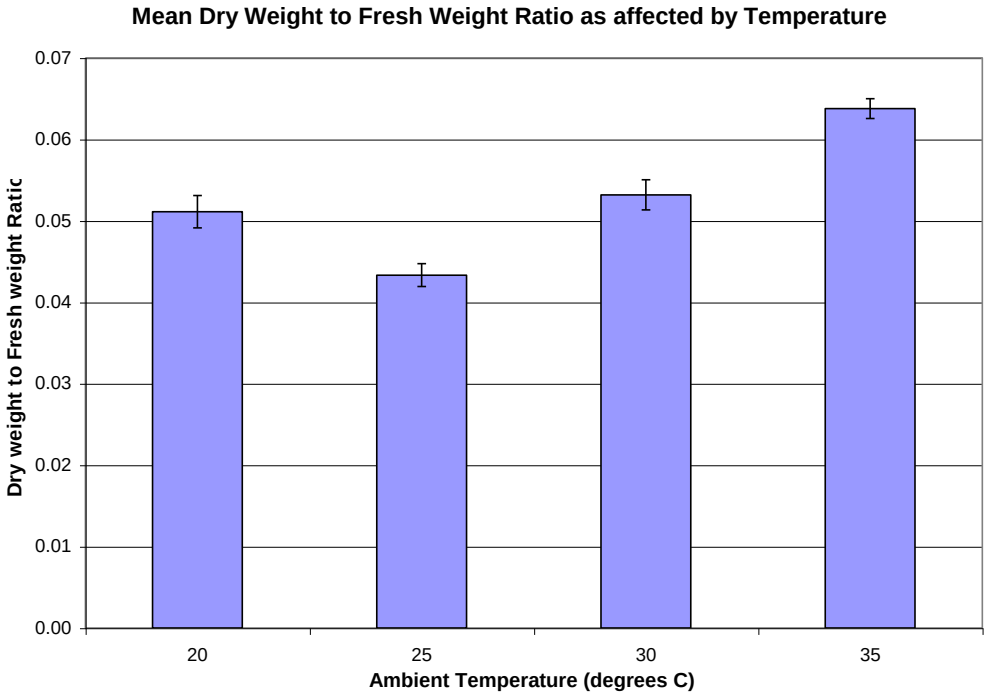


Figure 3-7: Effect of temperature on DW to FW ratio. Error bars show plus and minus one standard error about the mean.

The results for DW/FW were as expected only for the 25°C condition, which had lower dry matter content than the 20°C condition. From 25°C on, DW/FW ratio increased with increases in ambient temperature. It can be seen from the error bars the dry matter content at various temperatures was a stable property, with little variability between plants.

The data on which the charts are based are presented in Tables 3-12 and 3-13 below. Most of the comparisons suggested by the hypotheses are examined in t-tests in Table 3-14. In deciding what differences to consider significant, allowance should be made for the number of comparisons made at one time.

	Temperature Condition (deg. C)	No. Sample Units (SU) (n)	Av. Fresh weight per SU (g)	Av. Dry weight per SU (g)	Av. Ratio DW to FW per SU	Av. no. Plants per SU
	20	8	27.52	1.38	0.049	7.4
Rep 1	**25**	8	31.51	1.42	0.045	7.3
	30	8	20.51	1.17	0.057	7.3
	35	8	15.21	0.96	0.063	6.8
	20	8	27.47	1.46	0.053	7.3
Rep 2	**25**	8	30.11	1.24	0.041	7.0
	30	8	26.87	1.34	0.050	7.3
	35	8	10.53	0.68	0.065	4.6
	20	16	27.49	1.42	0.051	7.3
Combined	**25**	16	30.81	1.33	0.043	7.1
Reps	**30**	16	23.69	1.25	0.053	7.3
	35	16	12.87	0.82	0.064	5.7

Table 3-12: Mean Fresh and Dry weights and DW-FW ratios

	Temperature Condition (deg. C)	No. Sample Units (SU) (n)	SD of Fresh weight per SU	SD of Dry weight per SU	SD of Ratio DW to FW per SU	SD of No. Plants per SU
	20	8	7.78	0.59	0.010	1.92
Rep 1	**25**	8	7.87	0.35	0.004	1.04
	30	8	4.45	0.27	0.006	1.16
	35	8	1.95	0.14	0.003	1.16
	20	8	5.39	0.34	0.005	0.5
Rep 2	**25**	8	6.67	0.34	0.007	0.8
	30	8	7.72	0.42	0.007	1.4
	35	8	2.23	0.16	0.006	0.9
	20	16	6.47	0.47	0.008	1.4
Combined	**25**	16	7.08	0.34	0.006	0.9
Reps	**30**	16	6.92	0.36	0.007	1.2
	35	16	3.15	0.20	0.005	1.5

Table 3-13: Standard Deviations for Fresh and Dry weights and DW-FW ratios

	Temperature conditions compared	Fresh weight comparisons		Dry weight comparisons		DW to FW comparisons	
		Ratio of Means	p value (1-tail test)	Ratio of Means	p value (1-tail test)	Ratio of Means	p value (2-tail test)
	25 to 20C	1.15	0.007	1.03	0.361	0.92	0.311
Rep 1	25 to 30C	1.54	0.002	1.22	0.013	0.79	0.022
	20 to 30C	1.34	0.024	1.19	0.127	0.87	0.001
	30 to 35C	1.35	0.002	1.22	0.012	0.90	0.013
	25 to 20C	1.10	0.075	0.85	0.032	0.78	0.004
Rep 2	25 to 30C	1.12	0.407	0.93	0.281	0.84	0.033
	20 to 30C	1.02	0.095	1.09	0.259	1.07	0.021
	30 to 35C	2.55	0.001	1.96	0.004	0.77	0.000
	25 to 20C	1.12	0.001	0.94	0.109	0.85	0.006
Combin	25 to 30C	1.30	0.001	1.06	0.205	0.81	0.000
Reps	20 to 30C	1.16	0.040	1.14	0.054	0.96	0.332
	30 to 35C	1.84	0.000	1.53	0.001	0.83	0.000

Table 3-14. Result of t-tests for selected paired comparisons: p values and ratios

In this experiment small unguarded crops stands were produced, yet they were harvested quite young before edge effects had too great an effect, so it is of interest to examine productivity and light use efficiency. So far results have been presented for small rectangular areas of 9 cells; Table 3-15 below gives productivity and light use efficiency calculated in standard units. Calculations are presented both on an area basis and a plant basis. The calculations on an area basis give realistic figures for productivity in the 20, 25, and 30°C conditions. Crop duration was taken to be 17 days.

In thinning the stand to match flats, plant density was reduced unevenly in some cases, which would have depressed productivity if remaining plants were unable to intercept the light falling in the gaps left behind. To set an upper limit, calculations were also made assuming all cells in the flat produced at the rate of the average plant. The productivity of an un-thinned stand can be expected to lie between the per-cell and per-plant values.

	Temperature Condition (deg. C)	Av. no. Plants per SU	Plant Density (plnts m-2)	Av. Cell Fresh weight (g per cell)	Av. Cell Productivity (g m-2 d-1)	Light use Efficiency (g mol-1)	Av. Plant Fresh wght (g per plnt)	Av. Plant Productivity (g m-2 d-1)	Light use Efficiency (g mol-1)
	20	7.4	1200	3.06	263	19	3.80	327	23
Rep 1	**25**	7.3	1180	3.50	302	21	4.36	376	26
	30	7.3	1180	2.28	196	14	2.84	245	17
	35	6.8	1098	1.69	146	10	2.29	197	14
	20	7.3	1180	3.05	263	19	3.81	328	23
Rep 2	**25**	7.0	1139	3.35	288	20	4.28	369	26
	30	7.3	1180	2.99	257	18	3.69	318	22
	35	4.6	753	1.17	101	7	2.29	198	14
	20	7.3	1190	3.05	263	19	3.80	328	23
Both	**25**	7.1	1159	3.42	295	21	4.32	372	26
Reps	**30**	7.3	1180	2.63	227	16	3.27	281	20
	35	5.7	925	1.43	123	9	2.29	197	14

Table 3-15. Productivity and light use efficiency in the final harvest

Time was taken to weigh leaf blade material separately from stems and petioles in harvesting one of the reps in this experiment, and to obtain fresh and dry weights on this basis. These data are of interest because they give a rough estimate of the proportion of the harvest that is saleable as baby spinach in fresh cut packages.

It was expected the highest proportion of shoot fresh weight in leaf blade would be found in the lowest temperature conditions, and this was the case. 76% of the fresh weight was in leaf blade in the 20°C condition, and this fell to 71% in the 30°C condition. All comparisons were significant. (The blade proportion was 78% in the 35°C condition, but here the plants were stunted and productivity was very low, so it is not a useful finding.) These trends can be seen in Table 3-16 below. In this table a calculation is made to show the effect on the relative performance under the different temperature conditions if just the leaf blade part of the plant is considered. Whereas in terms of total shoot, the 20C condition only produces 89% that of the 25C condition, if the economic part of the plant is considered, it is raised to 93%.

Temperature Condition	Ratio of leaf blade to whole shoot (Rep2)		FW Whole Shoot	Ratio: Total FWs to 25C Total FW	FW of leaf blade only	Ratio: Blade FWs to 25C Blade FW
	FW	DW				
20C	0.76	0.844	27.49	0.89	20.88	0.93
25C	0.73	0.832	30.81	1.00	22.46	1.00
30C	0.71	0.798	23.69	0.77	16.90	0.75
35C	0.78	0.820	12.87	0.42	10.04	0.45

Table 3-16. Proportion of the whole shoot in leaf blade

The dry matter content of leaf tissue was found to be almost twice that of petiole and stem tissue, as expected for young herbaceous plant material. (See Table 3-17 below) As a consequence, the proportion of total plant dry weight found in leaves was considerably higher than the proportion of total fresh weight found in leaves. It was 84% in the 20°C condition falling to 80% in the 30°C condition. In the case of dry matter, although the trends were the same as for fresh weight, not all comparisons were significantly different. (See Table 3-16 above)

Temperature Condition	DW/FW ratios			Comparison of DWtoFW ratios to the 25C Temperature Condition		
	Leaf blade	Petioles	Whole Shoot	Leaf blade	Petioles	Whole Shoot
20C	0.059	0.034	0.053	1.25	1.32	1.28
25C	0.047	0.026	0.041	1.00	1.00	1.00
30C	0.055	0.035	0.050	1.17	1.33	1.19
35C	0.068	0.053	0.065	1.44	2.03	1.56

Table 3-17. Dry weight to fresh weight ratios by plant part

Dry weight to fresh weight ratios for petioles and leaf blades showed the same U-shaped function with temperature as illustrated for the whole shoot earlier. It appeared the differences correlated with temperature were greater for petioles than for leaf blade tissue, as illustrated in the right-hand columns of Table 3-17 above where, for instance, the DW-FW ratio for petioles in 20°C and 30°C conditions is over 30% greater than in the 25°C conditions, but for leaf blade tissue it is only 17 to 25% greater than in the 25°C condition.

In statistical tests for differences between DW-FW ratios, the only time when ratios are not significantly different is when the height on the legs of the U-curve is the same.

Temperature Condition Compared	Ratio of leaf blade to whole shoot weight		DW/FW ratio comparisons		
	FW	DW	Leaf blade	Petioles	Whole Shoot
		p values for 1-tailed t-tests			
20C-25C	0.000	0.423	0.002	0.023	0.002
25C-30C	0.006	0.047	0.016	0.035	0.021
20C-30C	0.000	0.000	0.045	0.377	0.033
30C-35C	0.000	0.025	0.002	0.000	0.000

Table 3-18. Statistical comparisons for ratios of leaf blade to whole shoot and DW/FW

Discussion. When one looks at a stand of spinach, what one sees is not well expressed in simple measures of biomass such as fresh or dry weight. In this experiment, the best fresh leaf biomass production by weight was from the 25°C condition. However, although spinach is sold by fresh weight (rather than dry weight or

individual plant), fresh weight does not necessarily reflect the best looking spinach for attracting the consumer, the most manageable spinach for harvest, the highest dry weight production, or the greatest leaf area. In this experiment, the most attractive and easiest to harvest spinach came from the 20°C condition, without a doubt, because the leaves were well formed and flat, the petioles were short, and the habit was upright. The tendency of the leaves to break under handling is also an issue in packaging spinach, and in this case, the turgidity and dry matter of the leaf are controlling factors in the pliability of the leaf. The dry matter content of the leaf blade was higher in the 20°C condition than either the 25 or 30°C condition, an additional advantage of this temperature.

Temperature Experiment 4: Effect of Root Versus Shoot Zone Temperature in a Commercially Applicable Range

Rationale. The experiments described showed that variation in ambient air temperatures in the range 20 to 30°C had potent effects on seedling emergence, plant morphology at harvest, and leaf-tissue water content. An air temperature of 35°C with corresponding root zone temperature around 30°C stunted growth to a high degree, and is out of the question. In the cultivar Alrite, the best results in terms of quality of product were in a 20°C ambient temperature condition, in which the water temperature was also approximately 20°C. Slightly more whole plant fresh weight was obtained at 25°C air temperature with root zone at c. 22°C, but at the cost of some sacrifice in quality. At 30°C air temperature (root zone c. 25°C), quality suffered considerably (i.e., in leaf shape and curvature) although there was little difference in biomass from the 20 and 25°C air temperature conditions.

These experiments had validity for low-tech hydroponic production in that root zone temperature of nutrient solution is often allowed to find its own level in relation to ambient air temperature. However, we envisage controlling root zone temperature separately from air temperature if there is an advantage to doing so, and would like to understand the separate effects of temperature in these zones. This was the prime objective of this experiment.

Several changes were made in the design of the current experiment. The venue shifted to the greenhouse from growth chamber, a step closer to true commercial production conditions. A higher daily light integral could be achieved, and was. A cultivar better adapted to warm temperatures and long photoperiods was used (Eagle). The root zone temperature was actively controlled separately from the air temperature. Edge effects were reduced by using reflective barriers to shut out side lighting and define the growing area precisely, which permitted accurate calculation of productivity (g m^{-2} d^{-1}) and light use efficiency (g mol^{-1}). And harvests were made both of the whole plant and just what would be included in a commercial cut – namely material above a suitable cutting plane, as would be the case in machine harvest.

In order to obtain all the desired combinations of aerial and root conditions, the experiment was restricted to three aerial conditions in combination with two root conditions for six treatments overall. The root zone was maintained at either 20°C or 25°C and aerial zone was maintained at either 20-20C (20°C day: 20°C night), 25-20C, or 25-25C.

Hypotheses. Pilot work with a similar type of cultivar suggested that *Eagle* might be able to hold quality at 25°C air temperature, at the same time as increasing productivity on both a fresh and dry weight basis. It was also expected the higher root zone temperature would encourage faster growth in this cultivar. Thus the prediction was that highest fresh and dry weights would be produced at 25-25:25C (25°C daytime air temp, 25°C nighttime air temp: 25°C nutrient solution temp), and the lowest at 20-20:20, with the 25-20 air temperature values falling between. It was not expected there would be large differences in plant quality as far as leaf shape and appearance, but petiole lengths and leaf dimensions were expected to change under the different conditions, and distribution of weight between plant parts. Air temperature was not expected to affect dry matter content in this range, but the higher root zone temperature was expected to decrease the dry weight (DW) to fresh weight (FW) ratio (DW:FW ratio) on the basis of previous experiments.

Methods. This study employed 3 greenhouse sections, each equipped with two independent hydroponic ponds 1m x 2m in dimension and approximately 0.35m deep. Each pond contained a circulation and distribution system. The solution was mixed continuously by being routed through an external pump, and kept oxygenated by the continuous use of air stones. Water temperature was controlled manually by regulating flow of cold water through a "cold finger" at the bottom of the pond, and use of immersion heaters when necessary. EC, pH, water temperature, dissolved oxygen level and nutrient solution level were monitored daily and adjusted when required. Cross contamination between ponds through shared use of sensors was avoided by withdrawing solution samples then discarding them after testing. Level was adjusted by replenishment with solution of the starting EC.

The three air temperature conditions were applied in the three matched greenhouse sections. One of the two ponds in each greenhouse section was assigned to each of the nutrient solution temperature conditions -- either 25°C or 20°C. A 16-hour photoperiod (and thermoperiod in one pair of treatments) was used with a target integral of 20 mol m^{-2} d^{-1}. Supplementary light was added according to computer-implemented rules to reach this integral as nearly as possible. Light, air temperature, humidity, and various control functions were monitored and logged on a two-minute basis.

Seeding took place on the afternoon of Thursday April 10, 2003, Julian day (JD) 100, Expt Day 0, using de-coated seed of the cultivar Eagle. Six full 242-cell flats (Speedling, 2.5 inches high, 11x 22 cells, 0.2308 m^2) were used, one per treatment. Rapid imbibition of the flats was started at 4.30 PM. The gravimetric moisture content of the medium in which seeding took place was c. 3.25 (g H_20 per g dry medium). Flats

were set out in a dark place and insulated against temperature fluctuation due to drafts; they were rotated periodically. Air temperature was set to 24°C for the first 48 hours, and then dropped to hold the seedlings during the third day. Seedlings were covered continuously through first 48 hours, but natural light was admitted on the third day to avoid excessive stretching of emerging hypocotyls. Flotation of flats was at 4.00 PM April 13, after 72 elapsed hours. (Assignment was: flats 4 and 2 to greenhouse Section B, 20-20C air temperature, flats 3 and 1 to Section C, 25-20C air temperature, and flats 6 and 5 to Section E, air temperature 25-25C.)

Seedlings were evaluated thoroughly on day 5. No stand correction was made. The 1st biomass harvest was on Day 20 of the experiment, Wednesday May 30, JD-120, and the 2nd and final biomass harvest was on Day 23, JD 123. First, an outer perimeter row of guard plants was removed. Then 6 sample units (SUs), each consisting of 9 cells in a square, were taken in the first biomass harvest. Nine guarded SUs were taken in the second harvest. In these harvests, plants in the sample unit were cut off just below the cotyledon (through the top part of the hypocotyl), and weighed collectively. Then the cotyledons were stripped off and the plants laid on their side on a cutting block and the bottom 4 cm from the base was cut off. The plants in the sample unit were thus divided into "base and petioles" and "commercial cut". Dry weights were obtained for the separate components of the plants. Special, very detailed plant-by-plant harvests, which included leaf areas by component leaf parts, were taken after the biomass harvests. These harvests were used to examine plant morphology differences between the treatments.

Results. Seed germination happened normally and with sufficient uniformity, that no stand adjustment was considered necessary. After 5 elapsed days, in a given 242-cell flat, 4 plants were missing (1.7% no-shows), 4 were pop-ups (1.6%), 29 plants had something wrong with them, such as deformed or trapped leaves, or were stunted (12%), and the remaining 85% of plants were rated as "good". Some of the 85% good plants might have been late emerging, but they started well. The range of "good" plants per flat in the different treatments when starting out with application of treatments was 200 to 213.

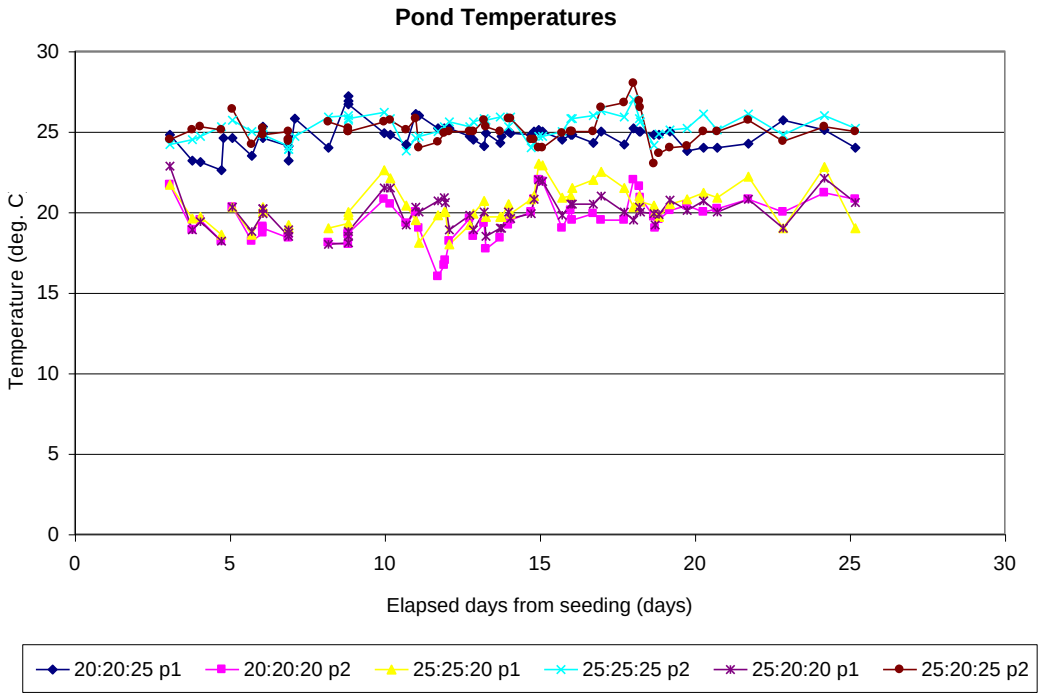


Figure 3-8: Pond temperatures

As can be seen in Figure 3-8 above, water temperature fluctuated somewhat. Average difference between warm and cold ponds was close to 5.0 degrees in all cases.

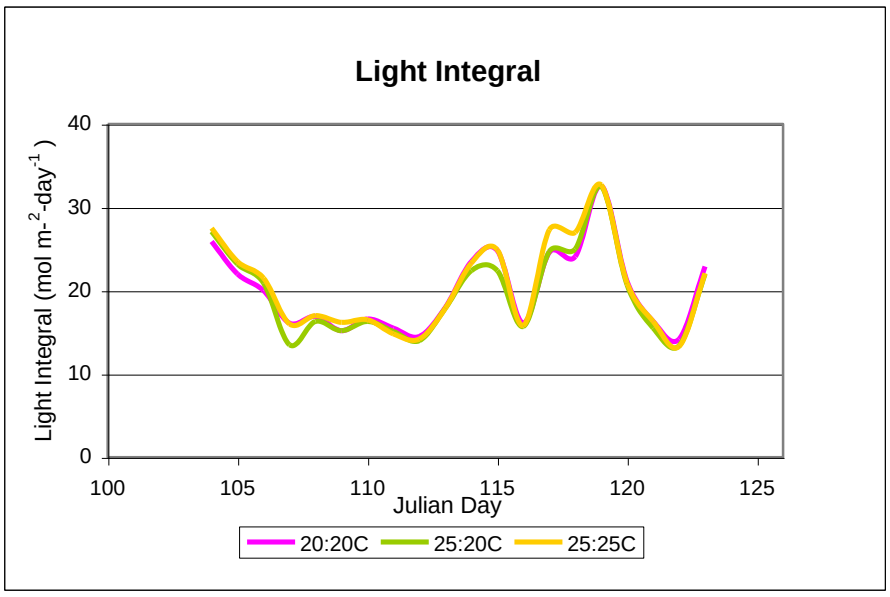


Figure 3-9: Light integral

The average daily light integrals in the three-air temperature conditions (20-20C, 25-20C, and 25-25C respectively) were 20.1, 19.7 and 20.4 mol m^{-2} d^{-1} at the end of day 20, and 20.4, 20.2 and 21.0 mol m^{-2} d^{-1} at the end of day 23, computing from the end of day 3. The above figure shows good uniformity across greenhouse sections but also the kind of variation that can happen during the transition seasons (13 thru 30 mols per day). Retractable shade was not available for this experiment.

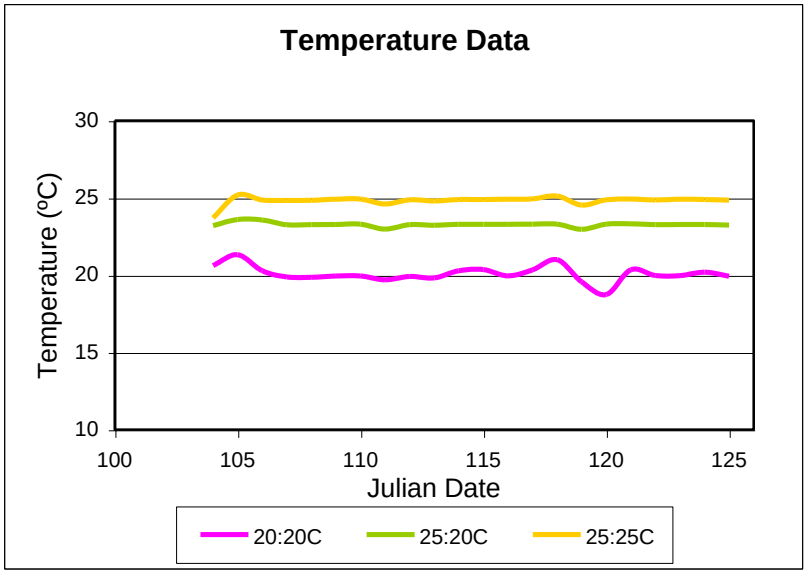


Figure 3-10: Temperature data

Apart from a deviation in the 20-20C condition at the time of the first harvests, air temperature control was excellent, as shown in Figure 3-10.

The first biomass harvest was conducted over a 4-hour period in the evening of Day 20. Slightly more than 20 days had elapsed since imbibition of seeds. The harvest was by sample-unit areas of 0.00858 m^2, and included however many plants were found in the 3x3 matrix of cells of the sample units. Results are presented in terms of productivity (g m^{-2} d^{-1}), (or the yield of the sample-unit (g m^{-2}) divided by the number of days from seeding). In this case, we will assume the same elapsed time of 20.2 days for all SUs harvested, and the same area for all 20.2 days. To give a feel for plant size, yield per cell has been tabulated also. This figure underestimates the actual plant size because usually some cells were empty within an SU (a mental factor of 242/205 representing total cells to cells filled with good plants may be applied).

Day 20 Harvest: Commercial cut and Whole Plant Productivity						
n = 6 Sus	Air temp: 20:20		Air temp: 25:20		Air temp: 25:25	
	20C water	25C water	20C water	25C water	20C water	25C water
Cell yield, FW (g)	3.9	4.8	4.3	4.8	4.3	5.2
			Fresh Weight Productivity (g m-2 d-1)			
Whole Plnt FW	203	250	223	248	222	269
Commercial cut, FW	134	160	145	168	147	180
Other FW	69	90	78	81	75	89
			Dry Weight Productivity (g m-2 d-1)			
Whole Plnt DW	11.1	11.1	12.2	12.3	12.2	13.2
Commercial cut, DW	8.1	7.8	8.8	9.3	9.0	9.8
Other DW	3.0	3.3	3.4	3.1	3.2	3.4
			Ratio DW to FW			
Commercial cut	0.060	0.049	0.061	0.055	0.061	0.054
Stem and Cotyledons	0.043	0.036	0.043	0.038	0.043	0.038
Whole Plnt	0.054	0.044	0.055	0.050	0.055	0.049
% in Com. Cut	0.66	0.64	0.65	0.68	0.66	0.67

Table 3-19: Day 20 Harvest - Commercial cut and whole plant productivity

The first striking thing about the Day 20 harvest (see Table 3-19 above) is that in all three air temperature conditions (ATCs), water temperature had a large effect on fresh weight, of both whole plant and commercial cut. Fresh weight was higher in the warmer temperature ponds on the order of 20% (23, 11, and 21%). On the other hand, water temperature appeared to have little effect on dry weight within any given air temperature environment; dry weights were identical across water temperature conditions in two of the three ATCs, and only 8 % higher in the warm pond of the 25-25C ATC (non-significant); at least at this stage of growth, water temperature did not greatly affect dry matter accumulation. Thus, the mechanism by which fresh weight was increased was primarily through alteration of the water content of the plant. The ratio of dry weight to fresh weight, the most stable index underlying the FW differences, was significantly higher in the 20°C water temperature in all 3 comparisons between different water temperature conditions within ATCs. Although the FW differences were large, the individual comparisons of FW within each air temperature treatment just missed significance at the 0.05 level due to the small sample size (n = 6) and large variability of spinach. Under any combination of pond data across air temperature conditions, the difference was easily significant, however.

In 20°C ponds, increasing air temperature from 20-20C to 25-20 or 25-25C increased dry matter production by about 10%in both cases. In 25°C ponds increasing air temperature from 20-20C to the higher regimes increased dry matter 11% and 19%. Thus air temperature appeared to have a consistent overall effect on

dry weight. However, it was not significant at the .05 level; combining data over both pond temperatures to increase n, and comparing the 20-20C condition to the 25-25C condition gave a p value for a paired t-test of 0.07.

The commercial cut proportion of 66%, was consistent with previous results reported for Alrite, as one might expect; in this case the cotyledons were completely excluded, and probably there were offsetting effects of including some petiole and excluding some small leaves in a cut at 4 cm. Quality of harvest was good in all treatments in terms of leaf appearance and shape (a photographic record is available), and overall plant productivity was reasonable at circa 250 g m^{-2} d^{-1} FW. It will be shown below that leaf size was also reasonable. Petiole length was a little longer than desirable for the larger leaves.

The second and final biomass harvest was conducted 3 days later, after almost exactly 23 elapsed days from seeding. This time 9 SUs were harvested. Data are presented in Table 3-20 below. The roots of the plants in the warm pond of the 25-20C ATC were light brown in color whereas roots were white in all other 5 experimental conditions. This was taken to indicate incipient disease, and the data for this condition (25-20:25C) though quite reasonable, will not be considered beyond this observation: the data were almost identical to the 25-20:20C condition in terms of FW and DW.

The pattern of results in the Day 23 harvest was similar to the Day 20 harvest. There was an even larger difference in fresh weight between water temperature conditions within the same ATCs than in the Day 20 harvest (here 32 and 33%). Again, the dominant effect of pond water temperature was on tissue water content, and thus on fresh weight. However, in this case there was also some apparent effect of water temperature on dry matter accumulation. In the 20-20C ATC, dry matter was 9% higher in the 25C pond than the 20C pond, but not significant. The effect was 19% and significant in the 25-25C ATC. The warmer pond temperature appeared to result in both increased dry matter production and increased tissue water content in this air temperature, resulting in a 33% increase in FW.

In 20°C ponds, increasing air temperature from 20-20C to 25-20 or 25-25C increased dry matter production an insignificant 6% and 3%. In 25°C ponds, increasing air temperature from 20-20C to the 25-25C regime increased dry matter 12%. Thus air temperature continued to have a minor positive effect on dry biomass in the Day 23 harvest.

Day 23 Harvest: Commercial cut and Whole Plant Productivity						
n = 9 SUs	Air temp: 20:20		Air temp: 25:20		Air temp: 25:25	
	20C water	25C water	20C water	25C water	20C water	25C water
Cell yield, FW (g)	6.0	7.9	6.4	6.6	6.0	7.9
Fresh Weight Productivity (g m-2 d-1)						
Whole Plnt FW	274	361	292	301	272	361
Commercial cut, FW	190	266	211	221	197	269
Other FW	85	95	81	80	75	93
Dry Weight Productivity (g m-2 d-1)						
Whole Plnt DW	14.6	15.9	15.5	15.1	15.0	17.8
Commercial cut, DW	11.3	12.9	12.5	12.2	11.8	14.3
Other DW	3.4	3.0	3.0	2.9	3.2	3.5
Ratio DW to FW						
Commercial cut	0.060	0.049	0.061	0.055	0.061	0.054
Stem and Cotyledons	0.043	0.036	0.043	0.038	0.043	0.038
Whole Plnt	0.054	0.044	0.055	0.050	0.055	0.049
% in Com. Cut	0.69	0.73	0.72	0.73	0.72	0.74

Table 3-20: Day 23 Harvest - Commercial cut and whole plant productivity

The commercial cut was taken at the same height of 4 cm above the base of the plant, and not surprisingly, the proportion of plant weight increased, now to 72%. Although attractive in appearance, many of the leaves in this harvest would have to be considered too big to count as baby spinach, and their petioles would have been too long.

Day 21 Sampling for Quality Evaluation

Representative samples of plants from each of the conditions were taken on Day 21 to characterize plants from the different conditions as to commercial suitability for sale as baby spinach. The average weight of the sample plants from the different conditions was matched.

An ideal baby spinach leaf is between the size of a tablespoon and a serving spoon, or somewhat less than 2.75 inches length by 2.0 inches width in the blade, in an oval shape, with a petiole less than ¾ inches long, and never longer than 1.5 inches. (In cm, the blade would be 7 cm long, 5 cm wide, and with a petiole 2 cm to 4 cm). It is desirable to have a mix of sizes; a few larger leaves are acceptable, and many smaller. Blades larger than 7 cm x 10 cm would be excessively large. In terms of area, the average size should be less than 27.5 cm^2, and anything over 55 cm^2 is excessively large. Tiny pieces are not very desirable either.

Day 21 Plant FW Characteristics	Air temp: 20:20		Air temp: 25:20		Air temp: 25:25	
	20C water	25C water	20C water	25C water	20C water	25C water
Averages of 7 plants. Fresh Weights (g)						
Whole plant	**6.42**	**7.26**	**6.60**	**7.29**	**6.27**	**7.56**
Standard Deviation	1.19	1.88	1.36	1.33	0.75	1.30
Cotyledons	0.82	1.03	0.73	0.89	0.77	0.81
1st true leaves	3.46	4.14	3.14	3.34	3.08	4.05
2nd true leaves	1.57	1.42	1.78	1.84	1.56	1.65
3rd true leaves	0.41	0.40	0.71	0.87	0.64	0.74
Remnant	0.10	0.20	0.16	0.24	0.18	0.26
Proportions by plant part						
Whole plant	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
Cotyledons	0.13	0.14	0.11	0.12	0.12	0.11
1st true leaves	0.54	0.57	0.48	0.46	0.49	0.54
2nd true leaves	0.24	0.20	0.27	0.25	0.25	0.22
3rd true leaves	0.06	0.06	0.11	0.12	0.10	0.10
Remnant	0.02	0.03	0.02	0.03	0.03	0.03

Table 3-21: Day 21 Plant FW characteristics

Seven medium-to-large plants were sampled from complete rows of plants, and overall weights of different plant parts were obtained rapidly as shown in the table above. These weight data were normalized (also shown) and a series of statistical comparisons made between proportions of the plant found in different leaf types in relation to air temperature condition. Examining first the effect of ATC, and going by leaf type, the cotyledons in the 20-20 air temperature condition (ATC) were significantly heavier than in the other ATCs. The first true leaves in the 20-20 ATC were significantly heavier than those in the 25-20 ATC, and approaching significance as heavier compared to the 25-25 ATC. On the other hand, third true leaves and remnant leaves were significantly smaller in the 20-20 ATC than the two warmer ATCs. In the case of the second true leaves, the 20-20 ATC leaves were also smaller than the 25-20 ATC, but no different than the 25-25 ATC leaves. These findings were consistent with the growth chamber temperature study using the cultivar Alrite reported earlier, at which time they were explained in terms of sink development.

Statistical comparisons were also made to examine the effect of pond temperature on the three main leaf types within a given ATC. Of all 18 comparisons made, only one showed a significant difference (2^{nd} true leaves in the 20-20 ATC), which supports what a visual scan of the data suggests. Although pond temperature had a very large effect on tissue water content and some effect on dry matter accumulation, it did not appear to affect distribution of weight within the plant.

Subsequent to the fast overall weight-taking, dimensions and weights of each leaf blade and petiole of all the measurable leaves on the plant were obtained. The size distribution of leaves is shown below, expressed as cumulative percentage.

Average leaf size	Air temp: 20:20		Air temp: 25:20		Air temp: 25:25	
(cm2)	20C water	25C water	20C water	25C water	20C water	25C water
Percentages						
0 to 7.4	14	17	7	7	10	7
less than 17.4	36	45	26	24	36	38
less than 27.4	67	62	52	52	64	62
less than 37.4	74	71	88	79	90	79
less than 47.4	95	83	98	95	98	86
less than 57.4	100	93	100	100	100	98
less than 67.4	100	93	100	100	100	100
less than 77.4	100	100	100	100	100	100

Table 3-22. Frequency distribution of leaves by size (area) at Day 21

One can see that one day after the first biomass harvest, almost all leaves in all conditions were below the upper acceptable limit (55 cm^2), and as many as 67% were of ideal size or smaller (27.5 cm^2) in one condition (20-20:20C). Thus, the Day 20 harvest was probably suitable for commercial purposes. A Day 21 harvest with correspondingly improved productivity may also have been acceptable.

When the spinach plant was broken down for analysis, leaves were treated as the same within pairs, and average characteristics of all 1st true leaves were compared with those of 2nd and 3rd true leaves. (See Table 3-23 below.) At Day 21, the 1st true leaves still dominated in terms of weight and size and area. There were significant differences corresponding to treatment temperatures, but typically, the primary or 1st true leaves accounted for 60 to 70% of the total blade weight, the 2nd true leaves 20 to 30%, and the 3rd true leaves just 10%.

For a successful harvest, one would like as much uniformity as possible in petiole length, both within and between 1st and 2nd true leaves, and secondarily, some separation between the top of the 3rd pair of leaves and the base of the blades of the 1st and 2nd true leaves. One also would like a thick and heavy leaf rather than a thin and light leaf. On day 21, in terms of <u>average</u> heights there was a clear cutting plane above the top of the third true leaves, and below the bottom of the blades of the 1st and 2nd true leaves. The average gap varied from 1.3 to 2.5 cm. If this plane can be located for a first harvest cut, it allows for re-growth of the plant for a second harvest with minimal damage to the 3rd true leaves, and also avoids inclusion of too much petiole in the harvest, or little bits of leaf tips. The biomass harvests were all done at 4 cm, but with the detailed data obtained on petiole and leaf dimensions, it can be seen in the Table 3-23 below the best cutting height varied with conditions, probably from about 5 to about 7.5 cm. (Compare "Leaf length" of 3rd true leaves with "Petiole length" of 2nd true leaves).

Day 21 Leaf details		Air temp: 20:20		Air temp: 25:20		Air temp: 25:25	
		20C water	25C water	20C water	25C water	20C water	25C water
Plant averages	n	7	7	7	7	7	7
Plant weight (g)		6.42	7.26	6.60	7.55	6.27	7.56
Plant stem length (cm)		0.59	1.25	0.84	1.29	0.84	1.53
Single leaf averages	n	14	14	14	14	14	14
Petiole weight (g)	1st true	0.55	0.53	0.50	0.50	0.48	0.59
	2nd true	0.30	0.30	0.40	0.42	0.33	0.39
	3rd true	0.03	0.05	0.10	0.15	0.09	0.13
Petiole length (cm)	1st true	8.8	7.8	9.2	9.4	8.3	9.4
	2nd true	6.5	7.2	9.8	10.8	9.2	9.7
	3rd true	1.2	1.4	2.6	3.7	3.0	3.5
Pet. wt/lnth (g cm-1)	1st true	0.062	0.067	0.054	0.053	0.058	0.063
	2nd true	0.046	0.042	0.041	0.039	0.036	0.040
	3rd true	0.027	0.036	0.038	0.039	0.031	0.038
Blade weight (g)	1st true	1.17	1.52	1.02	1.16	1.04	1.41
	2nd true	0.47	0.40	0.50	0.48	0.44	0.42
	3rd true	0.17	0.15	0.25	0.28	0.23	0.24
Blade length (cm)	1st true	9.4	10.5	9.1	9.6	8.6	10.1
	2nd true	6.5	5.9	7.2	7.1	7.0	6.5
	3rd true	3.6	3.5	4.8	5.3	4.6	5.0
Blade width (cm)	1st true	5.3	5.8	5.0	5.2	4.8	5.3
	2nd true	4.0	3.8	4.2	4.3	4.0	4.1
	3rd true	2.8	2.5	3.6	3.6	3.1	3.4
Blade lngth to wdth	1st true	1.8	1.8	1.8	1.9	1.8	1.9
	2nd true	1.6	1.6	1.7	1.6	1.9	1.6
	3rd true	1.4	1.5	1.4	1.5	1.6	1.5
Area (calcd) (cm2)	1st true	39.2	48.6	35.6	39.1	33.0	42.5
	2nd true	22.1	19.1	24.3	24.3	22.0	21.3
	3rd true	9.1	8.2	15.0	16.1	12.6	13.7
Spec. leaf Wt (g cm-2)	1st true	0.030	0.031	0.029	0.030	0.032	0.033
	2nd true	0.021	0.021	0.021	0.020	0.020	0.020
	3rd true	0.018	0.018	0.017	0.018	0.018	0.018
Leaf length (cm)	1st true	18.2	18.4	18.3	19.0	16.9	19.5
	2nd true	13.0	13.1	16.9	17.9	16.1	16.2
	3rd true	4.8	4.9	7.4	9.1	7.6	8.4

Table 3-23: Day 21 leaf details

Comparisons of the six treatments' effects on plant morphology (the goal of the special sample analysis) focused on the effect of water temperature, of which there were three pairs of comparisons to make, one for each air temperature condition, and on the effect of air temperature within a given water temperature.

The examination of effects of water temperature led to some generalizations, but was also somewhat confusing. Higher water temperature increased plant stem length substantially (~+ 0.75 cm) and significantly, an indication of some inducement towards flowering. This small stem extension was of interest but not great concern.

The most dominant effect of water temperature was on the primary leaf blades. Weight, length, width and area of these leaf blades were consistently larger/greater in the warm water conditions of all ATCs than in the cool water conditions of the same ATCs, and the effect was substantial, particularly in the 20-20 and 25-25 ATCs. Primary leaf petioles did not follow the pattern, however. Petioles were shorter in the warm water condition of the 20-20 ATC, of similar length in the 25-20 ATC, and substantially larger in the 25-25 ATC. Primary leaf petiole weights followed the same pattern as the lengths.

The effect of water temperature on second true leaf development was a little more complicated. Petioles were longer in all three ATCs in the warm water condition than in the cool water condition, but leaf blade characteristics (weight, length, width, area) either declined in the warm water condition (e.g., 20-20 ATC) or remained the same in both water conditions.

As far as third true leaf and higher, there was a consistent increase in petiole length and weight in the warmer water temperatures compared to the cool water temperatures, but water temperature appeared to have no effect on leaf blade parameters.

Neither water temperature nor air temperature conditions appeared to have any effect on specific leaf weight (SLW) within a leaf type. SLW was highest in the oldest leaves.

The impression one has is that most of the gain in FW as a result of using a 25°C pond temperature instead of a 20°C temperature was through increased leaf area, and overall weight of the primary leaves. Although the area per unit of weight ($cm^2\ g^{-1}$) did not change on a fresh weight basis, presumably the same amount of dry matter projected more leaf area, and weight was made up in water, since the harvest for biomass indicated the dry weight to fresh weight ratio of leaf blades did change considerably. (DW: FW ratios were not available in this sample.)

When comparing plants at the same water temperature but with increasing air temperature, interestingly, the size of primary leaf blades (length, width, and area) trended down in the cool water temperatures as air temperature increased. Blade weight also either fell or stayed the same. Petiole length remained similar. There was also a decline in leaf blade parameters between the 20-20 ATC and the 25-20 ATC in warm water, after which there was little change. The same was not true for the 2^{nd} and 3^{rd} true leaves, where if anything petiole and leaf blade increased in size with air temperature; of the three air temperature treatments, largest 2^{nd} and 3^{rd} leaves occurred in the 25:20 treatment, and second largest in the 25:25 treatment when water temperature was 25°C. When water temperature was 20°C, largest 2^{nd} and 3^{rd} true leaves were still found in the 25-20 treatment, but they were about the same size in the 20-20 and 25-25 ATCs. Again, these results are consistent with a sink development explanation.

Petiole lengths of 2nd and 3rd leaves appeared to be strongly affected by both water and air temperature, leading to substantial differences in length between conditions.

The implication of these morphological trends, should they prove to be consistent, is that if one is to exploit the benefit of increased fresh weight production that accompanies a warmer water temperature, better size distribution of leaves will be found in air temperatures of 25:20 or 25:25 rather than 20:20. However, if one plans a second cut, better separation of 2nd and 3rd leaves occurs in a 20:20 air condition.

Spacing Experiment 1: Effect of High to Very High Plant Densities on Yield, Whole-Plant Productivity, and Commercial-Cut Productivity of Contrasting Cultivars of Spinach

Rationale. At the outset of the project, it was understood that if high productivity were to be obtained in production of baby spinach, a very high plant density would be needed. It would be necessary to make up in numbers for what was lost in size. Early in the project, plant spacing studies were performed with the cultivar Alrite in growth chambers, using small but carefully guarded high-density stands. These studies indicated that it would be possible to use very high density planting to achieve high levels of productivity with the baby spinach crop, similar to those of lettuce, but they also demonstrated the deleterious effects of too long a duration at too close spacing.

The problem with very high plant densities is that plant quality falls off rapidly once plants begin to seriously crowd each other. In the growth chamber studies, at very high densities petioles became highly elongated and brittle and lower leaves began to lose color even though the top layer of leaves in the canopy looked good. Spinach can become so fragile in high-density plantings it becomes questionable whether it can be handled without breakage, disfigurement, and loss of market value. Ideally, the desired size of leaf for a particular market will be attained in the highest plant density possible that does not unduly impair quality, for this will maximize productivity and minimize cost of production through efficient space and light use. For each level of plant density there is an appropriate crop duration that may not be exceeded without sacrificing quality. The crop duration required to achieve a given leaf size is not dependent on just physical environmental factors (amount of light applied, temperature, etc); plant density itself affects the length of time required to reach target leaf size because of mutual shading by adjacent plants; in addition it alters several leaf characteristics such as water content, brittleness, petiole length and leaf length and shape.

This study was designed to make a preliminary survey of the issues surrounding plant spacing. It was conducted in the greenhouse, where light levels fluctuate and the plant experiences a variety of intensities, as will be the case under production; two cultivars were employed of contrasting growth habit; three plant densities were used, ranging from a conservative level of c. 1000 plants m^{-2} to the very high density of

3000 plants m^{-2}; and harvests were repeated over a period of 4 days to evaluate effects of time of harvest on yield and commercial acceptability of the cut product.

Hypotheses. In general, it was expected that higher plant density would result in higher yield in the same amount of time for both whole-shoot and commercially-useful parts of the plant, but that at the limit higher density would force the harvest to be taken earlier in order to retain desirable leaf characteristics, and thus offset the yield advantage. Greater stretching of petioles and taller plants were expected at higher density and, as a general rule, poorer quality in terms of leaf appearance and handling characteristics.

In the early cultivar Alrite, commercial size was expected to be achieved sooner than in the northern-latitude-adapted Eagle, and to present a narrower window of opportunity for harvest in optimum condition. It remained to be seen whether Eagle, being a slower growing cultivar, could match productivity with Alrite either through use of a longer crop cycle or a higher density.

Methods. Two greenhouse sections were used, one for each replicate of this experiment. Each greenhouse contained two ponds, and each pond was devoted to one of the two cultivars, and contained three flats of plants of that cultivar at different plant densities (approximately 1000, 2000 and 3000 plant m^{-2}). The cultivars were Alrite, a Japanese cultivar that is very fast growing early in the growth cycle but prone to bolt, and Eagle, a slower growing northern-adapted cultivar tolerant of long photoperiods. A 16-hour photoperiod and was used, with a target daily light integral of 20 mol m^{-2} d^{-1}, and day-night temperature settings of 24-19°C. Aerial conditions were logged on a two-minute basis. Nutrient solution temperature was measured periodically to be sure it matched across ponds. Pond temperature was typically c. 25°C in both ponds and was adjusted when necessary. The different plant densities were obtained by planting one, two, or three seeds per cell in 242-cell Speedling-type Styrofoam seedling trays (area 0.2308 m^2, height 2.5 inches). Seedling production followed standard procedures described previously. Flotation was 48 hours from seeding.

On day 8 of the experiment, reflective barriers were installed around each flat, and on day 9, partitions were inserted between segments of the flats designated for harvest at different times. Progress of the crop was monitored photographically using a digital camera with reference scale included.

Harvest Procedures. In the first replicate, destructive harvests were made to represent each cultivar at each plant density at three stages of growth. The first harvest was timed to take place slightly before plants had achieved ideal market size; it was on day 16 from seeding. Further harvests were spaced one or two days apart. In all, there were 18 separate harvests, 9 for each cultivar and 3 for each plant spacing. In initial harvests, rows at the ends of the flats were harvested separately to quantify any edge effects that might remain despite the use of reflective barriers.

All three plant densities were sampled (1/3 of a flat per sample) in each cultivar on day 16 of the experiment. The less dense plantings appeared to be the more advanced in terms of leaf size. On day 17, plants were harvested from the two less dense plantings for each cultivar (1000 and 2000 plants m^{-2}). On day 18 plants were harvested from 1000 and 3000 plants m^{-2} densities. A final harvest was made of 2000 and 3000 plants m^{-2} densities on day 20; however, only the data of the Eagle harvests were taken in detail on this day, because the Alrite plants were clearly past useable size and condition. In all harvests, data were taken for both whole shoot and commercially useful part of the shoot (hereafter called the "commercial cut", CC). To permit analysis of the harvest from the point of view of leaf and petiole size and leaf quality, large sub-samples of the commercial cut were laid out flat and photographed from a fixed position.

On the first day of harvest (day16), plants were harvested individually in each cultivar at the 1000 plants m^{-2} density. Each plant in turn was cut below the hypocotyl, immediately weighed, then laid out and cut at a measured height above the base. The top part constituted the "commercial cut"; it was also immediately weighed. All plants at the 1000 plants m^{-2} density were harvested individually on subsequent days as well.

In the first harvest at 1000 plants m^{-2}, both Alrite and Eagle were cut at 1.5 inches above the base to facilitate a direct comparison of plant characteristics across cultivars. This height was judged to be optimum for Eagle. It resulted in inclusion of too much petiole in the taller growing Alrite. In subsequent harvests at 1000 plants m^{-2}, the height at which to make the commercial cut was determined in such a way as to avoid having any leaf stalk (petiole) remaining attached to the leaf longer than 1.5 inches in either cultivar. Cotyledons were left in place during all cuts, as they would be during machine harvest. For obtaining dry weights, plants were grouped into sample units comprised of 6 cells. There were 9 such sample units of strictly interior plants.

It was found extremely difficult to disentangle plants from one another at the higher densities of 2000 and 3000 plants m^{-2} even in the earliest harvest. After harvest of the 1000 plants m^{-2} crop on Day 16 insufficient time was available to harvest plants in the higher densities on a single plant basis, and they were harvested in bulk. In the two remaining harvests of plants at the higher plant densities (2000 and 3000 plants m^{-2} on subsequent days), the area to be harvested was divided from above into 6 rectangular areas representing sample units. For each sample unit area, the commercial cut portion of the plants was sheared off at the determined cutting plane. Plant bases/ remnants were then recovered and weighed and dried separately, also by sample unit. This procedure was followed because of the near impossibility of disentangling plants at the higher densities in a timely fashion. With the tops removed, the plant bases became readily accessible to complete the whole-plant harvest. However, some dislocation between top and bottom of the plant was possible.

For the second rep, pond assignment was reversed and the harvest procedure was simplified. All plants of Alrite were harvested at the same time, on day 21 from seeding, at a cutting height of 4.5 inches. Average plant height was 8 inches. A detailed plant-by-plant sub-sample was taken from the 1000 plants m^{-2} density (PD) condition; otherwise, flats were divided into 4 SUs.

The bulk of the Eagle harvest was taken 5 days later, on day 26 at the same cutting height of 4.5 inches. A small sample of Eagle in the 1000 PD condition was also taken on day 21 with the Alrite harvest. Cutting height at this time was 2.5 inches and average plant height was 5.5 inches. The later harvest in the replication was due to lower light conditions during the crop cycle.

Results

Introductory Matters. The areas taken in each harvest were roughly the same in all harvests, comprised of 7 rows of cells at the ends of the flats and 8 cells from the middle of the flat, and thus 77 to 88 plants potentially. Plants on the edges of the area harvested were examined separately in some harvests to determine if there were significant edge effects despite the use of reflective barriers. Since little if any edge effect was detected, all plants are included in data analysis wherever possible.

For better appreciation of the data, results are tabulated in terms of yield (kg m^{-2}), productivity (g m^{-2} d^{-1}), and light use efficiency (LUE, g mol^{-1}). "Yield" gives an idea of increase in plant size day by day and is useful on that account. "Productivity", because it takes crop duration into account, allows direct comparison of performance by different crops in different crop durations or the same crop at different stages. It helps determine if it is worth leaving the crop to grow further, because even when yield continues to increase, productivity as yield per day may be static or in decline. Productivity is affected by amount of light the crop receives; LUE tells what light input was required to achieve a given productivity, and thus is of great interest in terms of the economics of the crop.

To give a reference point, the lettuce crop has a whole plant productivity of 300 g m^{-2} d^{-1} fresh weight basis when grown as head lettuce in a 35-day crop cycle in the greenhouse, and taking into account efficiencies achieved by transplant and respacing steps. The yield on day 35 is 10.5 kg m^{-2}. Using an average daily light integral of 16.5 mol m^{-2} d^{-1} in the greenhouse (and counting light used in seedling production as well), the LUE is 18 g mol^{-1}.

In the following calculations for yield, productivity and LUE of the spinach crop, it was assumed the same area was occupied by the crop from seeding to harvest, and the actual measured light impinging on the plants was used in calculation of LUE. (This is a conservative approach since some space and light use efficiencies are possible during the first two to three days of the crop cycle by stacking plant trays, and will be incorporated in commercial production.)

Seedling emergence was normal. Emergence was 97% for both cultivars in the 1000 plants m^{-2} density. At time of harvest, over 90% of plants in the single-seeded Alrite flat weighed more than one gram, and over 84% Eagle plants weighed more than one gram. (Eagle de-hulled seed typically had more defects from the de-hulling process than Alrite, and this result was as expected.)

The average plant size in the first harvest was c. 5 g. Plants below 1g in size contributed very little if anything to the commercial cut, and may be considered to be failed plants. Thus, we may take the figures of 90% and 84% as a "success rate", representing the proportion of seeds sown successful in contributing to stand establishment. Applying these proportions to the starting seeding densities of 1049, 2098, and 3147 seeds m^{-2}, one may estimate the *de facto* plant densities for Alrite were 945, 1890, and 2835 plants m^{-2}, and for Eagle they were 885, 1770, and 2655 plants m^{-2}.

There is a wealth of data to be examined. Data are available for both whole shoot and commercial-cut fresh and dry weight, and have been computed as yield, and productivity and light use efficiency. An attempt was made to adjust the height of the cut so that most leaves were acceptable commercially in terms of petiole length, and the amount of petiole left on the leave was the same in all the harvests.

Effect of Timing of Harvest

1000 Plants m^{-2}. Special attention is given to the 1000 plants m-2 harvests since data were taken in greatest detail at this PD. In the accompanying figures it can be seen that while whole plant yield increased over succeeding days in both cultivars as expected (8% per day for Alrite, 19%and 7% per day for Eagle), in Alrite productivity had nearly reached a maximum by day 16 and was steady in all three harvests (244, 244, and 253 g m^{-2} d^{-1}). In Eagle productivity increased between the first and second harvests, in correspondence with a large increase in yield, after which it was steady (204, 227 and 231 g m^{-2} d^{-1}).

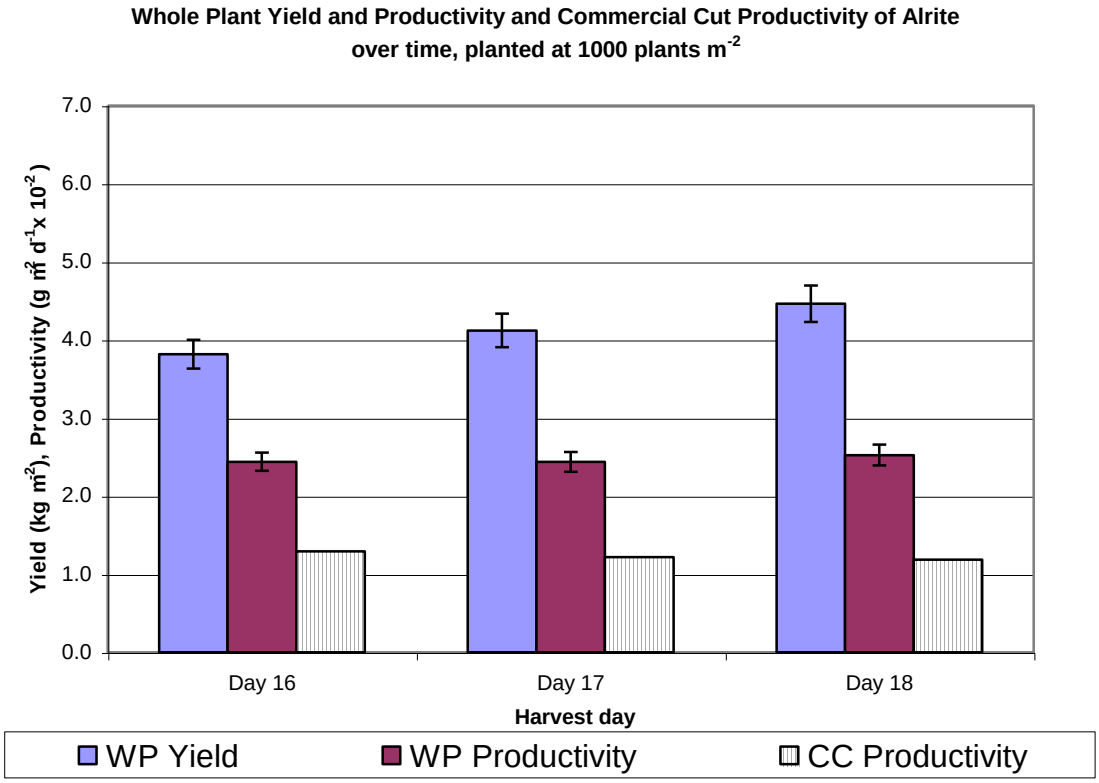


Figure 3-11: Whole Plant (WP) Yield and Productivity and Commercial Cut (CC) Productivity of Alrite over time, planted at 1000 plants m^{-2}

The differences in yield and productivity observed in Eagle between day 16 and day 17 were significant. The productivity of Alrite was significantly greater than that of Eagle in the day 16 harvest (but not in subsequent harvests).

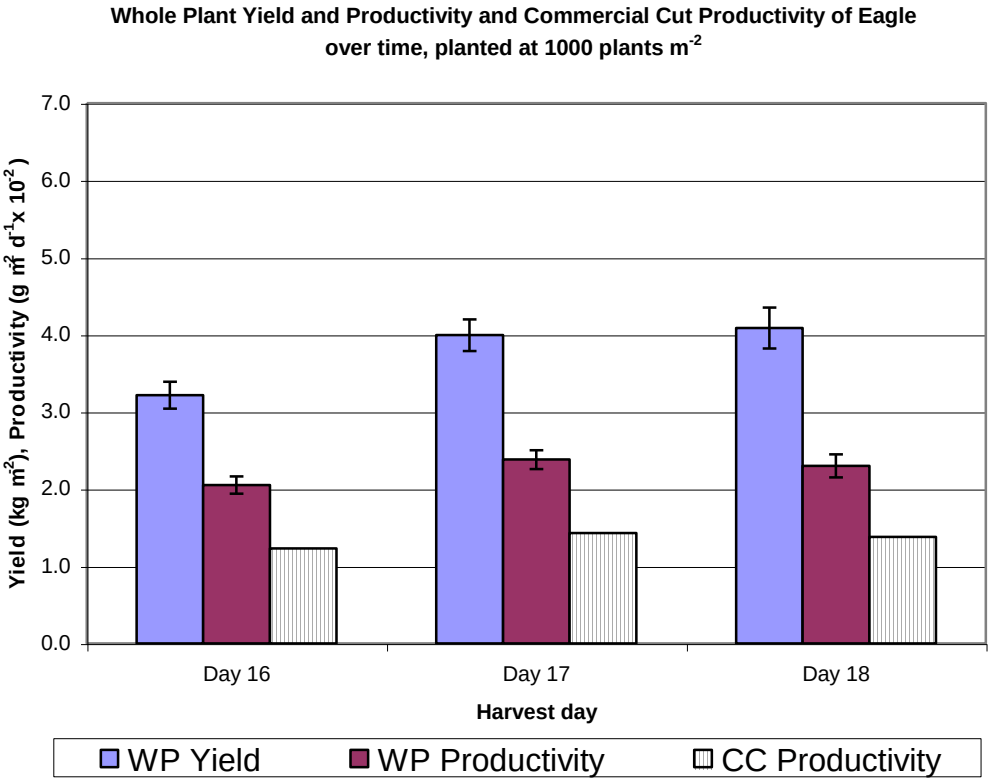


Figure 3-12: Whole Plant (WP) Yield and Productivity and Commercial Cut (CC) Productivity of Eagle over time, planted at 1000 plants m^{-2}

It will be observed there was great variability in the day 18 harvest of Eagle, and less increase in yield than one would expect. As chance would have it, an excessive number of plants had "failed" in the day-18 part of the flat. A truer picture of relative plant performance over time may be gained by considering the median plant yield in each succeeding harvest, or by removing outliers, as shown in the figures below.

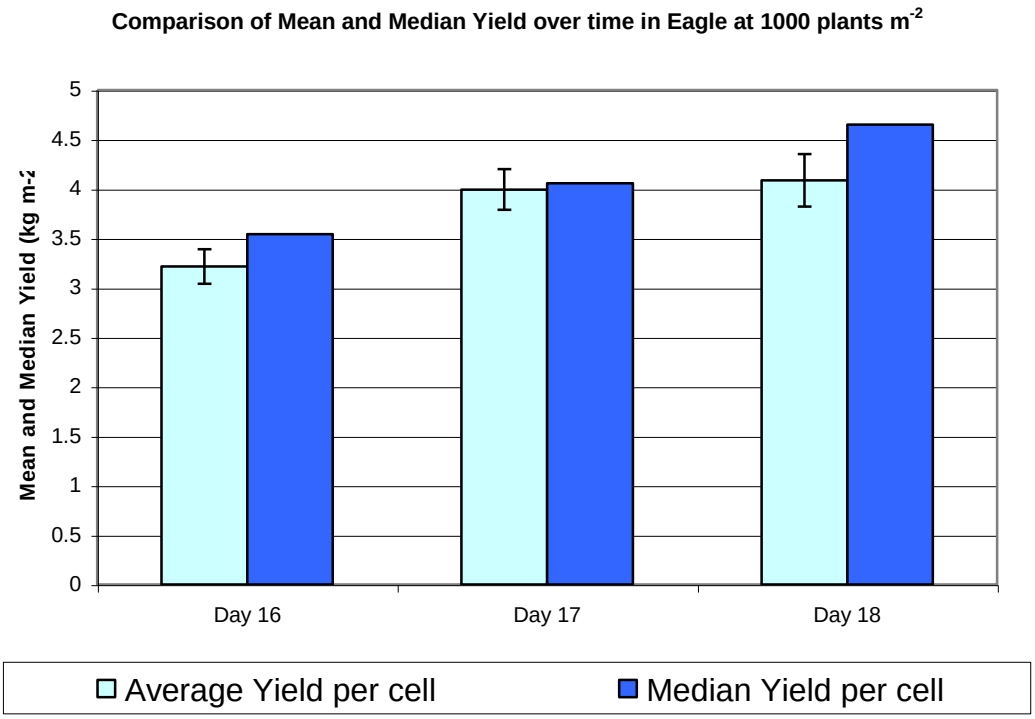


Figure 3-13: Comparison of Mean and Median yield over time in Eagle at 1000 plants m^{-2}

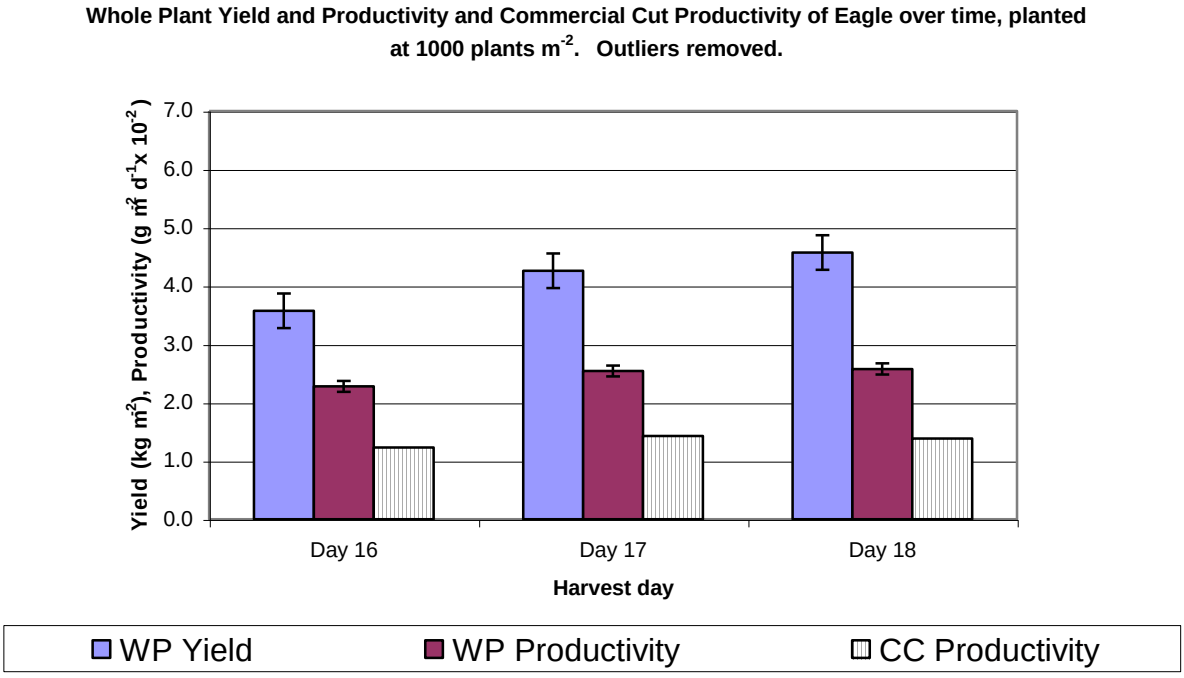


Figure 3-14: Whole Plant (WP) Yield and Productivity and Commercial Cut (CC) Productivity of Eagle over time, planted at 1000 plants m^{-2}: Outliers removed.

In terms of the commercial cut portion of the harvest, in Alrite approximately 50% of the plant could be used; in contrast 60% could be used in Eagle. It was clear early in the crop cycle that Eagle was lower-growing than Alrite, with correspondingly less of the plant devoted to leaf stalk, and this characteristic persisted through harvest. As a consequence, the commercial cut productivity for Alrite ranged from 122 to 125 g m^{-2} d^{-1} while for Eagle it ranged from 122 to 139 g m^{-2} d^{-1}. Over the three-day period of harvest, there was a slight (but insignificant) decline in commercial cut productivity in Alrite, but a significant increase in productivity in Eagle. Thus despite whole plant yield of Eagle always being less than that of Alrite, by timing the harvest appropriately, the commercial cut productivity of Eagle exceeded that of Alrite. There also appeared to be a small decline in the proportion of the whole plant legitimately includable in the commercial cut over the three-day harvest period, but the determination of where to make the commercial cut was too imprecise to be sure about this.

To summarize: For Alrite plants at 1000 PD, although whole plant yield increased 8% per day and significantly over the three day harvest period (days 16 to day 18, July 2 to 4), productivity was static. In absolute terms, whole plant productivity for the 16-day old crop was excellent at 245 g m^{-2} d^{-1}, and LUE was also good at 16 to 17 g mol^{-1}. Commercial-cut productivity was 50% of whole-plant productivity.

The story was different for Eagle plants at this density. In the day-16 harvest, whole-plant yield and productivity measures for Eagle were significantly lower than those of Alrite. However, the plants were still growing rapidly, and yield, productivity and LUE increased substantially over the 3-day period, closely matching those of Alrite by the 3rd harvest. Eagle almost equaled Alrite on commercial cut productivity in the first harvest since 60% of the plants were useable instead of 50%; it substantially surpassed Alrite in terms of commercial cut productivity by the third harvest. (Figures on day 18 were 151 and 111 g m^{-2} d^{-1} for Eagle and Alrite respectively, a significant difference).

2000 Plants m^{-2}. For this plant density, Alrite whole-plant yield increased 17% and productivity 9%, between the first and second harvests (day 16 to day 17, July 2 to July 3) with considerable increase in LUE also. Values for productivity and LUE were in excess of those for lettuce on a WP basis in these two harvests (305 and 332 g m^{-2} d^{-1}, and 20.1 and 22.1 g mol^{-1}). However, the commercial cut was estimated to be only 40% of the whole shoot weight in both harvests, and as a consequence was little different than in the 1000 PD condition despite increased whole-plant yields. At 2000 PD, commercial cut productivity was estimated at 118 and 137 g m^{-2} d^{-1} for the two harvests (compared to c.122g m^{-2} d^{-1} in equivalent harvests at 1000 PD). By July 6 (day 20), the crop was past marketable stage, plants having stretched excessively.

For Eagle in 2000 PD, even though plants grew substantially between July 2 and July 6, productivity and LUE were static or slightly declining (See Figure 3-16 below). Thus, there was little advantage to delaying harvest other than the exact appearance and composition of the commercial cut, which surely did not improve over time. The increase in yield between successive harvests was significant for both whole plants and the commercial cut part of the yield, but there was no difference in Productivity. Whole plant LUE ranged from 19 to 21 g mol^{-1}. To facilitate comparison between cultivars, the first commercial cut level was made high at 3.5 cm. In subsequent harvests, the cut was made at 2.5 and 3.0 cm. The measured proportion of the plant in the commercial cut was 52% and 48% in the second and third harvests. It was estimated to be 55% in the first harvest.

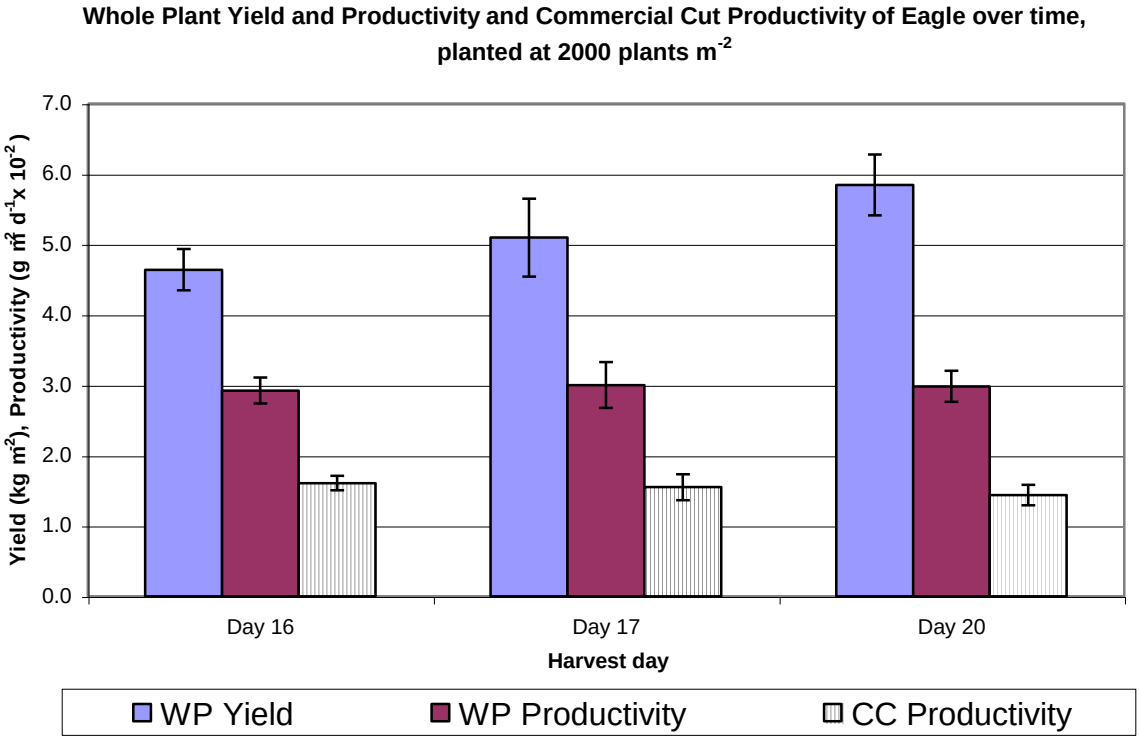


Figure 3-15: Whole Plant (WP) Yield and Productivity and Commercial Cut (CC) Productivity of Eagle over time, planted at 2000 plants m^{-2}

3000 Plants m^{-2}. In 3000 PD Alrite, productivity and other measures were static between day 16 and day 18 (July 2 and 4), even though they were absolute highs. However, commercial cut was only marginally better than at other PDs, and quality questionable in comparison.

In 3000 PD Eagle, yield was marginally greater than at 2000 PD, but CC productivity did not match that at 2000 PD. Plants grew overall, but appeared to be static in terms of productivity over the day 16 to day 18 harvest period. LUE matched or exceeded that of lettuce in the 2000 and 3000 PD conditions.

Conclusions. Taken as a whole one can make a coherent account of these data (with one exception, the performance of Alrite at 2000 plnt m^{-2}).

In terms of whole plant performance, each cultivar's whole-plant yield increased with both increasing plant density and increased duration of crop cycle, as one would expect. Increases were more modest for Alrite than for Eagle because presumably it had become crowded sooner.

In terms of commercial cut performance, productivity in Alrite only slightly increased with plant density, and only slightly increased, if at all, with time of harvest. In Eagle, the highest commercial cut productivity was in the earliest harvest at 2000 plnt m^{-2}, after which it declined over time. It was poorer at 3000 plants m^{-2} than at 2000 plants m^{-2}. The day 17 harvest at 1000 plants m^{-2} closely matched that a 2000 plants m^{-2} on day 16.

All in all, densities between 1000 and 2000 plants m^{-2} probably were nearly equally good in terms of commercial cut productivity, although optimal timing of harvest was dependent on the particular plant density, and the effect of plant density interacted with cultivar. However, it was possible that a better plant density lay between the values examined here, and with this in mind plant densities intermediate between 1000 and 2000 plnt m^{-2} were examined in a subsequent experiment to be described.

Photoperiod-Cultivar Experiment 1

Introduction. This experiment examined the effect of continuous light on two spinach cultivars strongly contrasting in terms of response to day length. It was a photoperiod cultivar study conducted in the greenhouse under conditions simulating possible future commercial production in floating hydroponics. Two photoperiods were used, 16-hr and 24-hr, and two cultivars, Alrite and Eagle which are oriental and occidental types exhibiting high and low photoperiod sensitivity, and short and long critical photoperiods for flowering. The intent of the experiment was to determine the effect of very long photoperiods on spinach when harvested as a baby leaf crop. Harvests were conducted over a period of several days to determine the most propitious time to harvest.

Design. Each treatment (photoperiod-cultivar combination) was represented by one 242-cell flat of plants with an area of 0.2308 m^2; thus nominal plant density was 1049 plant m^{-2}. The photoperiod treatments were imposed in widely separated greenhouse sections to avoid light contamination. The sections were equipped with identical arrays of HPS lamps to provide supplementary lighting to define the photoperiods and equalize the daily light integral across the sections. Instantaneous light level, temperature and humidity

were continuously logged. The supplementary lighting was also used to compensate for day-to-day variation in the natural lighting received by the greenhouse complex. One pond was used in each photoperiod condition, so that the two cultivars shared the same nutrient solution. The nutrient solution was maintained in a nearly identical state in the two-photoperiod treatments, in terms of temperature, EC, pH, dissolved oxygen (DO) concentration, and rate of mixing. Target water temperature was 25°C. EC was that of a standard half-strength Hoagland-type mix (as recommended by Sonneveld for lettuce production; c. 1300 micro Siemens cm^{-1}). pH started at 5.8. DO was maintained above 7.0 mg l^{-1} at all times by the use of airstones. Otherwise, the nutrient solution was continuously circulated for mixing. The status of the nutrient solution was checked frequently and manually adjusted as required. The harvest was in three parts. Harvest was started day 14 and it was completed days 16 and 17.

Method. Polystyrene flats were seeded with de-hulled seed, using established procedures described elsewhere, between 1.30 PM and 3.30 PM, July 23. After two days germination in the dark at 25°C, the flats were floated 9.30 PM July 25, and the photoperiod light conditions and temperatures were imposed immediately.

Temperature settings corresponding to photoperiods were 24°C: 19°C day: night in the 16-hr photoperiod, and 22.3°C in the 24-hr period providing the same number of degree-hours in each photoperiod. Each crop received 8 hours of supplementary light from an array of HPS lamps. This was scheduled during the night period, 9:06 PM to 5:06 AM, for the 24-hour photoperiod condition. It was split into 4-hour blocks for the 16-hr condition, 5:06 AM to 9:06 AM, and 5:06 PM to 9:06 PM. To even out fluctuations in daily light integral due to day-to-day weather changes across the duration of the experiment, lights were turned on when instantaneous level fell below 200 micromoles m^{-2} s^{-1}, and turned off when it exceeded 400 micromoles m^{-2} s^{-1} in the period 9:15 AM to 5:06 PM. The daily light integral target was 18 mol m^{-2} d^{-1}.

On day 8 of the experiment, a 4.0-inch high reflective barrier was erected around each flat. At this time, the crop was approximately 2 inches in height. This was used to control quantity of light received by the plant stands - in particular amount of side lighting - and to restrict and define the area in which the crop was growing. By time of harvest, plants had grown slightly higher than the barrier.

Harvest Method. The floats were arranged in the ponds with the long axis east west. Six rows of 11 cells (out of 22 rows) were harvested in each of the first two harvests, counting from the east. The last harvest was of ten rows. The east end of the reflective barrier was removed for each of the first two harvests then reinstalled immediately after.

1st Harvest. The first harvest on day 14 was intended to establish growth performance, so whole-plant fresh weight (FW) was taken, for each plant. The 16-hr treatments were harvested first, starting with Eagle, 2.10 PM to 2.40 PM, then Alrite, 2.40 PM to 3.10 PM. Thus, almost exactly 14 days had elapsed from

seeding for the harvest of plants from the 16-hr treatments. The harvest of plants from the 24-hr treatments commenced with Alrite at 3.40 PM. It was completed for Eagle by 5.30 PM. Thus, approx.14 days and 2 hours had elapsed for the 24-hr conditions. Stem extension of plants in the 24-hr treatment was measured in two of the 6 rows of each cultivar harvested in addition to FW, since stretching was evident. For obtaining dry weights (DW), plants were bulked by row throughout.

(In comparing results, one can say plants in the 24-hr condition in this first harvest received 3.5 hours more supplementary light than plants in the 16-hr condition because the standard 8-hour daily supplementary lighting allotment was only half completed for the 16-hr condition at time of harvest -- the remainder was due at day's end. Since the 24-hr plants were harvested second, they also had approximately two hours extra growing time under daylight.)

2nd Harvest. The second harvest was intended to evaluate the effect of photoperiod on stretching of stem and petiole and other physical plant characteristics. The second harvest was taken during day 16 in two parts: first the 24-hr condition, then the 16-hr. Harvest commenced at 2.30 PM in the 24-hr conditions with Alrite; it was finished in Eagle by 5.00 PM. The harvest of 24-hr treatments was thus 16 days and about 1-hour elapsed time from seeding. For two rows, 9 and 10, overall plant height (taken to end of longest leaf), stem length from base of cotyledons to growing tip, and major and minor diameters of the largest leaf were obtained for each plant, in addition to whole-plant fresh weight. Plants were bulked by row for DWs.

Harvest of the 16-hr conditions was started after the night period, at 6.30 AM, towards the end of day 16. The whole operation was completed by 8.50 AM. In terms of elapsed time, this harvest was 16 days and 16 hrs after seeding, but in terms of daily light allotment it was little different than the 24-hr treatment, since originally, the 24-hr condition got a head start on supplementary lighting. However, the 16-hr plants did receive a small amount of extra lighting relative to the 24-hr condition: they got about two hours extra supplementary lighting on the morning of harvest, and 3 hours or so of extra daytime light the night before. Detailed data on plant physical characteristics were collected for two rows of plants in the 16-hr photoperiod, as in the 24-hr condition.

3rd Harvest. The 3rd harvest was commenced immediately following the 2nd harvest towards the end of day 16. The focus in the third and final harvest was on determining commercially viable biomass, not just total biomass. In the case of the two Eagle plant stands, FW was also taken by individual plant, to provide statistically analyzable growth and yield data. Work commenced on the 16-hr treatments with Eagle at 9.30 AM; it was finished in Alrite by 11.40 AM. (16 days and 20 hours). Work on the 24-hr treatments started with Alrite at 11.45 AM and ended with Eagle at 2.30 PM. (16 days and 22 hours). After whole plant weights were taken, the "commercial cut" was determined by cutting off the base of the plant at an appropriate height and weighing the top of the plant. The height of cut was determined by visual evaluation of a whole row of plants, after which it was fixed for the rest of the harvest of the particular cultivar and

photoperiod treatment. To provide additional information, a second cut of the plant base was made and weighed so that the effect of different heights of cut could be determined. For Eagle in the 16-hr condition, the primary cut was at 3 inches above soil line. For the 24-hr condition, it was 4 inches. Commercial-cut yields were determined by row for ten rows, along with stem data.

The method used for harvest of Alrite differed in the two photoperiod treatments. In the 16-hour condition, four groups of plants were harvested, with a commercial cut at 4.5 inches above the soil line. In the 24-hour condition, where plants had run to flower, whole plant FW was obtained for 5 groups of plants, but no attempt was made to determine commercial cut. No DWs were obtained for the third harvest. Post harvest analysis of amount of stem included in the commercial cut was conducted. Leaves were categorized by the length of attached petiole.

Results. To help make comparisons of the four treatment combinations, results are presented both as average weight of plant biomass per cell, and average weight per plant. This is because there was a difference in ultimate plant density between the cultivars. Eagle seed is new in the de-hulling process, and this seed lot did not withstand the process as well as Alrite. Consequently, although seed emergence rates were high and simultaneity of emergence was good in both cultivars; eventual stand of good quality seedlings was not the same in Eagle as in Alrite. Overall, plant stand of good quality plants was about 93% in Alrite, but only 80% in Eagle (for a detailed breakdown see Table 3-36 following). For Eagle, there was complete failure of plant growth in 8 out of the 66 cells harvested in each of the 16-hr and 24-hr treatments of the 1st harvest. For Alrite, by contrast, there were no blanks in the 16- hr condition, and just one in the 24-hr condition. Stand establishment of plants in the second harvest showed the same pattern as in the first harvest.

1st Harvest Results. The most striking finding was a difference in fresh weight yield between the 16-hr and 24-hr crops in both cultivars: in both cases the 16-hr yield was only ~75% of the 24-hour yield, and the difference was highly significant in t-tests on the 66 cells involved. (p values were $1.2E^{-4}$ and $1.4E^{-2}$ for 2-tailed tests of Alrite and Eagle respectively). A summary of fresh weight data is given in Table 1. It must be kept in mind there were slight differences in amount of light received in the two conditions, and also in length of growing time. These considerations will be accounted for when productivity and light use efficiency (LUE) are calculated (see below).

	16-hr	24-hr
Average cell yield after 14 days (g FW)		
Alrite	**3.06**	**3.95**
Eagle	**2.66**	**3.49**
Average plant yield after 14 days (g FW)		
Alrite	**3.06**	**4.01**
Eagle	**3.09**	**4.04**
Ratio of Eagle to Alrite		
Cell yield	**.87**	**.88**
Plant yield	**1.01**	**1.01**
Ratio of 16-hr to 24-hr cell yield		
Alrite	**.77**	
Eagle	**.76**	
Stem lengths (cm)		
Alrite	n/a	**4.00**
Eagle	n/a	**1.74**

Table 3-24: Day 14 Harvest - Fresh weights

There was also a difference in yield between cultivars on an area basis (per cell); Eagle yield was approximately 87% of Alrite in both photoperiods. It has long been believed Japanese cultivars initially grow faster than American cultivars, so this was not unexpected in an early harvest. The difference was significant when the photoperiods were pooled ($p = 0.012$). It just missed significance at the 0.05 level in each photoperiod taken separately (p values of 0.050 and 0.077). However, if yield *per plant* is considered, Eagle performance exactly matched Alrite. (See Table 3-24. Since some compensation for missing plants occurs by expansion of adjacent plants, the true values of Eagle yield for comparison with Alrite lies between cell and plant yield.)

Stem extension was measured in the 24-hour harvests, because it was obvious some extension had occurred. Average stem length of 24-hr Eagle plants on day 14 was 1.74 cm based on 18 plants (contents of two rows) versus 4.00 cm for Alrite based on 22 plants. This difference was highly significant ($p = 7.6E^{-10}$). Neither cultivar was noticeably "stretched" in the 16-hr treatment. Dry weight data for the Day 14 harvest are summarized in Table 3-25. The sample unit for dry weight data was the row, and thus n was 6 for this first harvest. Dry weight data provided an extraordinary reversal of the findings suggested by the fresh weight data. The results are sufficiently unexpected and implausible to suggest a possible error in procedure such as mixing of bags from the oven, though none has so far been found. They are analyzed as though real and confrimed through replication of the experiment.

The dry weight of Alrite plants in the 16-hr photoperiod was 11% greater than that of Alrite in the 24-hr condition even though the FW was 24% less. The dry weight of Eagle in the 16-hr conditions was 34% greater than that of Eagle in the 24-hr photoperiod even though FW was 24% less. Pooled across cultivars, this difference was nearly significant at the 0.05 level in a two tailed test ($p = 0.052$). The reversal of biomass data resulted from a large difference in dry-matter/water content of the tissue in the two

photoperiods. The proportion of dry matter in the fresh product fell by over 30% in the 24-hr condition from what it was in the 16-hr condition in both cultivars (see Table 3-25). These differences were large and highly significant, even in samples of 6 units (p = 0.0016 and p = 0.0010 for Alrite and Eagle respectively).

		16-hr	**24-hr**
Average cell yield after 14 days (g DW)			
	Alrite	**.142**	**.128**
	Eagle	**.117**	**.087**
Average plant yield after 14 days (g FW)			
	Alrite	**.142**	**.130**
	Eagle	**.135**	**.100**
Average ratio of DW to FW			
	Alrite	**.046**	**.032**
	Eagle	**.044**	**.025**
Ratio of 16-hr to 24-hr cell yield			
	Alrite	**1.11**	
	Eagle	**1.34**	
Ratio of Eagle to Alrite			
	Cell yield	**.82**	**.68**
	Plant yield	**.95**	**.77**

Table 3-25: Dry weights in the Day 14 harvest

The difference in yield between cultivars (per cell) was clearer on a dry matter basis than on a FW basis; Eagle was approximately 82% of Alrite in the 16-hr photoperiod and 68% in the 24-hr photoperiod (compared to values of 0.87 and 0.88 on FW basis). These differences were individually significant (p = 0.045 and 0.017 respectively for Alrite and Eagle, n of 6). The difference was also significant when the photoperiods were pooled (p = 0.012). However, if yield per plant is considered, Eagle performance nearly matched Alrite in the 16-hr treatment. The difference in the 24-hr condition though larger, was not significant at the 0.05 level in a 1-tail test.

2nd Harvest Results. In this harvest, photoperiod had very little effect on fresh weight yield, and what effect there was was not significant, which may have been in part a consequence of the order of harvest. In the first harvest, the order of harvest favored the 24-hr condition as mentioned earlier. Order of harvest was reversed in the 2nd harvest, and favored the 16-hr treatment somewhat.

As to cultivar, Alrite did out yield Eagle again, and by the same amount. The difference was significant for the 24-hour photoperiod and for the combined photoperiods, but just missed significance for the 16-hr photoperiod by itself (p 0.08, n 66). The difference between cultivars more or less disappeared when computed on a per plant basis. For all the above, see Table 3-26-FW below.

		16-hr	24-hr
Average cell yield after 16 days (g FW)			
	Alrite	**5.00** (3.06)	**5.34** (3.95)
	Eagle	**4.42** (2.66)	**4.47** (3.49)
Average plant yield after 16 days (g FW)			
	Alrite	**5.24** (3.06)	**5.42** (4.01)
	Eagle	**5.12** (3.09)	**5.18** (4.04)
Ratio of Eagle to Alrite			
	Cell yield	**.88** (.87)	**.84** (.88)
	Plant yield	**.98** (1.01)	**.96** (1.01)
Ratio of 16-hr to 24-hr cell yield			
	Alrite	**.94** (.77)	
	Eagle	**.99** (.76)	

Table 3-26-FW: Day 16 Harvest: Fresh weights (Day 14 values in parentheses)

The lack of differentiation in fresh weight yield between photoperiods was also seen in the dry weights, which implies this time there was not a large difference in dry matter content between the photoperiod treatments. Table 3-26-DW indicates this to be the case. There was no difference in dry weight to fresh weight ratio in either cultivar based on photoperiod. However, Alrite appeared to have a larger dry matter content than Eagle in both photoperiods. The difference (in 2-tailed tests) is significant if the photoperiods are pooled (n = 12, p=0.006), significant for the 24-hr period (n=6, p=0.014) and close to significant for the 16-hr period (n = 6, p=0.051).

		16-hr	24-hr
Average cell yield after 14 days (g DW)			
	Alrite	**.210** (.142)	**.233** (.128)
	Eagle	**.160** (.117)	**.148** (.087)
Average plant yield after 14 days (g FW)			
	Alrite	**.220** (.142)	**.237** (.130)
	Eagle	**.185** (.135)	**.172** (.100)
Average ratio of DW to FW			
	Alrite	**.042** (.046)	**.044** (.032)
	Eagle	**.036** (.044)	**.033** (.025)
Ratio of 16-hr to 24-hr cell yield			
	Alrite	**0.90** (1.11)	
	Eagle	**1.08** (1.34)	
Ratio of Eagle to Alrite			
	Cell yield	**.82**	**.68**
	Plant yield	**.95**	**.77**

Table 3-27-DW: Dry weights in the Day 16 harvest (Day 14 values in parentheses)

In the cultivar comparison, dry matter in Alrite on an area basis was significantly greater than in Eagle in both photoperiods (p=0.012 and 0.001 in one-tailed tests, n = 6). Even on a per plant basis, the cultivar difference in yield approaches significance in this harvest. In summary: in regard to photoperiod comparisons, no difference in fresh weight was evident at Day 16 although there was a large difference at

Day 14, nor did dry matter ratio show any response to photoperiod at Day 16, whereas it had at day 14. In regard to cultivar comparisons, Alrite had a weight advantage over Eagle in both harvests and for both fresh and dry weights when considered on an area basis. This difference was strengthened in the Day 16 harvest.

A main objective in the second harvest was to compare plant characteristics as affected by photoperiod and cultivar. Physical measures were taken on plants from two rows (22 cells) in each condition. Stem extension from the base of the cotyledons to the growing tip was measured, the height of the plant was measured to the tip of longest leaf, and the major and minor axes of the largest leaf were measured, from which area of largest leaf was calculated, treating the leaf as an ellipse. Results are presented in Tables 3-27 and 3-28 below.

	Stem Length (cm)	Plant Height (cm)	Leaf Dimensions Major (cm)	Minor (cm)	Area (cm^2)	Fresh Weight (g)
Alrite 16-hr	**2.31**	**20.8**	**9.1**	**4.2**	**119**	**4.97**
Alrite 24-hr	**8.99**	**24.1**	**7.5**	**4.0**	**96**	**5.41**
t-test, 1-tail p	.000	.003	.009	.120	.015	.249
Eagle 16-hr	**0.92**	**18.8**	**10.1**	**5.0**	**159**	**5.55**
Eagle 24-hr	**2.69**	**19.7**	**9.8**	**5.0**	**157**	**5.92**
t-test, 1-tail p	.000	.076	.258	.467	.441	.279

Table 3-28: Plant characteristics - comparing photoperiods

	Stem Length (cm)	Plant Height (cm)	Leaf Dimensions Major (cm)	Minor (cm)	Area (cm2)	Fresh Weight (g)
Alrite 16-hr	**2.31**	**20.8**	**9.1**	**4.2**	**119**	**4.97**
Eagle 16-hr	**0.92**	**18.8**	**10.1**	**5.0**	**159**	**5.55**
t-test, 1-tail p	.000	.000	.001	.000	.000	.129
Alrite 24-hr	**8.99**	**24.1**	**7.5**	**4.0**	**96**	**5.41**
Eagle 24-hr	**2.69**	**19.7**	**9.8**	**5.0**	**157**	**5.92**
t-test, 1-tail p	.000	.001	.002	.000	.000	.247

Table 3-29: Plant characteristics - comparing cultivars

All comparisons showed significant differences except plant weight and leaf dimensions in the case of Eagle.

Stem extension was also measured at Day 14. It was 4.0 cm and 1.7 cm for Alrite and Eagle respectively in the 24-hour treatment (See Table 3-24). In the intervening two days, Alrite stem length more than

doubled to 9.0 cm, and flower buds were visible to the naked eye in some plants at this time. Eagle also made substantial extension to 2.7 cm.

The difference in stem length for Eagle in 24 versus 16-hr conditions and the change from Day 14 to Day 16 suggests Eagle was beginning to bolt in the 24-hr condition by Day 16. This was also suggested by the difference in plant stand height and by differences in petiole lengths determined in harvest 3. However the extent of bolting in Eagle in the 24-hr treatment was not very great; it was on a par with that of Alrite under a 16-hr photoperiod. Notably, there was no difference in leaf dimensions of the largest (and usually first) leaf, and plant height was not greatly different from the 16-hr photoperiod.

3rd Harvest Results. The third harvest was of a large block of plants - 110 cells - and it was conducted right after the harvest of the 16-hr treatments in the second harvest. The harvest was fast (4 hrs start to finish), so comparisons can be made on overall yield, although the main purpose of the harvest was to look at "commercial cut".

By the third harvest, crops were extending above the reflective barrier more or less depending on treatment. Alrite in the 24-hr condition was quite tall, and thus able to garner light from the side better than shorter crop stands. On the other hand, the possible effects of side lighting on the Eagle crop were analyzed by comparing guarded interior plants with the whole stand; in this cultivar, edge effects on biomass were not evident in either 16 or 24-hr photoperiods. (See below). Whole-plant performance is examined first.

	16-hr	24-hr
Average cell yield after 17 days (g FW)		
Alrite	**5.07** (5.00)	**7.12** (5.34)
Eagle	**4.57** (4.42)	**5.39** (4.47)
Average plant yield after 17 days (g FW)		
Alrite	**c5.10** (5.24)	**c7.15** (5.42)
Eagle	**4.83** (5.12)	**5.87** (5.18)
Ratio of Eagle to Alrite		
Cell yield	**.90**	**.76**
Plant yield	**.95**	**.82**
Ratio of 16-hr to 24-hr cell yield		
Alrite	**.71**	
Eagle	**.85**	

Table 3-30: Day 17 Harvest - Fresh weights (Harvest^{-2} values in parentheses)

In the photoperiod comparison, 24-hr yield is significantly greater than 16-hr yield in each cultivar, and is a large effect. Thus, these results more nearly resemble the first harvest than the second in this respect. The best basis for comparison of photoperiod performance, given the differences in lighting schedule and greenhouse sectional differences, is probably in terms of grams per mol (light use efficiency, LUE), or grams per meter squared per day (productivity) if the lighting regime is well matched. This type of analysis is performed at the end of the Results section.

Alrite was again ahead of Eagle in cultivar comparisons, on an area basis, and again this advantage was reduced on the per plant basis. The difference between cultivars was stronger in the 24-hr condition. T-test probabilities of the cultivar differences being spurious on an area basis were 0.06 and 0.001 for 16-hr and 24-hr treatments respectively. (Sample sizes were small for the Alrite treatments - the sample units were large to expedite harvest.)

Note there was little difference between third and second harvest values for the 16-hr treatment (the second harvest values are given in parentheses). The closeness of the figures is as it should be because the third harvest immediately followed the second in this condition. For the 24-hr treatments, the second and third harvests were the better part of a day apart, and considerable growth evidently took place in the meantime.

All cell contents were individually weighed in the Eagle harvests. Since the plants were arranged almost in a square, it permitted analyzing how interior plants performed compared to outer plants, which is always a concern. Results are presented in Tables 3-30 and 3-31.

Average fresh weight of whole plants in grams.

	16-hr photoperiod	**24-hr photoperiod**
Av for 110-cell block	**4.64**	**5.46**
Av for 72-cell inner block	**4.56**	**5.46**
Av for 35-cell innermost block	**4.54**	**5.33**
Av for the 104 plants of 110-cell block	**4.91**	**5.95**
Av for the 66 plants of 72-cell block	**4.98**	**6.05**
Av for the 32 plants of 35-cell block	**4.96**	**6.22**

Table 3-31: Performance of nested blocks of plants in Eagle, day 17

Average fresh weight of whole plants in grams.

	16-hr photoperiod	**24-hr photoperiod**
Inner core of 6 cells	**4.78** (6 plant)	**4.88** (6 plant)
Next perimeter of 14 cells	**3.14** (11plt)	**5.57** (12plt)
Next perimeter of 22 cells	**5.18** (18plt)	**4.65** (19plt)
Next perimeter of 30 cells	**4.74** (28plt)	**6.11** (28plt)
Outer perimeter of 38 cells	**4.78** (38plt)	**5.47** (36plt)

Table 3-32: Performance of concentric rings of Eagle plants on day 17

There did not seem to be any systematic position effect based on these averages. Where there was a difference it is sometimes explainable by the number of plants missing from the stand e.g., for the low value 3.14 g in Table 3-31, 3 of 14 cells were empty, and otherwise explainable by the high variability of

spinach plants. The coefficient of variation in the 110 cellblocks was 0.48 in the 16-hr treatment and 0.58 in the 24-hr treatment.

The main purpose of the 3rd harvest was to determine the commercial yield and its characteristics. A commercial cut was taken in both Eagle treatments and in the 16-hr Alrite treatment. The 24-hr Alrite had virtually gone to flower and was beyond use for commercial purposes. Commercial cut is shown in relation to whole plant cut in Table 3-32. As plants stretched due to induction of flowering and crowding, a smaller proportion of the whole plant could be included in the commercial cut.

	16hr			**24-hr**		
	ComCut	Whl. Plant	CC/WP	ComCut	Whl. Plant	CC/WP
Eagle						
Height of cut	7.6 cm (3 in.)			10 cm (4 in.)		
Av. Cell yld (g)	2.66	4.57	**0.58**	2.82	5.39	**0.52**
Av. Plant yld (g)	2.81	4.83	0.58	3.07	5.87	0.52
Alrite						
Height of cut	11.4 cm (4.5 in.)					
Av. Cell yld (g)	2.19	5.07	**0.43**	n/a	7.12	
Av. Plant yld (g)	n/a	n/a		n/a	n/a	

Table 3-33: Commercial Cut versus Whole Plant fresh weight, Day 17

Judgment was used in where to make the cut. The idea was to keep all petioles shorter than 4 cm (1.5 inches) on the one hand, and waste as little leaf blade as possible on the other. All plants within an experimental condition were cut to the same height once the height was decided upon. In order to see how successful the judgment was, and to assess the variability in petiole length under the different treatments, the whole commercial cut was sorted by petiole length as summarized in Table 3-33.

Categories of cut leaf	No.	Weight (g)	% of lvs	Cum.%
Eagle: 16-hr. Cut at 3 in.				
Petiole less than 2 cm	336	245.3	88.7	88.7
Petiole 2 to 4 cm (1-1.5 in)	26	11.3	6.9	95.5
Petiole 4 to 5 cm (1.5 to 2 in)	17	9.8	4.5	100.0
Petiole 5 to 6 cm	0	0.0	0.0	
Petiole 6 to 7 cm	0	0.0	0.0	
Trash (cotyledons mostly)		19.8	0.0	
	379	286.2	100.0	
Eagle: 24-hr. Cut at 4 in.				
Petiole less than 2 cm	264	226.2	69.8	69.8
Petiole 2 to 4 cm (1-1.5 in)	27	10.0	7.1	77.0
Petiole 4 to 5 cm (1.5 to 2 in)	32	14.6	8.5	85.4
Petiole 5 to 6 cm	31	18.4	8.2	93.7
Petiole 6 to 7 cm	24	18.7	6.3	100.0
Trash (cotyledons mostly)		14.1	0.0	
	378	302.0	100.0	
Alrite: 16-hr. Cut at 4.5 in.				
Petiole less than 2 cm	320	169.3	77.1	77.1
Petiole 2 to 4 cm (1-1.5 in)	34	20.6	8.2	85.3
Petiole 4 to 5 cm (1.5 to 2 in)	41	26.0	9.9	95.2
Petiole 5 to 6 cm	14	8.3	3.4	98.6
Petiole 6 to 7 cm	6	2.8	1.4	100.0
Trash (cotyledons mostly)		1.8	0.0	
	415	228.8	100.0	

Table 3-34: Petiole lengths in the commercial cut

The 16-hr Eagle stand was cut so that only 5% of leaves had petioles longer than 4 cm (a somewhat lucky choice). One cm higher and none would exceed 4 cm. The 24-hr Eagle was cut so that 23% of leaves had petioles longer than 4 cm, which is probably unacceptable. One would have to cut an additional 3 cm to eliminate petioles longer than 4 cm. This would probably cut into leaf blade and reduce yield. As it was cut, 15% of Alrite petioles exceeded 4 cm. If the Alrite were cut 1 cm higher, 95% of leaves would have petioles shorter than 4 cm.

	16hr			**24-hr**		
	ComCut	Whl. Plant	CC/WP	ComCut	Whl. Plant	CC/WP
Eagle						
Height of cut	7.6 cm (3 in.)			12.7 cm (5 in.)		
Av. Cell yld (g)	2.66	4.57	**0.58**	2.13	5.39	**0.40**
Av. Plant yld (g)	2.81	4.83	0.58	2.32	5.87	0.40
Alrite						
Height of cut	12.7 cm (5 in.)					
Av. Cell yld (g)	1.92	5.07	**0.38**	n/a	7.12	
Av. Plant yld (g)	n/a	n/a		n/a	n/a	

Table 3-35: Commercial Cut estimate to give 95% petioles less than 4cm long in a Day 17 crop

A reasonably good estimate of the commercial cut weight if the 24-hr Eagle and 16-hr Alrite were cut so that 95% of leaves had petioles less than 4cm is/was possible, based on data collected. Results of this calculation are presented in Table 3-34.

The commercial cut cell yield for the 24-hr Eagle which, at 2.82g/cell was the highest of the three treatments evaluated, now falls to 2.13g/cell, below that of the 16-hr Eagle, and is similar to the 16-hr Alrite yield, which is also well below the 16-hr Eagle yield. What this probably means is that it is critical to take the 24-hr crop early, probably day 14 or earlier, before substantial differential stretching creates too much variability in petiole lengths.

Environmental Log Data. Light, temperature, relative humidity, and whether supplementary lights were on, were logged continuously and subsequently condensed and plotted on a ten-minute-average basis for each day, and inspected for anomalies. Precise estimates of moles received by the crop based on exact time of harvest have been developed for each harvest and treatment within harvest where appropriate. In the table below, daily averages for the various parameters are given for 13 days following transfer into the greenhouse ponds, measured midnight to midnight.

	Target or Setpoint	16hr pp 24-hr Av.	24hr pp 24-hr Av.	Range of Avs.
Daily light integral (mol m^{-2} d^{-1})	18	**18.2**	**18.4**	12 to 22
Temperature (Deg. C)	24&19 or 22.3	**23.2**	**23.3**	22.4 to 24.5
Duration of supp. Lighting (hrs)	>8	**9.5**	**9.8**	8.0 to 14.9
Relative humidity (%)	70	**75**	**75**	64 to 86
PPFD (micromol m^{-2} s^{-1})	313/208	**317(for 16hrs)**	**213(for 24hrs)**	

Table 3-36: Daily averages for 13 days in the greenhouse, day 3 to day 15.

Within the constraints of the photoperiods, only 8 hours in the middle of the day were left for discretionary light supplementation. The maximum duration of light supplementation could have been 16 hours; the most used was 15 hours on one occasion. Temperature averaged one degree above set point and matched well across sections, a satisfactory result for the hottest time of year.

Stand Establishment. It has been established on many occasions that both Alrite and Eagle de-hulled seed emerges fast and with high percentage if given the right temperature and soil moisture content, so this particular aspect of seedling performance was just visually noted. In this experiment, stand quality was evaluated on day 4 of the experiment, with the results shown in Table 3-36 below.

	Alrite-24-hr	Eagle-24-hr	Alrite-24-hr	Eagle-24-hr
Number of seeds	242	242	242	242
Visible seedlings	241	237	241	239
% Emergence	100	98	100	99
Clear misses	1	5	1	3
Pop-ups	0	2	0	3
Failed plants	5	30	N/A	N/A
All poor plants	17	46	13	41
Good plants	224	190	228	198
% Good plants	**93**	**79**	**94**	**82**

Table 3-37: Seedling evaluation on day 4

It can be seen over 90% of Alrite seedlings had good prospects at day 4, but only 80% of Eagle. It was for this reason both per plant and per cell data were presented.

Productivity and Light Use Efficiency. Decisions are needed on how to calculate productivity and LUE. In spinach, the first two days are used to germinate the crop before any light is needed. It is not necessary or desirable to use lighted greenhouse space during this time. There is the future possibility of efficiently stacking seed trays during this period, reducing the space required. In that case, productivity will increase, since space required will be reduced. However, it will still be necessary to carefully control temperature and avoid temperature stratification during these two days, and thus special space and equipment are still required, so it is not an entirely cost-free phase of production. To be on the conservative side, productivity is calculated counting time from seeding, which coincides with the start of imbibition. Productivity for the crop just when it is in the greenhouse is also calculated. Depending on space use efficiency during the two days of germination, productivity will fall closer to one or the other of these values.

To simplify presentation, only per-cell yield is used as the basis for calculations: it should be borne in mind, performance of Eagle is probably underestimated a small amount in so doing, due to incomplete stand establishment in this cultivar in this experiment. Whole plant figures are presented which makes possible direct comparison with lettuce. It should be noted that spinach grows faster than lettuce over the first 14 days, so it is a matter of how soon productivity and LUE reach the same level as lettuce.

Five tables follow, presenting calculation of productivity and LUE for each of the harvests on fresh and dry-weight bases (where available).

	16-hr Alrite	24-hr Alrite	16-hr Eagle	24-hrEagle
Days from seeding	14.00	14.08	14.00	14.08
Days from floating	11.73	11.81	11.73	11.81
Cum. light from seeding (mol m^{-2})	219.8	228.4	219.	228.4
Av daily integral from floating (mol m^{-2} d^{-1})	18.7	19.3	18.7	19.3
Yield per cell (g)	3.06	3.95	2.66	3.49
Yield per m2 (g m^{-2})	3209	4142	2789	3659
Productivity from seeding (g m^{-2} d^{-1})	229	294	199	260
Productivity in GH (g m^{-2} d^{-1})	274	351	238	310
LUE from seeding (g mol^{-1})	14.6	18.1	12.7	16.0

Table 3-38: Whole Plant Fresh Weight Productivity and LUE. Day 14 harvest

	16-hr Alrite	24-hr Alrite	16-hr Eagle	16-hrEagle
Days from seeding	16.69	16.00	16.69	16.04
Days from floating	14.42	13.73	14.42	13.77
Cum. light from seeding (mol m^{-2})	268.2	267.9	268.2	270.3
Av daily integral from floating (mol m^{-2} d^{-1})	18.6	19.5	18.6	19.6
Yield per cell (g)	5.00	5.34	4.42	4.47
Yield per m2 (g m^{-2})	5243	5599	4635	4687
Productivity from seeding (g m^{-2} d^{-1})	314	350	278	292
Productivity in GH (g m^{-2} d^{-1})	364	408	321	340
LUE from seeding (g mol^{-1})	19.6	20.9	17.3	17.3

Table 3-39: Whole Plant Fresh Weight Productivity and LUE. Day 16 harvest

	16-hr Alrite	24-hr Alrite	16-hr Eagle	16-hrEagle
Days from seeding	16.85	16.88	16.79	16.96
Days from floating	14.58	14.60	14.52	14.69
Cum. light from seeding (mol m^{-2})	270.3	276.8	269.4	278.5
Av daily integral from floating (mol m^{-2} d^{-1})	18.5	19.0	18.6	19.0
Yield per cell (g)	5.07	7.12	4.57	5.39
Yield per m2 (g m^{-2})	5316	7466	4792	5652
Productivity from seeding (g m^{-2} d^{-1})	315	442	285	333
Productivity in GH (g m^{-2} d^{-1})	365	511	330	385
LUE from seeding (g mol^{-1})	19.7	27.0	17.8	20.3

Table 3-40: Whole Plant Fresh Weight Productivity and LUE. Day 17 harvest

	16-hr Alrite	24-hr Alrite	16-hr Eagle	16-hrEagle
Days from seeding	14.00	14.08	14.00	14.0
Days from floating	11.73	11.81	11.73	11.81
Cum. light from seeding (mol m^{-2})	219.8	228.4	219.	228.4
Av daily integral from floating (mol m^{-2} d^{-1})	18.7	19.3	18.7	19.3
Yield per cell (g)	0.142	0.128	0.117	0.087
Yield per m2 (g m^{-2})	149	134	123	91
Productivity from seeding (g m^{-2} d^{-1})	10.6	9.5	8.8	6.5
Productivity in GH (g m^{-2} d^{-1})	12.7	11.4	10.5	7.7
LUE from seeding (g mol^{-1})	0.68	0.59	0.56	0.40

Table 3-41: Whole Plant Dry Weight Productivity and LUE. Day 14 harvest

	16-hr Alrite	24-hr Alrite	16-hr Eagle	16-hrEagle
Days from seeding	16.69	16.00	16.69	16.04
Days from floating	14.42	13.73	14.42	13.77
Cum. light from seeding (mol m^{-2})	268.2	267.9	268.2	270.3
Av daily integral from floating (mol m^{-2} d^{-1})	18.6	19.5	18.6	19.6
Yield per cell (g)	0.21	0.23	0.16	0.16
Yield per m2 (g m^{-2})	220	244	168	171
Productivity from seeding (g m^{-2} d^{-1})	13.2	15.3	10.1	10.7
Productivity in GH (g m^{-2} d^{-1})	15.3	17.8	11.6	12.4
LUE from seeding (g mol^{-1})	0.82	0.91	0.63	0.63

Table 3-42: Whole Plant Dry Weight Productivity and LUE. Day 16 harvest

Further Discussion and Conclusions. Productivity and yield are linearly related to daily light integral in spinach as shown by Both et al. As a result, it is necessary to know how much light is being used to achieve a given productivity to assess its economic significance. Light use efficiency (LUE), measured as grams biomass per mole of light delivered to the crop, is an index of performance that takes light into account, but it omits time and space-use aspects of production. Since time and space-use based costs are a large part of the fixed costs of greenhouse production (or production in isolated confined environments), how long it takes and how much space it takes to produce the crop is important, just as amount of supplementary light used is important. Potentially one can achieve very high LUEs during low-light conditions in the winter. However, since it takes a long time and/or much space to obtain a given yield under winter conditions without light supplementation, it is not desirable to adopt maximizing LUE as a sole goal.

Spacing of plants, timing of harvest and height of the cut also have a direct impact on productivity and light use efficiency. In this experiment, the commercial cut analysis was done on day-17 plants, planted at 1049 plants per m^2, and cut at different heights depending on canopy height. This was a reasonably good time for the harvest in the case of Eagle in the 16-hr photoperiod, but in the other treatments it was a little late (probably), and too much variability in petiole length had arisen, so while whole plant productivity was up, in, say, Eagle in the 24-hr condition, the percentage of the whole plant that could be used in the commercial cut was down, and what could be cut contained an unacceptable proportion of long petioles.

Essentially all the leaves of spinach arise at the same point or within a 1cm zone, unless the plant has bolted; as long as petiole lengths to the base of the leaf blades are the same, a uniform commercial cut can

be made. But as plants get overcrowded and or begin to bolt the newer leaves shoot through the older, and when a harvesting cut is made through a horizontal plane, it results in a proportion of the petioles being too long for commercial acceptability. There is also a difference in length of leaf blade, the newer leaves being shorter and smaller than the older, and this exacerbates the problem. In lettuce, for head production of Boston lettuce, spacing and timing of harvest were adjusted to maximize productivity of a commercially acceptable sized head. In spinach, the goal is to maximize productivity of cut leaves acceptable for sale as baby salad spinach. Both the size of the leaves and the length of petiole included must fall within acceptable limits. This means timing of harvest cannot be used to increase productivity beyond a certain point, and closer spacing and increased lighting or other environmental manipulations become the tools of use. For instance, based on this experiment, a lengthened photoperiod may be useful in increasing productivity in some cultivars, even though more variability in petiole lengths is likely to occur. From this and previous experiments it has become clear that under light integrals of around 18 moles per day, it would be desirable in terms of product size and quality to harvest no later than 14 days from seeding, and perhaps earlier if the photoperiods are longer. If harvest is this early, the plant density can be increased without adverse effect; previous research suggests the optimal density would be approximately 1500 plants m^{-2}. Previous work has also suggested that root temperature exerts a high degree of control over shoot biomass production and perhaps also shoot morphology. The precise root zone temperature that is optimal for each cultivar is not known. Plants did perform significantly better at an average temperature of 25°C than one of 20°C, so the optimum probably lies between these two.

Lettuce has a fresh weight LUE of **18.5** g mol^{-1} and a productivity of **313** g m^{-2} d^{-1} at day 35. In this experiment, the cultivar Alrite achieved a LUE of **18.1** g mol^{-1} and productivity of **294** g m^{-2} d^{-1} by day 14 under 24-hr light, and a LUE of **19.6** g mol-1 and productivity of **313** g m^{-2} d^{-1} by day 16 under 16-hr light. Unfortunately in terms of commercial cut for supermarket sales, probably only 50% of these crops would be saleable (See Table 3-34 above). In terms of the current US consumption patterns, LUE for a commercial cut of baby spinach appeared to be about half that of lettuce in this experiment. One of the positive outcomes of this experiment was a good start was made on development of a procedure for determining the correct height for the commercial cut in terms of petiole length, and for evaluating the effect of different heights of cut on yield. One problem detected was in the method of drying spinach leaves, especially groups of leaves. If cell walls burst, as they tend to, some of the cell contents that should be in the dry weight, and would be in a slow drying process, are lost.

Photoperiod-Cultivar Experiment 2: Effect of a 24-Hr Versus a 16-Hr Photoperiod on Performance of Contrasting Spinach Cultivars at Multiple Plant Densities and Times of Harvest. Replication of Photoperiod-Cultivar Experiment 1 with Plant Density as an added variable

Introduction. In the first cultivar photoperiod experiment, described above, it was uncertain that even a day 14 harvest was early enough for best quality of baby spinach leaves, especially in the longer photoperiods when a strong bolting tendency was induced. In the replication of the photoperiod study here described, earlier harvests and higher plant densities were explored, in addition to repeating the earlier experiment, using the same two cultivars, the same photoperiods, and the same basic experimental design. Four harvests were conducted over a six-day span in each cultivar in each photoperiod. Harvesting commenced at day 11 in the case of Alrite under the 24-hour photoperiod. Next harvested were Eagle in the 24-hour photoperiod and Alrite in the 16-hour photoperiod on day 12. The first harvest of Eagle under the 16-hr photoperiod was on day 13. Four progressively higher plant densities were examined starting with that used in the earlier study, 1050 plants m^{-2}, and going up to 1700 plants m^{-2}. Additional data collection and post harvest analyses of the commercial cut were conducted to better evaluate the quality of the commercial cut portion of the harvest.

In controlled environments it is possible to manipulate spinach development to emphasis primary leaf development over development of subsequent leaves (or vice versa), and, to some degree, control petiole elongation to advantage. However, in all the cultivars we have tested, the primary leaves eventually grew too big to be saleable as baby spinach leaves. This happened before the second true leaves reached a great size, and well before the 3rd pair of true leaves are able to contribute to the harvest. Thus, for purposes of producing baby spinach we believe our main focus must be on obtaining the commercial product from the first and second pairs of true leaves, harvesting them before they have become too large.

The nicest looking commercial product contains whole leaves without an excessive amount of petiole. If harvest is made by cutting in a horizontal plane, there are two times during early plant development when a cutting plane may exist that will yield only whole leaves. (Cotyledons inevitably will be included in any early cut). The first time is before leaves of the second pair of true leaves have elongated enough to overlap the leaf blades of the primary leaves. The second time is when the base of the blade of the second pair of true leaves has drawn level with the base of the primary leaves, and the third pair of true leaves has not yet reached this high.

In this study as in the last, attention was given to when was the best time for harvesting from the point of view of cutting as little as possible into the blade of the leaf of either the first or second true leaves.

Methods. The greenhouse sections in which the photoperiods were applied were reversed for this replication. In each greenhouse section two ponds were employed, each containing four flats of spinach, one at each plant density. The flats were all of the same overall external dimensions of depth width and breadth, but contained different numbers of cells, in matrices of 11x22, 12x24, 13x26, and 14x28 cells (242, 288, 338, and 392 cells per flat), corresponding to potential plant densities of 1050, 1250, 1460, and 1700 plants m^{-2}. The area of the flats was 0.2308 m^2. (The flats were manufactured by Beaver Plastics of Canada). Within each pond all plants were of the same cultivar.

Environmental controls were applied in the same manner as previously described, in such a way that the daily light integral and the degree-hours were the same in each photoperiod condition on any given day.

Since the experiment employed a 2x2x4 factorial design, of 2 photoperiods, 2 cultivars and 4 plant densities, there were 16 experimental conditions (combinations of independent variables) to be examined at each time of harvest. There were 4 different times of harvest. The harvest was typically conducted one pond at a time, cutting approximately a quarter of the plants from each of the flats representing the different plant densities, as rapidly as possible. The reflective barrier was removed at the edge of the plant stand that was to be harvested, then replaced immediately after the required number of plants had been removed. Harvests were conducted in the period between early afternoon and evening.

To facilitate harvest operations, the Eagle crop was seeded one day later than the Alrite crop, and half of the harvests then could be staggered to spread the workload.

The first harvest in each of the cultivar-photoperiod combinations was made just before primary leaves reached a useful size for commercial purposes, with the intent of bracketing the best time/times for commercial harvest. Additional harvests were made at two-day intervals until the plants were past prime condition for commercial purposes. Use of a 24-hr photoperiod encourages rapid early growth. In both cultivars, the first harvest of plants subjected to a 24-hr photoperiod was made a day ahead of plants in the 16-hr photoperiod. The Japanese cultivar Alrite is known for fast early growth and was harvested one day ahead of the slower growing Eagle cultivar. Thus the first harvest was of Alrite in a 24-hour photoperiod, 11 days after seeding. A day later, 12 days after seeding, Eagle in the 24-hour photoperiod and Alrite in a 16-hr photoperiod, were both harvested. Lastly, Eagle in a 16-hr photoperiod was harvested on day13 from seeding.

Several types of data were collected from the harvested material to serve different goals. One important goal was to determine fresh weight yield and productivity as accurately as possible. For this purpose as many plants as possible and as large an area as possible was included in each harvest. Data were obtained on the whole above-ground part of the plant, the shoot, because the whole plant is a well defined unit,

comparable across conditions, and for which objective repeatable measures can be expected. Whole-plant fresh-weight data were obtained on an individual plant basis.

A large sub sample of the plants harvested for fresh weight were oven dried individually to determine dry matter content. Dry matter content is of interest in several respects from a theoretical point of view; from a practical point of view it affects how fragile the plants are in handling, and it may have impact on storability of the commercial product.

It was considered important the biomass harvest should be completed in a reasonable length of time so that the fresh weight data for each of the plant densities represented within a pond were comparable. After fresh weight data had been taken plants were carefully packed away in cold storage for subsequent more detailed analysis.

Information on plant morphology was collected from plants in 2 rows out of the 5 or 5+ rows harvested for biomass. In most cases the data were collected on plants held under cold storage rather than just-harvested plants. This was done for each of the four different plant densities at which the cultivar was grown, and for each of the harvest dates. Just the plants that were large enough to be commercially useful were measured. (There was some incidence of failed plants that could not contribute to the commercial cut.) Plant height and stem height and data about the largest leaf, were obtained. The rationale for taking data just on the largest leaf was the commercial importance of leaf size in baby spinach, which is a large determinant of value. The petiole length and blade length and width were measured and recorded. Later, leaf area and shape were estimated based on the blade dimensions. In some harvests an additional sub sample was used to obtain information on the height relationship between the first true leaves and second true leaves, and to compare the size of leaves within pairs.

After data on plant morphology had been collected, we still needed to know how much of the plant would be included if a commercial cut were made in a fixed plane, and what qualities this cut product would have in terms of petiole length and damage to the leaves if the cut were made at different heights. Different techniques were used to obtain this information in different harvests. In the early harvests a very low cut was made, and then the product obtained was evaluated with a view to what would have been the effect of cutting higher. In later harvests an attempt was made to determine the best cutting height based on plant morphology data and make the initial cut at that height. In this case also, post harvest analysis of the cut material was then used to evaluate the result, and to estimate the effect of different cutting heights on petiole length etc.

RESULTS

Introduction. In evaluating the effect of the independent variables (cultivar, photoperiod, plant spacing, and timing of harvest) on biomass production, average plant size could be the basis of comparison. If we were only using one plant density, this would be a reasonable approach, and it was the one taken in the previous experiment. However, the interpretation of plant size becomes complicated when plant density is a variable. As plant density increases, plant size goes down even though yield increases. From the commercial production standpoint we are most interested in how much we can produce per day or per month, which is best reflected in the measure "Productivity", namely biomass produced per unit area per unit time. Although our starting point was sets of weights of individual plants we have standardized the results by converting weights first to Yield (weight per unit area) and then to Productivity (weight per unit area per day). We have calculated Productivity for the duration the plants were floating in the ponds. This is an accurate figure in a direct sense, since the seedlings were only just emerging from the soil when they were placed in the ponds and their weight was negligible. It is possible to calculate Productivity taking into account the space used outside of the ponds during incubation of seeds, and we have in fact done this for check purposes. However, space use during incubation is quite different from later space use; it is in the head house, the flats will be stacked ten high, and lighting is not needed. Because of the economical use of space during incubation the calculation of Productivity taking that space into account is little different from the values used here. In the interest of simplicity and utility, we consider Productivity calculated for the period of flotation the best choice.

The second critical matter of commercial interest is how much light energy is required to obtain a given level of productivity, since light must be paid for if more is required than is supplied by the sun. The amount of natural light available varies considerably with geographic location and climate and, consequently, so does the amount needed for supplementation. To determine cost of production, the amount of supplementary light used to sustain the given level of productivity does need to be known, but it is specific to location. For general application to analysis of commercial production in diverse locations, it is most useful to know the average daily integral required by the crop (mol m^{-2} day^{-1}) and also the Light Use Efficiency (LUE) - the amount of biomass produced by a unit amount of light. The latter is expressed as grams per mole of photosynthetically active radiation (g mol^{-1}).

In this experiment, there was only moderate day-to-day fluctuation in daily light integral received by the crop, and the integral in the two greenhouse sections representing the different photoperiods was nearly identical each day. The average daily integral received by the crop at each harvest is shown in Table 3-42 following. We have compared graphs in which productivity is plotted either against time of harvest or against cumulated light integral. Because the harvests were on a regular schedule and the daily light receipts were similar through the period of harvest, the shape of the curves and the relationships revealed

are very similar in both cases, and we have plotted productivity against time rather than accumulated light integral.

It generally took 2 to 3 hours to complete a single harvest of all four plant densities represented in one pond, in one photoperiod-cultivar combination. As a practical matter, a single time, the midpoint of the harvest, was used to calculate days elapsed between seeding and harvest for all plants in the harvest, and a single time was used to determine the cumulative total of light (the light integral) the crop had received to that point. Yield for the sample in the harvest was determined by calculating the exact area of the block of cells harvested, which differed somewhat for each plant density and each harvest according to how many rows were taken and the cell dimensions, and dividing the weight of shoot harvested by this area. Productivity was then determined by dividing Yield by time elapsed from flotation of the crop. Light use efficiency (LUE) was then determined by dividing Productivity by the average daily light integral, or, alternatively by dividing yield by the cumulative light integral from seeding.

Fresh Weight Yield, Productivity and Light Use Efficiency

Exact time from seeding to harvest for each harvest, cumulative light integrals for each harvest, and average daily light integrals while the crop was in the greenhouse are presented in Table 3-42. Fresh weight Yield and Light Use Efficiency are presented in Table 3-43, and Fresh weight Productivity in Table 3-44.

Cultivar	Photoperiod	Harvest Number			
		1	2	3	4
	Days elapsed from seeding				
Alrite	24hr	11.06	13.13	15.21	17.27
Alrite	16hr	12.17	14.20	16.25	18.88
Eagle	24hr	12.04	14.13	16.09	18.17
Eagle	16hr	13.09	15.13	17.17	19.11
	Cumulative light integral from seeding (mol m^{-2})				
Alrite	24hr	139.2	166.5	205.3	248.5
Alrite	16hr	158.8	193.8	229.0	267.8
Eagle	24hr	152.7	190.7	232.4	266.4
Eagle	16hr	178.4	213.2	247.2	272.4
	Average daily light integral (mol m^{-2} d^{-1}) in greenhouse				
Alrite	24hr	15.4	15.0	15.5	16.3
Alrite	16hr	15.6	15.9	16.1	15.9
Eagle	24hr	15.2	15.7	16.5	16.5
Eagle	16hr	16.1	16.2	16.3	15.9

Table 3-43: Age of plants at harvest, cumulated light, and average daily light received while in the greenhouse

Cultivar and Photoperiod	Harvest Number	Cumul. Integral (mol m^{-2})	**Nominal Plant Density** and Cells per flat							
			1049 m^{-2}, 242-cell		**1248 m^{-2}**, 288-cell		**1464 m^{-2}**, 338-cell		**1698 m^{-2}**, 392-cell	
			Yield	LUE	Yield	LUE	Yield	LUE	Yield	LUE
24-hour Alrite	**1**	139.2	**1504**	10.8	**1675**	12.0	**1983**	14.2	**2258**	16.2
	2	166.5	**2498**	15.0	**2666**	16.0	**3338**	20.0	**3633**	21.8
	3	205.3	**3802**	18.5	**3833**	18.7	**4056**	19.8	**4204**	20.5
	4	248.5	**4829**	19.4	**5101**	20.5				
16-hour Alrite	**1**	158.8	**1721**	10.8	**1713**	10.8	**2156**	13.6	**2238**	14.1
	2	193.8	**2460**	12.7	**2819**	14.5	**3212**	16.6	**3237**	16.7
	3	229.0	**3970**	17.3	**3721**	16.3	**4423**	19.3	**4403**	19.2
	4	267.8	**5352**	20.0	**5885**	22.0	**5642**	21.1	**5977**	22.3
24-hour Eagle	**1**	152.7	**1781**	11.7	**2068**	13.5	**2272**	14.9	**2258**	14.8
	2	190.7	**2947**	15.5	**3334**	17.5	**3732**	19.6	**3780**	19.8
	3	232.4	**3915**	16.8	**4145**	17.8	**4714**	20.3	**5102**	22.0
	4	266.4	**5288**	19.8	**5528**	20.7	**6176**	23.2	**5979**	22.4
16-hour Eagle	**1**	178.4	**1653**	9.3	**1916**	10.7	**2282**	12.8	**2500**	14.0
	2	213.2	**2739**	12.8	**2903**	13.6	**3602**	16.9	**3639**	17.1
	3	247.2	**3815**	15.4	**4491**	18.2	**4291**	17.4	**4850**	19.6
	4	272.4	**5027**	18.5	**4801**	17.6	**5486**	20.1	**5184**	19.0

Table 3-44: Fresh weight Yield and Light Use Efficiency (LUE) of whole shoot while in the greenhouse for all cells harvested. (Yield - g m^{-2}) (LUE - g mol^{-1})

Cultivar and Photoperiod	Days in Pond	Nominal Plant Density				Combined Densities		
		1049 m^{-2} 242-cell	1248 m^{-2} 288-cell	1464 m^{-2} 338-cell	1698 m^{-2} 392-cell	2 lower densities	2 higher densities	All densities
24-hour Alrite	8.85	**170**	**189**	**224**	**255**	**180**	**239**	**210**
	10.92	**229**	**244**	**306**	**333**	**237**	**319**	**278**
	13.00	**292**	**295**	**312**	**323**	**294**	**318**	**306**
	15.06	**321**	**339**	**n/a**	**n/a**	**330**	**n/a**	**n/a**
16-hour Alrite	9.96	**173**	**172**	**217**	**225**	**172**	**221**	**197**
	11.99	**205**	**235**	**268**	**270**	**220**	**269**	**245**
	14.04	**283**	**265**	**315**	**314**	**274**	**314**	**294**
	16.67	**321**	**353**	**339**	**359**	**337**	**349**	**343**
24-hour Eagle	9.87	**180**	**209**	**230**	**229**	**195**	**229**	**212**
	11.96	**246**	**279**	**312**	**316**	**263**	**314**	**288**
	13.92	**281**	**298**	**339**	**366**	**289**	**353**	**321**
	16.00	**331**	**346**	**386**	**374**	**338**	**380**	**359**
16-hour Eagle	10.92	**151**	**175**	**209**	**229**	**163**	**219**	**191**
	12.96	**211**	**224**	**278**	**281**	**218**	**279**	**249**
	15.00	**254**	**299**	**286**	**323**	**277**	**305**	**291**
	16.94	**297**	**283**	**324**	**306**	**290**	**315**	**302**

Table 3-45: Fresh weight Productivity of whole shoot while in the greenhouse, for all cells harvested (g m^{-2} d^{-1})

The effect of timing of harvest and plant density on Productivity is presented in Figures 3-17 to 3-20, based on Table 3-44. In the 3rd and 4th harvests of Alrite, the plants were well on their way to flowering, and especially in the higher plant densities had stretched beyond the reflective barriers. This is why some values are missing in the figures and tables for Alrite in the later harvests.

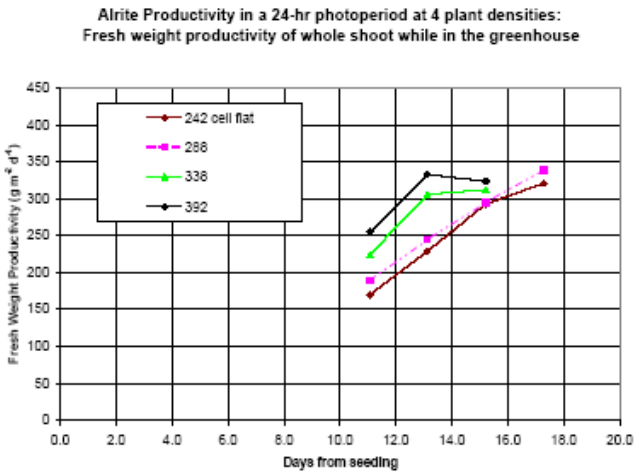


Figure 3-16: Alrite productivity in a 24-hour photoperiod at 4 plant densities: Fresh weight productivity of whole shoot while in the greenhouse

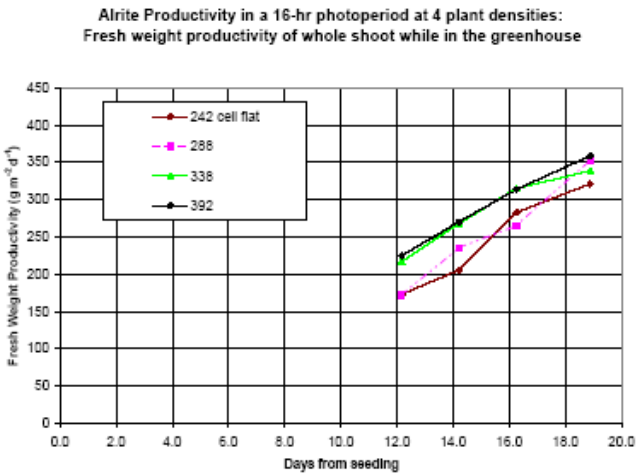


Figure 3-17: Alrite productivity in a 16-hour photoperiod at 4 plant densities: Fresh weight productivity of whole shoot while in the greenhouse

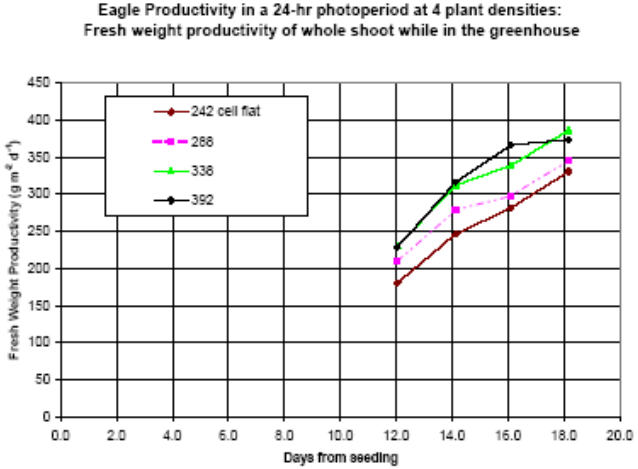


Figure 3-18: Eagle productivity in a 24-hour photoperiod at 4 plant densities: Fresh weight productivity of whole shoot while in the greenhouse

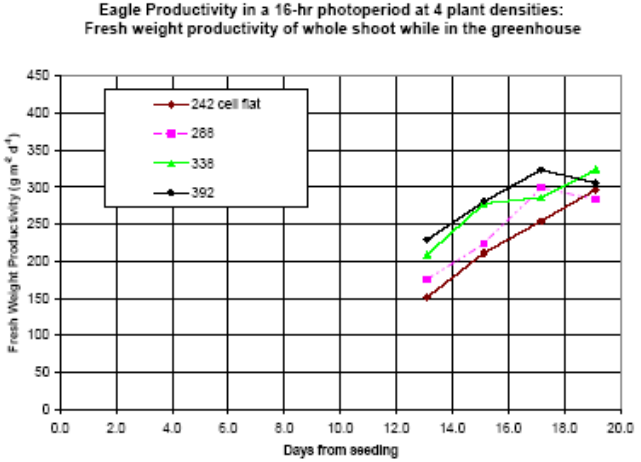


Figure 3-19: Eagle productivity in a 16-hour photoperiod at 4 plant densities: Fresh weight productivity of whole shoot while in the greenhouse

It can be seen that Productivity increased dramatically with each later harvest, although beginning to slow in some instances, andthere is a suggestion that Productivity increased with each increase in plant density. It is also apparent the differences in plant density were not sufficient, given the sample sizes and the natural variability found in the crop, to always produce clear separation or a significant difference of trend lines for different plant densities. Part of the problem here was simply that some cells contained failed plants, especially in the case of Eagle where germination was somewhat reduced, and the proportion of failed cells varied enough between samples to throw the productivity calculation off. Various means are available to clarify the data, such as tabulating data for just successful plants and recalculating area. This maneuver did straighten out the Eagle data considerably, as can be seen in Figure 3-21 (compared to Fig. 3-20) but is misleading in its own way, because it probably overestimates Productivity.

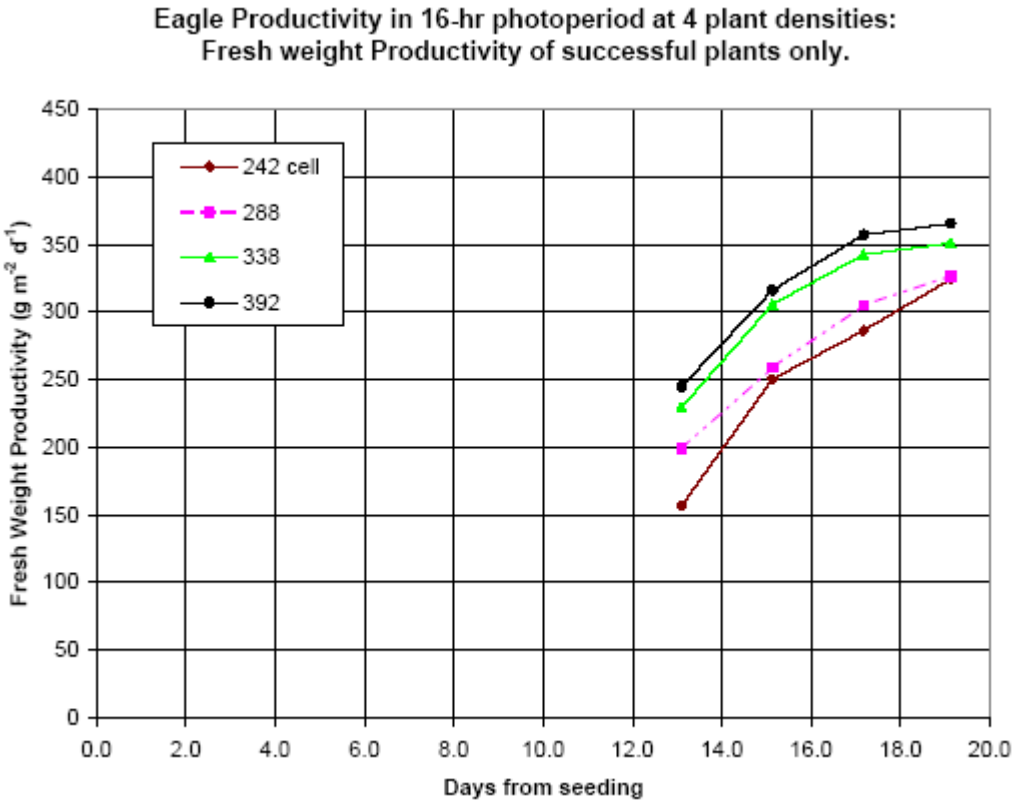


Figure 3-20: Eagle productivity in 16-hour photoperiod at 4 plant densities - fresh weight productivity of successful plants only.

To give a clearer indication of the effect plant density, data have been combined for the two higher plant densities and the two lower plant densities; the combined data are plotted in a separate set of graphs, Figures 3-22 to 3-25. Statistical tests performed on this set of combined data show virtually every comparison between points in the separate trend lines to be statistically significantly different at the 0.05 level or better.

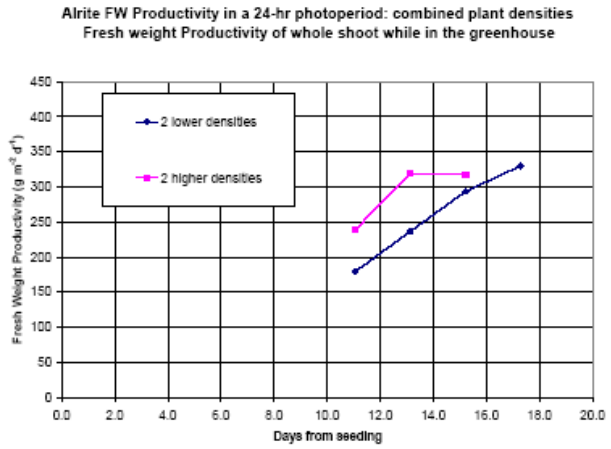


Figure 3-21: Alrite FW productivity in a 24-hour photoperiod - combined plant densities Fresh weight productivity of whole shoot while in the greenhouse

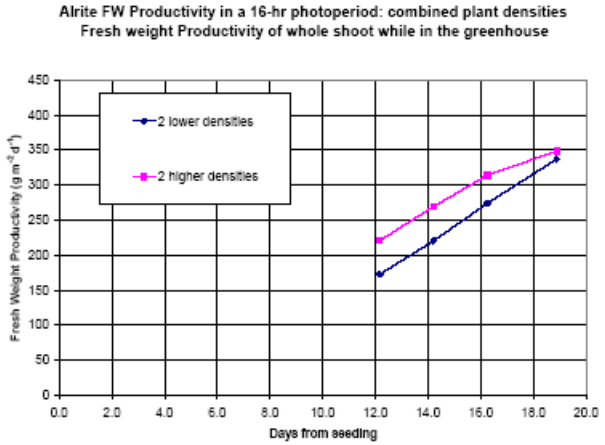


Figure 3-22: Alrite FW productivity in a 16-hour photoperiod - combined plant densities Fresh weight productivity of whole shoot while in the greenhouse

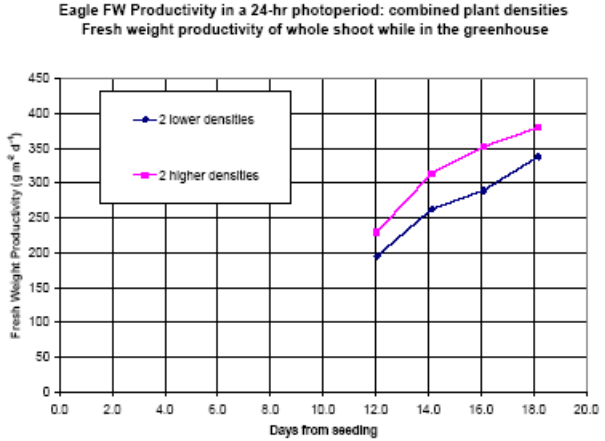


Figure 3-23: Eagle FW productivity in a 24-hour photoperiod - combined plant densities Fresh weight productivity of whole shoot while in the greenhouse

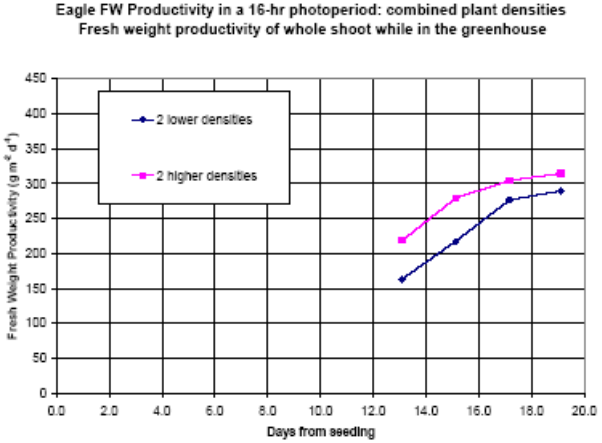


Figure 3-24: Eagle FW productivity in a 16-hour photoperiod - combined plant densities Fresh weight productivity of whole shoot while in the greenhouse

The Effect of Photoperiod on FW and DW Productivity

Photoperiod had a powerful effect on fresh weight. Data showing the effect of each photoperiod on fresh weight Productivity are juxtaposed in Figures 3-26 and 3-27, separately for each cultivar. The data supporting these graphs are provided in Table 3-44. The Eagle data are presented for all plant densities combined; the Alrite data are just for the lower two plants densities for which a complete set of data from 4 harvests is available.

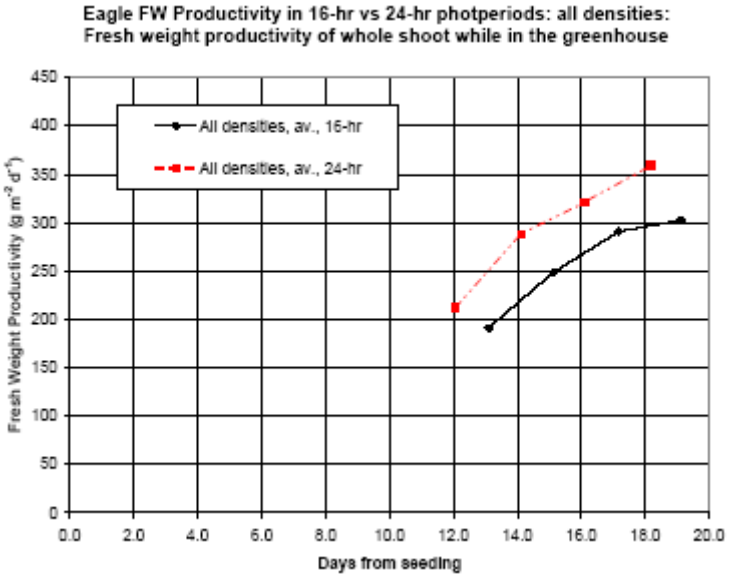


Figure 3-25: Eagle FW productivity in 16-hr vs 24-hour photoperiods - all densities - fresh weight productivity of whole shoot while in the greenhouse

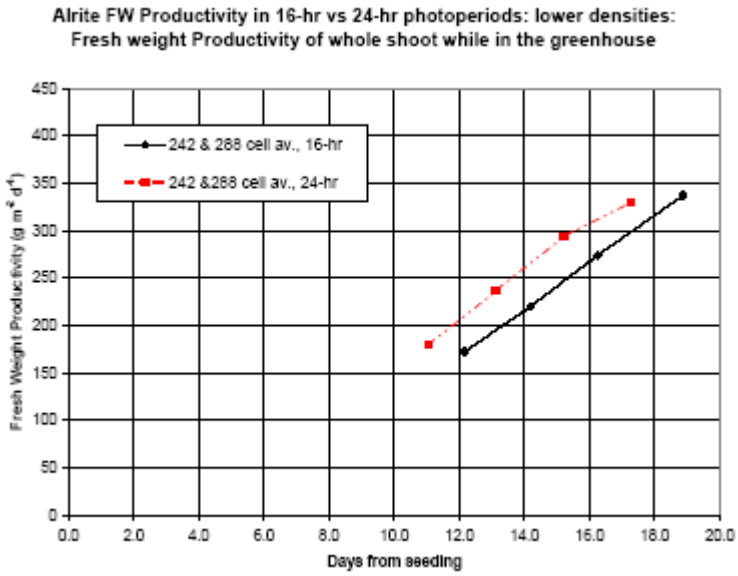


Figure 3-26: Alrite FW productivity in 16-hour vs 24-hr photoperiods - lower densities - fresh weight productivity of whole shoot while in the greenhouse

As shown on the graphs, the 24-hour crop was 18 to 25 % heavier than the 16-hr crop in each cultivar. The effect was slightly more pronounced in Eagle than in Alrite. This may be because Alrite was moderately induced to flower even in the 16-hr photoperiod (as will be proved later when stem extension is tabulated) whereas the northern- adapted Eagle is very slow to flower in a 16-hr photoperiod.

The effect of photoperiod is lessened when Dry weight Productivity is considered, especially in the case of Alrite, as can be seen in Figures 3-28 and 3-29.

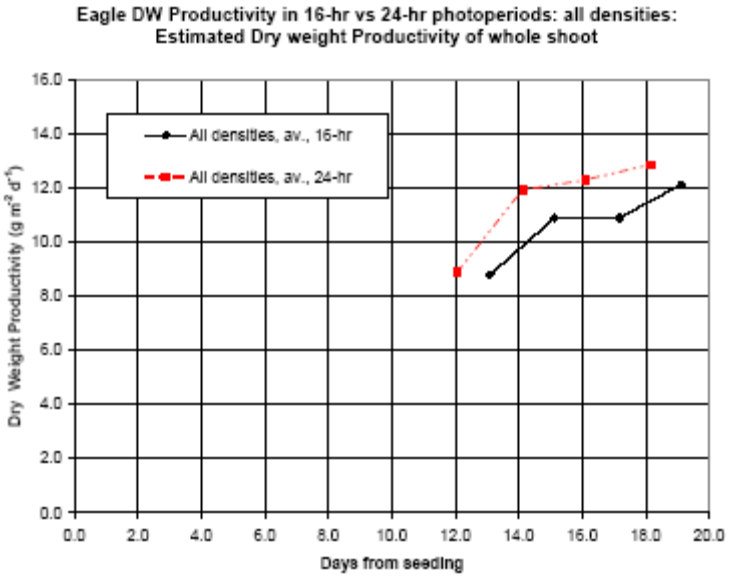


Figure 3-27: Eagle DW productivity in 16-hour vs 24-hour photoperiods - all densities - estimated dry weight productivity of whole shoot

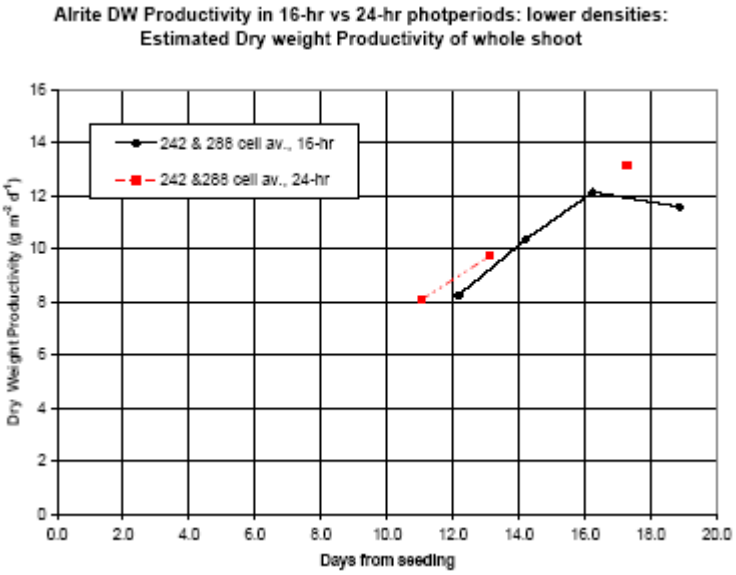


Figure 3-28: Alrite DW productivity in 16-hour vs 24-hour photoperiods - lower densities - estimated dry weight productivity of whole shoot

This is because there was a difference in dry matter content between plants in the two photoperiods at comparable points in time. In virtually all comparisons across photoperiods, the 16-hr crop showed a higher dry matter content (i.e., ratio of dry weight to fresh weight) than the 24-hour crop, even though the 16-hr measurements were always one day later. These relationships can be seen in Table 3-45.

Cultivar and Photoperiod	Days from Seeding	Nominal Plant Density				Combined Densities		
		1049 m^{-2} 242-cell	1248 m^{-2} 288-cell	1464 m^{-2} 338-cell	1698 m^{-2} 392-cell	2 lower densities	2 higher densities	All densities
24-hour Alrite	11.06	0.046	0.044	0.043	0.045	0.045	0.044	0.045
	13.13	0.043	0.040	0.039	0.038	0.041	0.039	0.040
	15.21							
	17.27	0.036	0.044	n/a	n/a	0.040	n/a	n/a
16-hour Alrite	12.17	0.047	0.049	0.045	0.047	0.048	0.046	0.047
	14.20	0.046	0.048	0.045	0.045	0.047	0.045	0.046
	16.25	0.045	0.043	0.044	0.044	0.044	0.044	0.044
	18.88	0.035	0.034	0.037	0.039	0.034	0.038	0.036
24-hour Eagle	12.04	0.043	0.042	0.043	0.040	0.043	0.042	0.042
	14.13	0.043	0.041	0.040	0.042	0.042	0.041	0.042
	16.09	0.042	0.041	0.037	0.035	0.041	0.036	0.039
	18.17	0.035	0.034	0.036	0.038	0.035	0.037	0.036
16-hour Eagle	13.09	0.051	0.049	0.046	0.041	0.050	0.043	0.047
	15.13	0.045	0.046	0.042	0.043	0.045	0.043	0.044
	17.17	0.041	0.035	0.040	0.036	0.038	0.038	0.038
	19.11	0.039	0.043	0.041	0.038	0.041	0.039	0.040

Table 3-46: Dry Matter Content of whole shoot on basis of sub sample: Ratio of Dry Weight to Fresh Weight (DW/FW)

Dry Weight Productivity

Table 3-45 of DW/FW ratios was derived from a sub sample of two rows of plants in each of the harvests. Each plant in the row was dried individually to obtain a measurement of variation.

In addition to the systematic effect of photoperiod on dry matter content, it can be seen the dry matter content consistently fell off with plant age. The effect was more pronounced in the lower densities. It is of some interest that plant density itself had no effect on dry matter content.

In Table 3-46, Dry weight Productivity has been calculated for the sub sample. Our best estimate of Dry weight Productivity is presented in Table 3-47, where the DW/FW ratios of Table 3-45 are applied to the FW productivity figures of Table 3-44. The DW/FW ratio is more stable under sampling than "weight

harvested" because it is less susceptible to the effect of missing plants. Sampling error is more apparent in these data than in the Fresh weight data, as one would expect. However, it can be seen the effect of plant spacing is still strong, as is the effect of time of harvest.

Cultivar and Photoperiod	Days from Seeding	Nominal Plant Density				Combined Densities		
		1049 m^{-2} 242-cell	1248 m^{-2} 288-cell	1464 m^{-2} 338-cell	1698 m^{-2} 392-cell	2 lower densities	2 higher densities	All densities
24-hour Alrite	11.06	**7.8**	**8.0**	**10.0**	**11.8**	**7.9**	**10.9**	**9.4**
	13.13	**9.7**	**10.2**	**13.2**	**13.4**	**9.9**	**13.3**	**11.6**
	15.21							
	17.27	**12.9**	**15.5**	**n/a**	**n/a**	**14.2**	**n/a**	**n/a**
16-hour Alrite	12.17	**8.8**	**9.2**	**10.5**	**11.0**	**9.0**	**10.7**	**9.9**
	14.20	**9.3**	**11.7**	**12.3**	**13.4**	**10.5**	**12.9**	**11.7**
	16.25	**14.0**	**10.8**	**13.3**	**14.3**	**12.4**	**13.8**	**13.1**
	18.88	**10.3**	**10.8**	**13.5**	**15.4**	**10.5**	**14.4**	**12.5**
24-hour Eagle	12.04	**7.5**	**9.0**	**10.9**	**10.4**	**8.2**	**10.6**	**9.4**
	14.13	**10.7**	**11.2**	**11.0**	**14.1**	**11.0**	**12.5**	**11.7**
	16.09	**13.5**	**9.6**	**14.0**	**13.9**	**11.6**	**13.9**	**12.8**
	18.17	**12.3**	**12.9**	**14.5**	**15.2**	**12.6**	**14.9**	**13.7**
16-hour Eagle	13.09	**6.9**	**8.4**	**8.8**	**8.9**	**7.7**	**8.8**	**8.2**
	15.13	**9.6**	**10.7**	**11.7**	**12.7**	**10.2**	**12.2**	**11.2**
	17.17	**9.1**	**10.6**	**12.0**	**11.6**	**9.9**	**11.8**	**10.8**
	19.11	**10.5**	**12.9**	**13.0**	**12.3**	**11.7**	**12.6**	**12.2**

Table 3-47: Sub-sample Dry Weight Productivity of whole shoot while in the greenhouse, (g m^{-2} d^{-1})

Cultivar and Photoperiod	Days from Seeding	Nominal Plant Density				Combined Densities		
		1049 m^{-2} 242-cell	**1248 m^{-2}** 288-cell	**1464 m^{-2}** 338-cell	**1698 m^{-2}** 392-cell	**2 lower densities**	**2 higher densities**	**All densities**
24-hour Alrite	11.06	**7.8**	**8.3**	**9.7**	**11.5**	**8.1**	**10.6**	**9.3**
	13.13	**9.8**	**9.7**	**12.0**	**12.8**	**9.7**	**12.4**	**11.1**
	15.21							
	17.27	**11.4**	**14.9**	**n/a**	**n/a**	**13.1**	**n/a**	**n/a**
16-hour Alrite	12.17	**8.1**	**8.4**	**9.8**	**10.7**	**8.2**	**10.2**	**9.2**
	14.20	**9.4**	**11.3**	**12.2**	**12.2**	**10.3**	**12.2**	**11.3**
	16.25	**12.8**	**11.4**	**14.0**	**13.8**	**12.1**	**13.9**	**13.0**
	18.88	**11.1**	**12.0**	**12.7**	**13.9**	**11.6**	**13.3**	**12.4**
24-hour Eagle	12.04	**7.8**	**8.8**	**9.9**	**9.2**	**8.3**	**9.5**	**8.9**
	14.13	**10.7**	**11.5**	**12.4**	**13.1**	**11.1**	**12.8**	**11.9**
	16.09	**11.7**	**12.2**	**12.6**	**12.8**	**11.9**	**12.7**	**12.3**
	18.17	**11.7**	**11.8**	**14.0**	**14.0**	**11.8**	**14.0**	**12.9**
16-hour Eagle	13.09	**7.7**	**8.6**	**9.7**	**9.3**	**8.1**	**9.5**	**8.8**
	15.13	**9.5**	**10.3**	**11.8**	**12.1**	**9.9**	**11.9**	**10.9**
	17.17	**10.3**	**10.4**	**11.5**	**11.5**	**10.4**	**11.5**	**10.9**
	19.11	**11.6**	**12.1**	**13.2**	**11.6**	**11.9**	**12.4**	**12.1**

Table 3-48: Estimated Dry Weight Productivity of whole shoot while in the greenhouse for the full harvest: FW Productivity of full sample x DW/FW ratio of sub sample. (g m^{-2} d^{-1})

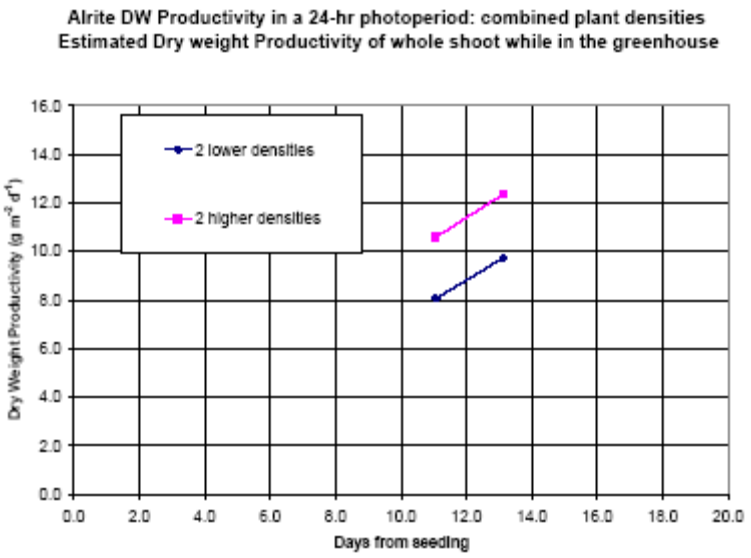


Figure 3-29: Alrite DW productivity in a 24-hour photoperiod - combined plant densities - estimated dry weight productivity of whole shoot while in the greenhouse

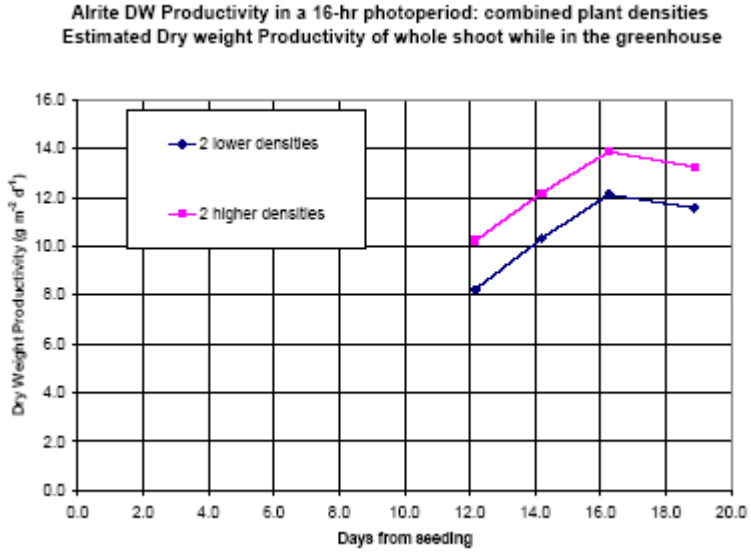


Figure 3-30 Alrite DW productivity in a 16-hour photoperiod - combined plant densities - estimated dry weight productivity of whole shoot while in the greenhouse

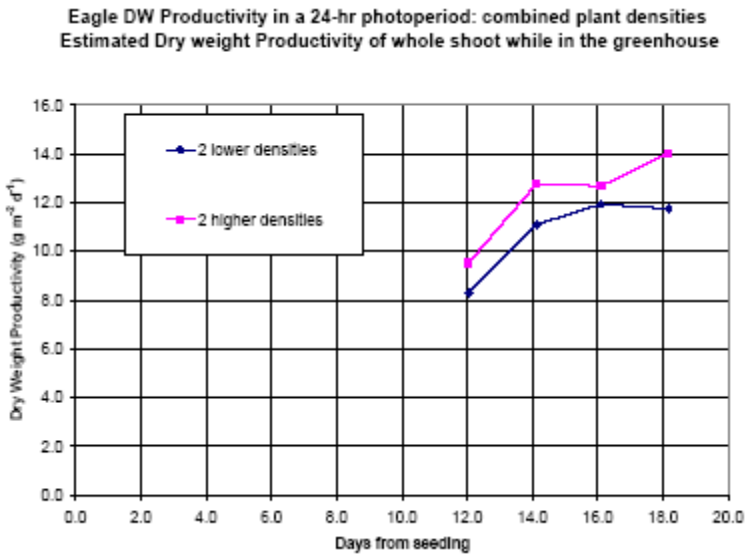


Figure 3-31 Eagle DW productivity in a 24-hour photoperiod - combined plant densities - estimated dry weight productivity of whole shoot while in the greenhouse

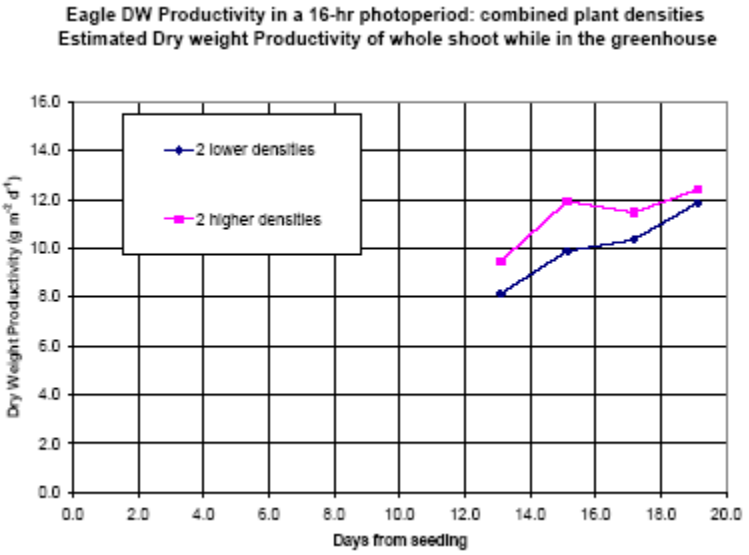


Figure 3-32 Eagle DW productivity in a 16-hour photoperiod - combined plant densities - estimated dry weight productivity of whole shoot while in the greenhouse

Dry matter Productivity appears to reach a plateau in the case of 16-hr Alrite and 24-hr Eagle, and be ascending slowly in 16-hr Eagle. The leveling off of Dry weight Productivity is not surprising considering the decline in dry matter content with age shown in Table 3-45.

Cultivar Comparisons

These two cultivars were selected for their differences in growth habit, particularly responsivity to photoperiod. It is commonly believed Alrite, and similarly adapted Japanese cultivars, show faster early growth than northern adapted American and European cultivars. In this study there was an opportunity to

directly compare the cultivars in terms of productivity over time *within each photoperiod*, as is illustrated in Figures 3-34 to 3-36.

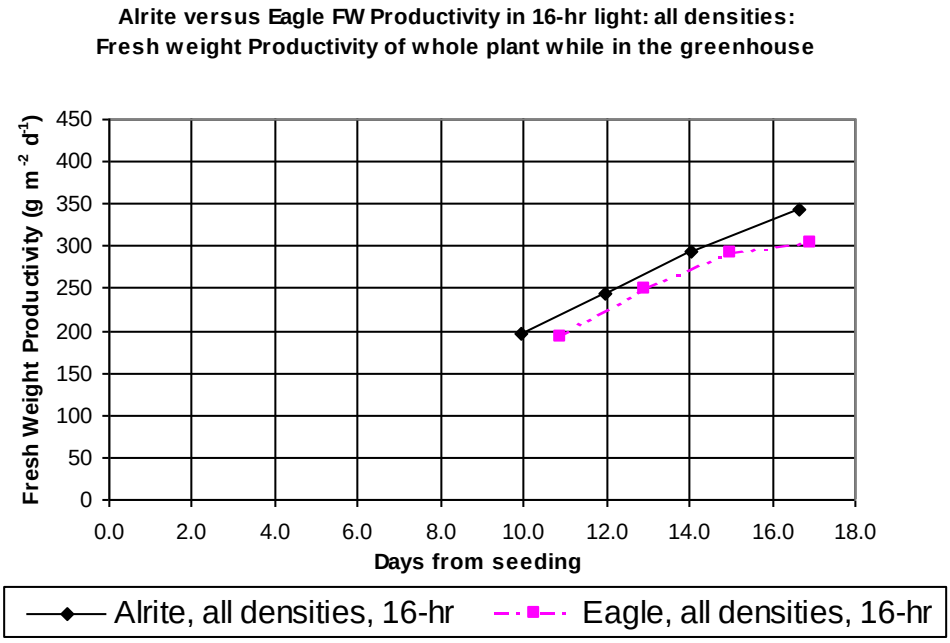


Figure 3-33. Alrite vs Eagle FW Productivity in 16-hr photoperiod: all densities combined

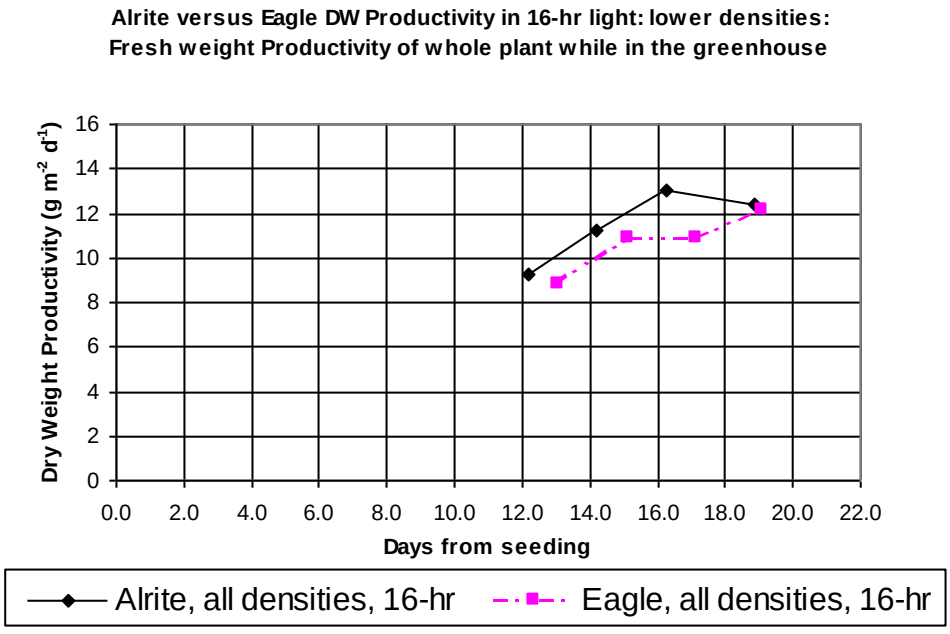


Figure 3-34. Alrite vs Eagle DW Productivity in 16-hr photoperiod: lower densities

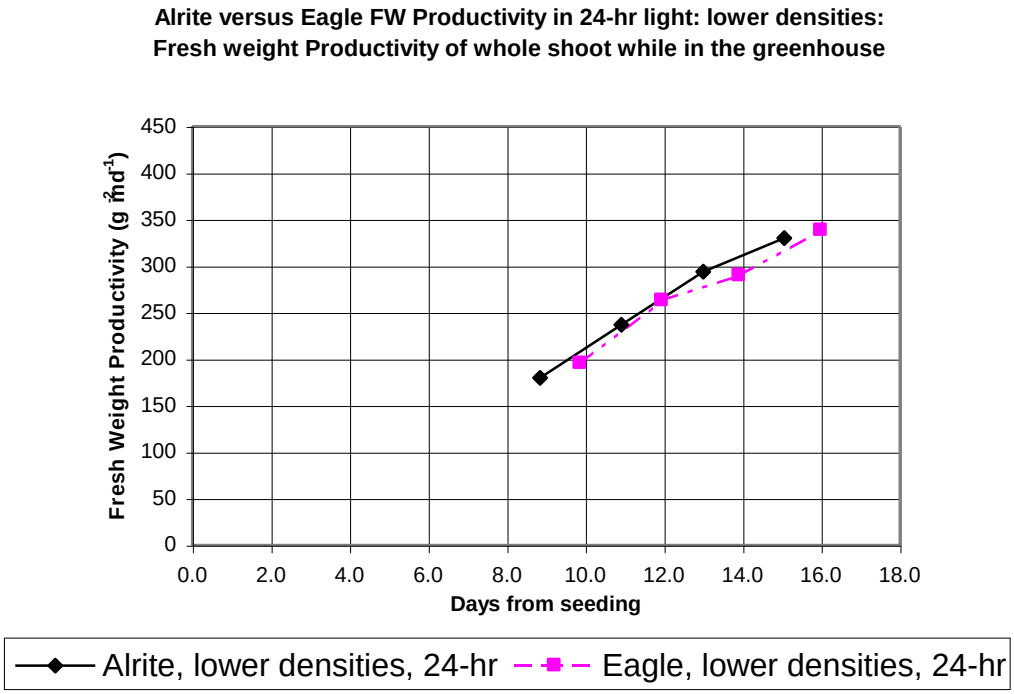


Figure 3-35. Alrite versus Eagle FW Productivity in 24-hr photoperiod: lower densities

A difference in FW Productivity of each cultivar can be seen in the 16-hr photoperiod, and Alrite was superior in all 4 harvest times. This result also held up when dry weights were considered. (See Figs 3-34 and 3-35). However, when Eagle was induced towards flowering, as was the case in the 24-hour photoperiod, there was no difference between the cultivars, despite the fact that Eagle stand establishment was somewhat poorer than that of Alrite (Fig. 3-36). Thus, it appears the common belief is true, but not because one cultivar is intrinsically faster growing than the other, rather, one surmises, because under field conditions where the comparison is usually made, the Asian cultivars are induced to flower whereas the temperate latitude cultivars are not.

Plant stand establishment was systematically smaller in Eagle than Alrite. We believe this was most probably the result of seed quality issues and not intrinsic to the plant type. De-hulled seed was used throughout this work. The de-hulling procedure was performed by Takii Seed Co. who holds the patent on the process. Takii have already perfected the de-hulling operation for the Japanese cultivar Alrite, and consequently it had a very high germination percentage and gave nearly full stand establishment. The other cultivar, Eagle, is of North American origin and we sent seed to Takii in Japan for de-hulling. Eagle is a large seed with different characteristics than Alrite. Only a limited quantity of seed was available for fine-tuning the de-hulling process. Being of a different size than Alrite, and no doubt varying in other ways,

Eagle did not fare well in de-hulling. As a result, stand establishment for Eagle was not as good as that of Alrite.

Table 3-7 below demonstrates the difference in seed quality as it affected plant stand. More than 93% of Alrite cells seeded were considered to have produced "successful" plants at time of harvest, but only 87% of Eagle cells were considered successful. The percentages of successful plants in each harvest recorded in Table 3-7 were based on a weight criterion applied to the all plants in the FW sample.

Cultivar and Photoperiod	Harvest Number	Nominal Plant Density				Combined Densities		
		1049 m^{-2} 242-cell	1248 m^{-2} 288-cell	1464 m^{-2} 338-cell	1698 m^{-2} 392-cell	2 lower densities	2 higher densities	All densities
24-hour Alrite	1	0.97	0.90	0.96	0.90	0.94	0.93	0.93
	2	0.85	0.89	0.91	0.94	0.87	0.92	0.90
	3	0.98	0.93			0.96		
	4	0.94	0.94			0.94		
16-hour Alrite	1	0.97	0.90	0.96	0.90	0.94	0.93	0.93
	2	0.85	0.89	0.91	0.94	0.87	0.92	0.90
	3	0.98	0.93	0.97	0.92	0.96	0.95	0.95
	4	0.94	0.94	0.92	0.89	0.94	0.91	0.92
All Alrite Harvests		**0.94**	**0.92**	**0.94**	**0.91**	**0.93**	**0.93**	**0.93**
24-hour Eagle	1	0.82	0.85	0.79	0.81	0.83	0.80	0.82
	2	0.84	0.93	0.94	0.91	0.88	0.92	0.90
	3	0.88	0.88	0.89	0.88	0.88	0.89	0.88
	4	0.82	0.92	0.90	0.84	0.87	0.87	0.87
16-hour Eagle	1	0.93	0.85	0.87	0.90	0.89	0.88	0.89
	2	0.82	0.83	0.88	0.86	0.83	0.87	0.85
	3	0.86	0.96	0.81	0.88	0.91	0.85	0.88
	4	0.89	0.85	0.90	0.82	0.87	0.86	0.86
All Eagle Harvests		**0.86**	**0.88**	**0.87**	**0.86**	**0.87**	**0.87**	**0.87**

Table 3-49. Proportion of cells containing successful plants in each harvest

Plant Morphology

One of the main purposes in collecting plant morphology data was to determine the size of the primary leaves. Generally these are close in size within the pair, so it was sufficient to gather data on one of them. Data were collected on the largest leaf. Stem height from the base to the apical tip was an indication of how far the plant had progressed towards flowering, and how rapidly it was changing in this direction. Data on plant morphology are presented in Tables 3-49 to 3-51, one table for each cultivar-photoperiod combination.

Plant Density (Nominal) (plnt m^{-2})	Sample Size (n)	FW of Whole Plant (g)	Plant Height (cm)	Stem Length (cm)	Dimensions of largest leaf				
					Petiole Length (cm)	Blade Length (cm)	Blade Width (cm)	Est. Area (cm^2)	Blade Lngth to Wdth ratio
1st harvest of Alrite in 34-hr light. Day 11									
1050	19	**1.65**	**11.74**	**0.67**	**5.64**	**6.09**	**2.99**	**14.63**	**2.12**
		0.08	*0.26*	*0.04*	*0.14*	*0.18*	*0.20*	*1.39*	*0.08*
1250	19	**1.50**	**11.71**	**0.69**	**5.93**	**5.78**	**2.71**	**12.34**	**2.15**
		0.05	*0.25*	*0.03*	*0.12*	*0.16*	*0.06*	*0.50*	*0.06*
1460	12	**1.54**	**11.68**	**0.73**	**5.69**	**5.99**	**2.56**	**12.49**	**2.36**
		0.16	*0.54*	*0.03*	*0.25*	*0.36*	*0.17*	*1.34*	*0.07*
1700	14	**1.29**	**11.63**	**0.88**	**6.25**	**5.38**	**2.43**	**10.37**	**2.22**
		0.06	*0.36*	*0.08*	*0.22*	*0.19*	*0.09*	*0.64*	*0.06*
2nd harvest of Alrite in 24-hr light. Day 13									
1050	13	**2.97**	**18.04**	**1.47**	**8.57**	**8.64**	**3.85**	**26.73**	**2.24**
		0.20	*0.74*	*0.12*	*0.36*	*0.40*	*0.17*	*2.13*	*0.05*
1250	12	**2.50**	**17.88**	**1.62**	**8.69**	**8.15**	**3.68**	**24.02**	**2.23**
		0.20	*0.62*	*0.19*	*0.21*	*0.37*	*0.19*	*2.25*	*0.07*
1460	13	**2.90**	**19.67**	**1.74**	**9.88**	**8.74**	**3.81**	**26.60**	**2.29**
		0.21	*0.72*	*0.13*	*0.34*	*0.41*	*0.15*	*2.02*	*0.07*
1700	16	**2.76**	**18.55**	**2.05**	**10.11**	**8.43**	**3.81**	**25.79**	**2.22**
		0.16	*1.21*	*0.18*	*0.22*	*0.34*	*0.15*	*1.93*	*0.04*
3rd harvest of Alrite in 24-hr light. Day 15									
1050	16	**4.95**	**22.44**	**3.63**	**10.45**	**10.43**	**4.30**	**35.51**	**2.43**
		0.20	*0.36*	*0.36*	*0.23*	*0.26*	*0.11*	*1.73*	*0.05*
1250	14	**4.64**	**21.49**	**3.61**	**10.24**	**9.94**	**4.21**	**33.03**	**2.38**
		0.22	*0.26*	*0.23*	*0.22*	*0.14*	*0.13*	*1.33*	*0.06*
1460		**n/a**							
		n/a							
1700	10	**3.76**	**25.15**	**5.59**	**12.00**	**9.96**	**4.38**	**34.57**	**2.28**
		0.28	*0.58*	*0.56*	*0.50*	*0.39*	*0.15*	*2.41*	*0.06*
4th harvest of Alrite in 24-hr light. Day 17									
1050	18	**6.11**	**24.39**	**7.77**	**11.68**	**10.36**	**4.53**	**37.15**	**2.30**
		0.52	*1.45*	*0.50*	*0.28*	*0.25*	*0.13*	*1.76*	*0.05*
1250	19	**4.97**	**25.59**	**8.25**	**11.75**	**9.67**	**4.36**	**33.55**	**2.24**
		0.35	*0.51*	*0.68*	*0.22*	*0.23*	*0.15*	*1.85*	*0.04*
1460		**n/a**							
1700		**n/a**							

Table 3-50. Alrite in a 24-hr Photoperiod: Average Plant and Leaf Dimensions of Successful Plants in each Harvest. Standard error is given in italics

Plant Density (Nominal) (plnt m^{-2})	Sample Size (n)	FW of Whole Plant (g)	Plant Height (cm)	Stem Length (cm)	Dimensions of largest leaf				
					Petiole Length (cm)	Blade Length (cm)	Blade Width (cm)	Est. Area (cm^2)	Blade Lngth to Wdth ratio
1st harvest of Alrite in 16-hr light. Day 12									
1050	10	**1.58**	**11.23**	**0.43**	**5.29**	**5.94**	**2.62**	**12.51**	**2.27**
		0.16	*0.64*	*0.02*	*0.30*	*0.38*	*0.14*	*1.32*	*0.10*
1250	11	**1.45**	**10.41**	**0.43**	**5.01**	**5.40**	**2.37**	**10.68**	**2.33**
		0.13	*0.69*	*0.01*	*0.30*	*0.41*	*0.20*	*1.34*	*0.09*
1460	13	**1.57**	**11.70**	**0.45**	**5.80**	**5.90**	**2.78**	**13.17**	**2.13**
		0.11	*0.40*	*0.02*	*0.20*	*0.24*	*0.13*	*1.02*	*0.04*
1700	12	**1.44**	**11.36**	**0.54**	**5.68**	**5.68**	**2.63**	**11.88**	**2.16**
		0.10	*0.44*	*0.03*	*0.25*	*0.23*	*0.09*	*0.80*	*0.06*
2nd harvest of Alrite in 16-hr light. Day 14									
1050	16	**2.88**	**16.24**	**0.83**	**7.52**	**8.34**	**3.73**	**24.51**	**2.24**
		0.15	*0.32*	*0.04*	*0.20*	*0.17*	*0.08*	*0.91*	*0.05*
1250	16	**2.84**	**16.16**	**1.02**	**7.34**	**8.42**	**3.75**	**24.84**	**2.26**
		0.12	*0.27*	*0.06*	*0.18*	*0.16*	*0.09*	*0.84*	*0.06*
1460	16	**2.57**	**16.88**	**1.05**	**7.91**	**8.38**	**3.79**	**25.02**	**2.25**
		0.13	*0.27*	*0.06*	*0.24*	*0.16*	*0.14*	*1.13*	*0.08*
1700	16	**2.33**	**16.84**	**1.04**	**8.05**	**8.14**	**3.38**	**21.85**	**2.43**
		0.19	*0.49*	*0.08*	*0.24*	*0.28*	*0.12*	*1.40*	*0.09*
3rd harvest of Alrite in 16-hr light. Day 16									
1050	17	**4.58**	**20.64**	**1.65**	**9.99**	**9.84**	**4.28**	**33.40**	**2.32**
		0.32	*0.39*	*0.12*	*0.18*	*0.29*	*0.15*	*1.92*	*0.06*
1250	15	**3.81**	**18.25**	**1.63**	**8.83**	**8.89**	**3.88**	**27.27**	**2.30**
		0.24	*0.38*	*0.13*	*0.28*	*0.22*	*0.10*	*1.26*	*0.05*
1460	17	**3.71**	**19.45**	**1.68**	**9.60**	**9.05**	**3.86**	**27.91**	**2.37**
		0.30	*0.47*	*0.14*	*0.27*	*0.28*	*0.17*	*1.98*	*0.07*
1700	18	**3.35**	**19.53**	**1.24**	**10.11**	**8.69**	**3.66**	**25.29**	**2.39**
		0.21	*0.46*	*0.08*	*0.25*	*0.24*	*0.12*	*1.46*	*0.05*
4th harvest of Alrite in 16-hr light. Day 18									
1050	17	**5.52**	**23.22**	**2.36**	**10.86**	**10.49**	**4.61**	**38.59**	**2.27**
		0.39	*0.63*	*0.23*	*0.25*	*0.38*	*0.14*	*2.40*	*0.04*
1250	14	**5.05**	**21.90**	**3.14**	**10.54**	**9.90**	**4.31**	**33.99**	**2.32**
		0.45	*0.73*	*0.29*	*0.30*	*0.36*	*0.19*	*2.37*	*0.07*
1460	16	**5.47**	**23.20**	**2.87**	**11.90**	**10.18**	**4.82**	**38.69**	**2.12**
		0.30	*0.45*	*0.33*	*0.28*	*0.28*	*0.11*	*1.65*	*0.06*
1700	16	**5.39**	**22.54**	**2.84**	**10.98**	**10.01**	**4.62**	**37.21**	**2.18**
		0.55	*0.62*	*0.27*	*0.26*	*0.42*	*0.22*	*3.27*	*0.06*

Table 3-50. Alrite in a 16-hr Photoperiod: Average Plant and Leaf Dimensions of Successful Plants in each Harvest. Standard error is given in italics

Plant Density (Nominal) (plnt m^{-2})	Sample Size (n)	FW of Whole Plant (g)	Plant Height (cm)	Stem Length (cm)	Dimensions of largest leaf				
					Petiole Length (cm)	Blade Length (cm)	Blade Width (cm)	Est. Area (cm^2)	Blade Lngth to Wdth ratio
Ist harvest of Eagle in 24-hr light. Day 12									
1050	12	**2.28**	**11.27**	**0.68**	**4.28**	**3.41**	**6.55**	**17.60**	**1.94**
		0.11	*0.30*	*0.04*	*0.10*	*0.13*	*0.24*	*0.98*	*0.08*
1250	15	**2.00**	**11.13**	**0.77**	**4.21**	**3.15**	**6.30**	**15.82**	**2.00**
		0.10	*0.24*	*0.03*	*0.08*	*0.11*	*0.23*	*1.04*	*0.03*
1460	13	**2.19**	**11.58**	**0.75**	**4.57**	**3.30**	**6.47**	**16.85**	**1.97**
		0.08	*0.24*	*0.03*	*0.13*	*0.09*	*0.19*	*0.79*	*0.05*
1700	16	**2.02**	**11.43**	**0.83**	**4.41**	**3.15**	**6.35**	**15.76**	**2.03**
		0.07	*0.24*	*0.03*	*0.13*	*0.08*	*0.15*	*0.62*	*0.05*
2nd harvest of Eagle in 24-hr light. Day 14									
1050	15	**3.22**	**15.14**	**1.21**	**5.84**	**4.00**	**8.36**	**26.47**	**2.10**
		0.21	*0.26*	*0.05*	*0.16*	*0.12*	*0.29*	*1.46*	*0.07*
1250	16	**3.12**	**15.53**	**1.50**	**6.19**	**3.92**	**8.25**	**25.58**	**2.11**
		0.15	*0.28*	*0.07*	*0.13*	*0.10*	*0.27*	*1.35*	*0.05*
1460	16	**3.11**	**15.80**	**1.51**	**6.54**	**4.01**	**8.07**	**25.57**	**2.03**
		0.11	*0.30*	*0.04*	*0.15*	*0.12*	*0.18*	*1.17*	*0.05*
1700	15	**2.76**	**15.19**	**1.45**	**6.29**	**3.49**	**7.57**	**21.05**	**2.17**
		0.17	*0.43*	*0.05*	*0.16*	*0.12*	*0.29*	*1.44*	*0.05*
3nd harvest of Eagle in 24-hr light. Day 16									
1050	20	**4.72**	**17.85**	**1.72**	**7.86**	**9.06**	**4.59**	**33.46**	**1.98**
		0.33	*0.54*	*0.09*	*0.21*	*0.39*	*0.18*	*2.40*	*0.06*
1250		**4.27**	**17.92**	**2.02**	**7.84**	8.873077	**4.38**	**31.50**	**2.05**
		0.41	*0.61*	*0.14*	*0.19*	*0.45*	*0.26*	*2.98*	*0.06*
1460	18	**4.59**	**18.89**	**2.03**	**8.62**	**9.04**	**4.48**	**32.26**	**2.02**
		0.28	*0.45*	*0.06*	*0.22*	*0.32*	*0.13*	*1.91*	*0.04*
1700	18	**4.33**	**19.23**	**2.44**	**8.68**	**9.11**	**4.56**	**32.79**	**2.01**
		0.24	*0.38*	*0.12*	*0.20*	*0.25*	*0.11*	*1.54*	*0.05*
4th harvest of Eagle in 24-hr light. Day 18									
1050	16	**6.67**	**20.24**	**2.88**	**8.31**	**10.34**	**5.32**	**43.73**	**1.96**
		0.58	*0.46*	*0.14*	*0.21*	*0.32*	*0.20*	*2.84*	*0.05*
1250	16	**6.45**	**21.32**	**3.65**	**8.98**	**10.46**	**5.28**	**44.13**	**1.99**
		0.58	*0.47*	*0.12*	*0.19*	*0.40*	*0.20*	*3.00*	*0.04*
1460	14	**6.09**	**21.96**	**3.85**	**9.67**	**10.30**	**5.21**	**42.63**	**2.00**
		0.46	*0.47*	*0.20*	*0.21*	*0.28*	*0.21*	*2.54*	*0.05*
1700	16	**5.54**	**20.03**	**4.13**	**8.99**	**10.28**	**5.03**	**41.32**	**2.07**
		0.42	*1.30*	*0.31*	*0.19*	*0.39*	*0.21*	*3.04*	*0.08*

Table 3-51. Eagle in a 24-hr Photoperiod: Average Plant and Leaf Dimensions of Successful Plants in each Harvest. Standard error is given in italics

Plant Density (Nominal) (plnt m^{-2})	Sample Size (n)	FW of Whole Plant (g)	Plant Height (cm)	Stem Length (cm)	Dimensions of largest leaf				
					Petiole Length (cm)	Blade Length (cm)	Blade Width (cm)	Est. Area (cm^2)	Blade Lngth to Wdth ratio
1st harvest of Eagle in 16-hr light. Day 13									
1050	16	**1.93**	**10.22**	**0.54**	**3.54**	**6.42**	**3.24**	**16.49**	**2.00**
		0.10	*0.15*	*0.01*	*0.08*	*0.15*	*0.11*	*0.87*	*0.05*
1250	16	**1.89**	**10.56**	**0.54**	**3.96**	**6.36**	**3.19**	**16.06**	**2.02**
		0.08	*0.14*	*0.02*	*0.09*	*0.15*	*0.11*	*0.79*	*0.07*
1460	16	**2.08**	**10.70**	**0.48**	**3.99**	**6.49**	**3.28**	**16.75**	**1.98**
		0.06	*0.16*	*0.02*	*0.09*	*0.15*	*0.06*	*0.58*	*0.04*
1700	16	**1.78**	**10.90**	**0.61**	**4.34**	**6.18**	**2.96**	**14.40**	**2.10**
		0.07	*0.17*	*0.02*	*0.11*	*0.13*	*0.08*	*0.53*	*0.06*
2nd harvest of Eagle in 16-hr light. Day 15									
1050	16	**3.35**	**14.03**	**0.67**	**5.80**	**8.06**	**4.08**	**26.33**	**1.99**
		0.24	*0.38*	*0.02*	*0.13*	*0.30*	*0.17*	*1.94*	*0.04*
1250	16	**3.16**	**13.77**	**0.60**	**5.34**	**8.15**	**3.95**	**25.64**	**2.06**
		0.18	*0.33*	*0.02*	*0.11*	*0.28*	*0.12*	*1.61*	*0.04*
1460	16	**3.51**	**15.38**	**0.64**	**6.35**	**8.74**	**4.09**	**28.36**	**2.16**
		0.18	*0.23*	*0.02*	*0.15*	*0.22*	*0.14*	*1.52*	*0.05*
1700	17	**3.16**	**15.11**	**0.76**	**6.38**	**8.46**	**4.01**	**26.78**	**2.12**
		0.13	*0.28*	*0.03*	*0.14*	*0.23*	*0.11*	*1.26*	*0.06*
3rd harvest of Eagle in 16-hr light. Day 17									
1050	16	**4.24**	**17.04**	**1.08**	**7.17**	**9.16**	**4.61**	**33.79**	**2.01**
		0.29	*0.47*	*0.05*	*0.21*	*0.34*	*0.20*	*2.35*	*0.06*
1250	17	**4.41**	**17.84**	**1.15**	**7.51**	**9.61**	**4.79**	**36.50**	**2.02**
		0.28	*0.28*	*0.03*	*0.15*	*0.26*	*0.15*	*2.05*	*0.05*
1460	16	**4.37**	**17.77**	**1.10**	**7.76**	**9.38**	**4.56**	**33.92**	**2.05**
		0.24	*0.34*	*0.05*	*0.17*	*0.26*	*0.12*	*1.73*	*0.03*
1700	19	**3.57**	**18.42**	**1.41**	**8.35**	**9.14**	**4.36**	**31.61**	**2.12**
		0.19	*0.31*	*0.06*	*0.13*	*0.27*	*0.15*	*1.82*	*0.07*
4th harvest of Eagle in 16-hr light. Day 19									
1050	15	**5.46**	**19.44**	**1.48**	**8.30**	**10.29**	**5.14**	**42.02**	**2.03**
		0.45	*0.35*	*0.07*	*0.19*	*0.26*	*0.22*	*2.70*	*0.06*
1250	15	**5.47**	**19.88**	**1.55**	**8.57**	**10.39**	**4.93**	**40.61**	**2.11**
		0.63	*0.58*	*0.07*	*0.37*	*0.38*	*0.15*	*2.47*	*0.06*
1460	15	**5.08**	**21.23**	**1.61**	**9.49**	**10.94**	**5.09**	**44.08**	**2.16**
		0.37	*0.38*	*0.05*	*0.20*	*0.27*	*0.15*	*2.22*	*0.03*
1700	16	**4.61**	**20.88**	**1.75**	**9.41**	**10.51**	**4.98**	**41.36**	**2.12**
		0.40	*0.37*	*0.07*	*0.28*	*0.31*	*0.14*	*2.07*	*0.06*

Table 3-53. Eagle in a 16-hr Photoperiod: Average Plant and Leaf Dimensions of Successful Plants in each Harvest. Standard error is given in italics

Stem Length. Even by the first harvest (day 11 or day 12) the stem was significantly longer in the 24-hour photoperiod than in the 16-hr photoperiod, in both cultivars, although it was still quite short (less than 1cm) in absolute terms. By the 4th harvest, 6 days later, in Alrite the average stem length was 8 cm and in Eagle it was 4 cm. Equivalent figures for the 4th harvest of the 16-hr photoperiod crops were 2.8 cm and 1.6 cm. In all harvests, the 16-hr crop was harvested one day later than the 24-hr crop, so the difference between photoperiod treatments was even greater than suggested by these figures. It is also of interest that stem extension was affected by plant density, being greater the higher the plant density, an effect visible even within these small sub samples. For the most part effects of plant density were not visible in the other parameters measured, or did not reach significance in these data, within a given harvest.

Leaf Size/Area. The first harvest was early enough in all cases to catch the crop before the primary leaves were bigger than optimal size for baby spinach. Two days later the largest of the two primary leaves was circa 25 cm^2. In terms of leaf size, the second harvest was timed nearly ideally for these cultivars under these inputs. The largest leaf was 30 to 35 cm^2 in the 3rd harvests. For Alrite in the 16-hr photoperiod the 3rd harvest was on day 16, and largest leaves averaged 28 cm^2. From the point of view of leaf size, we assume the second and third harvests would be most important commercially. Top price should be obtained with the crop in the second harvest. The first harvest might be of use for producing "microspinach", but the market for this crop is limited in size. The 4th harvest might be good for a different category of spinach than "baby" spinach. There is a larger-leaf "cooking" product already on the market since almost all spinach is now prepackaged. This product retails and wholesales at a lower price.

Leaf Shape. Leaf shape as reflected in the ratio of blade length to blade width was remarkably stable within each cultivar regardless of photoperiod, plant spacing, or timing of harvest. It was 2-to-1 in Eagle and 2.3-to-1 in Alrite. For the American market, the rounder leaved cultivars such as Eagle are probably preferable.

Commercial Cut

For each harvest and plant density, data were collected to determine the proportion of the plant suitable for saleable harvest if cuts were made in various different planes above the plant base, or if cotyledon material were included with the true leaves. The plane in which the blade of a mechanical harvester operates may be adjusted up or down to include more or less of the plant (particularly leaf petioles), affecting the weight and appearance of the commercial product.

Preferably the petiole should not be a noticeable feature of the leaf. Bigger leaves can have a little more petiole attached before they are noticeable in these leaves than in smaller leaves. In large leaves the cut may vary by a centimeter without much affecting leaf appearance. Also it makes a difference to the look of the product if the leaf blade blends gradually into the petiole or ends abruptly at the petiole. There are bound to

be a few petioles included in the cut. If they are just a few, their length does not matter much. It is probably preferable to have a few long petioles included in a mass of nearly perfectly cut leaves than to have short petioles on all leaves.

Analyses were made for each of the harvests from the point of view of commercial potential. However, it became apparent that growing Alrite in a 24-hour photoperiod is not a useful option because the product quality is poor in appearance under the strong induction to flower, and there is a very limited opportunity to make successful use of the crop before it bolts. We saw little point in harvesting Alrite in the later harvests and in the higher densities. For different reasons, growing Eagle in a 16-hr photoperiod is not very satisfactory either. Although crop appearance is excellent in the 16-hr photoperiod, productivity is slow to reach a high level compared to the same cultivar in a longer photoperiod. By the time the 16-hour crop reached adequate productivity for commercial purposes (which it eventually did) the leaves were too big to qualify as baby spinach. With reference to Table 3-44 it can be seen Eagle in a 16-hr photoperiod barely achieved a productivity of 300 g m^{-2} d^{-1} on day 17 from seeding in the third harvest, whereas Eagle in a 24-hr photoperiod had already achieve this level comfortably by day 14, in the second harvest. (As metric we were looking for productivity in excess of 300 g m^{-2} d^{-1} as an indication of commercial viability.)

Empirically determined Characteristics of the Commercial Cuts as made							Best Estimate of CC Productivity, Leaf size and Petiole length in ideal Commercial Cuts			
Plant Density	**Leaf area Largest Leaf** (cm^2)	**Petiole Length Largest Lf** (cm)	**Cutting Height from base** (cm)	**Proportion in CC with Cotyledons** (ratio)	**Proportion in CC only true leaves** (ratio)	**Whole Plant Productivity** ($g\ m^{-2}\ d^{-1}$)	**Idealized Proportion in CC** (ratio)	**CC Productivity** ($g\ m^{-2}\ d^{-1}$)	**Petiole Length Largest Lf** (cm)	**Leaf area Largest Leaf** (cm^2)
Harvest 1, Day 12										
1050 m^{-2}	13	**5.3**	4.0	0.80	0.43	173	0.45	**78**	0.5	13
1250 m^{-2}	11	**5.0**	4.5	0.78	0.37	172	0.45	**77**	0.5	13
1460 m^{-2}	13	**5.8**	5.0	0.77	0.39	217	0.45	**97**	0.5	12
1700 m^{-2}	12	**5.7**	5.0	0.76	0.37	225	0.45	**101**	0.5	12
Harvest 2, Day 14										
1050 m^{-2}	25	**7.5**	7.0	0.45	0.42	205	0.50	**103**	1.0	25
1250 m^{-2}	25	**7.3**	6.5	0.52	0.48	235	0.50	**118**	1.0	25
1460 m^{-2}	25	**7.9**	6.5	0.52	0.48	268	0.50	**134**	1.0	24
1700 m^{-2}	22	**8.1**	7.0	0.48	0.44	270	0.50	**135**	1.0	23
Harvest 3, Day 16										
1050 m^{-2}	33	**10.0**	7.0	0.54	0.49	283	0.50	**141**	1.5	33
1250 m^{-2}	27	**8.8**	7.0	0.53	0.51	265	0.50	**133**	1.5	28
1460 m^{-2}	28	**9.6**	8.0	0.51	0.49	315	0.50	**158**	1.5	28
1700 m^{-2}	25	**10.1**	8.0	0.49	0.49	314	0.50	**157**	1.5	25
Harvest 4, Day 18										
1050 m^{-2}	39	**10.9**	10.0	0.51	0.49	321	0.45	**145**	1.0	39
1250 m^{-2}	34	**10.5**	12.0	0.41	0.40	353	0.45	**159**	1.0	38
1460 m^{-2}	39	**11.9**	11.0	0.45	0.44	339	0.45	**152**	1.0	38
1700 m^{-2}	37	**11.0**	12.0	0.41	0.40	359	0.45	**161**	1.0	37

Table 3-54. Characteristics of the Commercial Cut (CC) and CC Productivity in Alrite grown in a 16-hr Photoperiod as a function of Plant Density, and Timing of Harvest

Analyses of the commercial cut for Alrite in a 16-hr photoperiod and Eagle in a 24-hr photoperiod are presented in Tables 3-53 and 3-54 respectively.

Appendix 3A. Harvest machine in action: sequence of 8 pictures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CHAPTER 4. SELECTION OF CULTIVARS

Project Task 4: Select cultivars for head and leaf CEA Spinach Production Systems

MEETINGS AND DISCUSSIONS WITH BREEDERS, GERMPLASM COLLECTION CURATORS, AND SPINACH SEED PRODUCTION COMPANIES

As part of this project, we visited a spinach breeder in Mount Vernon, Washington and toured local spinach seed production fields. We also contacted the US spinach germplasm collection curators in Ames Iowa, and identified and contacted the three major spinach seed production companies (Chriseed (Sakata subsidiary), Seminis Seed Co. (Peto subsidiary), and Vejo Seed Co.).

Our discussions with these spinach seed production companies led us to estimate that there are approximately 100 hybrid cultivars of spinach on the market worldwide, in addition to the 300 open-pollinated types in the germplasm collection (Johnson, 2000). The seed breeders utilize combinations of the germplasm collection accessions to develop their hybrids. However, different hybrids are available only to certain markets (e.g., Japanese hybrid cultivars may not be available for sale in the US.)

We learned from the seed companies that the driving force behind all current breeding efforts is the attempt to control the leaf pathogen, Blue Mold. Genetic resistance to Blue Mold is what drives the market for spinach seed, not seed quality.

Our invitations and attempts to persuade each of these spinach seed companies to work with Cornell CEA researchers in the development of improved, more uniform, high quality spinach seed were not successful. The companies did not perceive spinach seed as having a potential as a high-value seed. Compared to many other food crops, spinach seed is very inexpensive and seed quality is correspondingly low. Seed test germination rates between 80 to 90% are typical. However the uniformity of timing of germination is very poor (3 to 10 days) for purposes of a CEA commercial scale system.

It is possible to improve spinach seed quality, but we found that the current perspective of the seed production companies is that farmers can compensate for poor seed quality by over-planting with low-cost seed.

Later Contacts With Seed Companies

For the later stages of the project we worked with just two seed companies, Rijk Zwaan, a Dutch company (from whom we obtain lettuce seed and with whom we have an ongoing relationship), and Takii Seed company of Japan, who own the patent to the de-hulling process. Rijk Zwaan supplied bolt-resistant seed of European/American cultivars to us for evaluation, and later to Takii for de-hulling, and Takii sent that

and selected examples of fast growing Japanese cultivars to us directly. We would like to acknowledge the support given us freely by these seed companies.

SELECTION OF FINAL CULTIVARS EMPLOYED

For some years, we have compared performance of highly productive cultivars used in Europe and North America (such as Nordic and Whitney), against Japanese cultivars, which are adapted to shorter days in lower latitudes. In general, the Japanese cultivars have faster early growth but are very bolting-prone in photoperiods longer than 13 hours. The Northern cultivars are slower off the mark, but are much less bolting-prone, even in photoperiods in excess of 16 hours. In this project, we deemed it prudent to pick representative cultivars of each spinach type, and conduct research with both of them to have the benefit of observing contrasting growth habits, and to be sure not to miss capitalizing on productive potential. In the case of Japanese cultivars, Takii Seed had historically supplied us with several of the more important cultivars in commerce (e.g., Megaton, Dash, Banchu Park, Alrite), and we had already performed cultivar trials comparing them prior to this project (Katzman, 2000, MS thesis, Johnson-Rutzke,2002, PhD dissertation). *Alrite was* one of the best of these Japanese cultivars, and we decided to use that to represent the type in this research.

In the case of northern adapted cultivars we had expected to proceed with the cultivar Whitney, but it became discontinued. Thereafter we explored the cultivars provided us by Rijk Zwaan Seed company, including experimental lines, before settling on a short list of three cultivars, Eagle, Panther and Whale, all of which are excellent modern hybrids popular in commerce, and very bolt resistant. These cultivars differed little in productivity as far as we could determine through greenhouse cultivar trials. Ultimately, we obtained de-hulled seed for all three, and picked Eagle with which to carry forward final research studies.

FUTURE CULTIVAR SELECTION

At the end of this project it had become clearer what the desired characteristics in a cultivar are if it is to be used for baby spinach production. Ideally we would like to be able to grow the spinach plant to a point at which 4 true leaves are large enough, but not too large, for simultaneous harvest, and all have equal length petioles, standing upright enough that a cutting plane exists underneath them that will avoid damage to the harvested product. Currently we find that by the time the second pair of true leaves are contributing to the harvest, the first pair of true leaves tend to be too big. (More ideally, a rosette of 6 to 8 leaves of the same size would be present, but this is not in the growth habit of this species.) Another issue of importance is the size and habit of growth of the cotyledons. It is most satisfactory if these have a horizontal tendency and thus do not enter into the cut, since they are odd looking and undesirable in the final product. Small cotyledons are desirable, by the same token, so long as no penalty in early growth results.

Other variable features found in cultivars are the shape of the leaf and the degree of ruffling, which are a matter of consumer preference. At present it appears no definite leaf type is consistently used in the market place. Probably this is because requirements for disease resistance predominate over consumer preferences in breeding programs, as noted above.

Before committing to commercial test production, it would be well worthwhile collecting the best candidates for baby spinach production from all major breeding programs and evaluating them for the purpose intended.

References

Johnson, C. F. (2000). Genetic and environmental influences on the nutritive value of spinach, *Spinacia oleracea*, for humans. Ph.D. dissertation, Cornell University, Ithaca, NY.

CHAPTER 5. MARKETING CONSIDERATIONS

INTRODUCTION

Spinach has undergone a large change in how it is offered to the consumer in recent years. The old bunched spinach, tattered, dirty, and gritty from rain splash in the field, is offered only for a short period during the year in the East, and sold in small volume, whereas several newer spinach products are typically available year round in sealed packages, triple-washed and ready-to-eat, in various grades of leaf sizes, and sales are at a very high volume. Spinach is also sold as part of mixed-salad packages, again ready to eat, and sometimes in combination with carrot gratings. A package size of 6 oz. is dominant, but pack sizes vary from 5 ounces to a pound. Occasionally packaged spinach is offered at discounted rates. For the most part the retail sale rate is between $5 and $7 per pound for the highest quality baby spinach, but can be as low as $ 3 per pound for larger coarse leaf spinach. In all cases except bunched spinach, almost all of the petiole is excluded from the product. Usually a small number of cotyledon leaves are included with baby spinach. Packaged spinach appears to be marketed exclusively by very large operatives such as Fresh Express, who have the means to secure shelf space in supermarket chains, or else by the chain itself (e.g., Wegmans, a large northeastern chain, markets its own line of spinach).

The quality of fresh packaged spinach is by no means always good. Blackened partially rotted leaves can sometimes be found, with the rot starting where leaves have been broken, folded, or crushed during handling. Sometimes the ends of stems are ragged and brown. Sometimes the material looks old and limp, and has probably exceeded ideal shelf life. The highest grade of baby spinach is not available at all times of the year.

Apart from monitoring what is offered in a number of large supermarket chains, at what prices, most of our information and insight concerning spinach marketing has come from consultation with an expert in this area, David Schwartz.

EXPERT MEETINGS

We scheduled two formal meetings with David Schwartz, one in Ithaca, and one in Buffalo, at which the principals in the project attended. David Schwartz is a fresh produce market consultant who handles spinach from the west coast by the freight car load for some of his clients. At these meetings, we went over how the field crop is harvested, sold, transported, processed and packaged and retailed. On both occasions, Mr. Schwartz indicated that the best price obtainable by the California farmer for top quality baby spinach in bulk is on average $1.00/lb FOB. This price has not changed over the course of the project. About 30 cents/lb is added in bringing the product to the East coast to washing and packaging facilities. There are

only a very limited number of washing and packaging facilities, on the East coast. Evidently, the capital cost of the specialized washing equipment is extremely high.

The possible advantages of value-added features such as HACCP approval, pesticide-free labeling, organic certification, enhanced calcium availability, etc. were also discussed in these meetings. One of the difficulties of introducing a new or distinctively different value-improved product is the expense of launching it in diverse locations; national advertising campaigns are extremely expensive. Another difficulty is the reluctance of produce managers to have one product by implication show up another in a negative light. However, the growing tendency for stores to designate special areas for healthy food products such as organic produce is a positive sign, and could resolve this difficulty.

The major difficulty faced by hydroponic spinach producers attempting to enter the commodity market for spinach is that their cost of production (measured as dollars per pound of product) is considerably higher than the wholesale price paid to the field producers for the same product (see following section on production costs), and no other marketing system is in place. One suggestion David Schwartz had to address this problem was to aim towards a new product which already has some currency, namely a microwaveable spinach pouch, in which somewhat larger leaves can be included than in the salad product, and thus permit the greenhouse grower to capitalize on the extremely fast growth that is occurring at time of harvest in the baby leaf crop. However, we have not noticed the microwaveable pouch catching on as yet in the local chains. Another suggestion was to focus on selling the hydroponic product directly to institutional and commercial consumers, such as hospital and restaurants, and selling it in bulk packs. We regard this as a most feasible suggestion.

MARKET ACCEPTANCE OF THE HYDROPONIC SPINACH PRODUCT

Apart from some larger crop stands grown for testing the harvest machine, the quantities grown during the project were too small to offer to commercial outlets. It was considered a poor risk to attempt large-scale production of baby spinach for test marketing, given the unresolved status of disease control in the root zone. However, in several experiments, excesses of fresh product were available for distribution after data had been taken, and were given to a wide selection of consumers whose evaluations were sought. In the view of these consumers, the hydroponic product in every way matched or exceeded the commercial store product in desirability, and it was in high demand.

Because it requires no washing or processing, the hydroponic product tends to be in perfect condition, and because of the environmental conditions under which it is grown, also very tender. On those occasions where whole young plants, including leaf petioles, were given away for people to cook, it was found the cooked product was particularly valued for its tender texture. The cooked product usually available to the consumer is made from chopped frozen spinach, which is not nearly as desirable.

MARKET OPPORTUNITIES FOR HYDROPONICALLY PRODUCED SPINACH ON THE EAST COAST

As will be shown in the next chapter, as things stand the cost of production for hydroponically grown spinach ready to leave the greenhouse facility appears to be $2.50 to $3.00 per pound, which is two to three times that of the field-grown product ready to leave the farm in California. However, this cost comparison is deceptive. The field product from California must be transported cross-country to washing and packaging facilities serving Eastern markets. By the time the field product is transported, washed and packaged, it has more than doubled in wholesale price according to Mr. Schwartz. The hydroponic product on the other hand, does not require washing or processing and may be packaged ready for retailing as part of the harvest operation. Suitably placed, the growing facility will be close to eventual market outlets, and thus the long-distance transportation cost is avoided and freshness is enhanced. Thus, because transportation and processing costs are avoided in the greenhouse product, the wholesale price of the finished package ends up very similar to that for the field crop. However, marketing and distribution practices suited to the greenhouse product are not yet part of the current marketing system and will have to gain acceptance.

The hydroponic product has several advantages over the field crop that may make it especially attractive to certain consumers. Foremost of these are that the quality and supply are potentially much more consistent. As noted above, the supply of top quality baby spinach is somewhat erratic, as is to be expected given the vagaries of weather and season and multiple sourcing of supply that are features of the field crop. The quality is also variable; the washing operation is dangerous to the product because it requires rough handling; whenever the leaves are broken or crushed rot can quickly set in. Should the drying operation not be perfectly executed, again, rot will quickly set in. For some clients certainty of supply and guarantee of quality may be extremely valuable features, for which they will pay a premium. One thinks of restaurant food chains.

The hydroponic product on the East coast will tend to be at least two or three days fresher than the field product, if the latter is shipped from the west coast or Mexico for processing in the East. It will also tend to be more tender than the field product especially in grades including older and large sized leaves. Thus for customers on the East coast, the hydroponic product will become associated with tenderness and freshness which will enhance its desirability.

The hydroponic production process is easily manipulated to incorporate added-value features in the product. In particular, we think HACCP approval and enhanced calcium availability in the spinach may be worth pursuing. The nature of the highly automated production protocol for greenhouse spinach lends itself to easily obtaining HACCP approval, which is virtually impossible to obtain for the outdoor crop, and

we have a patent pending for a technique for enhancing calcium availability, a simple process that adds little cost to production. In both cases, customers need to be educated to the benefits of these added values to be willing to accept a larger price. At very least these added features might make the difference in which product is chosen if a choice is offered.

In Japan, hydroponic production of spinach is a substantial industry. There the whole plant is sold and consumed, usually cooked. In our production of baby spinach leaves, we discard half of the shoot, which then incurs a cost in disposal. Anyone who likes cooked spinach loves cooked hydroponic spinach because it is so tender and tasty, and there is no question about discarding the leaf stalks and cotyledons because they too are delicious. Many people do love cooked spinach but have no attractive alternative for obtaining it. To cook expensive packaged baby spinach is an extravagance, and the frozen product is not very appealing. Bunched spinach is a nightmare in preparation because of the grit it contains. The cost of production of whole-plant hydroponic spinach is literally half that of baby leaf spinach, the only difference being where the cut is made at harvest. Since the crop can be allowed to grow a little longer at a time when growth is at a maximum rate, cost of production may be further reduced. If it could be retailed as a cooking product, it would fill a market need and could be offered at a very attractive price, which would aid in consumer recognition.

SUMMARY AND CONCLUSIONS

Spinach grown in the CEA production system does not need to enter into the current marketing sequence for spinach because it does not require washing or long-distance transportation, and can readily be packaged for sale at time of harvest in the production facility. As such, it competes well in price with field-grown spinach after washing and packaging, and could potentially compete with the field product in the supermarket if grown in sufficient quantity and backed by a big enough name. Since access to shelf space in supermarkets is expensive and difficult to obtain, for the immediate future the best marketing opportunity would seem to be for the grower to deal as directly as possible with institutions and commercial consumers other than retail outlets.

The current supply of spinach leaves a lot to be desired. CEA spinach potentially offers clients with the special advantages of consistent high quality and steady availability. With little difficulty, added value features can easily be incorporated such as HACCP approval, and enhanced calcium availability, should it be profitable to do so. If consumer habits could be changed so that the whole plant was consumed instead of just small leaves, production cost would automatically be more than halved, and much better values could be offered the consumer.

CHAPTER 6. PRODUCTION COST ANALYSIS

INTRODUCTION

Most of the effort in developing a production system for baby spinach went into determining how best to grow the crop, how to get the highest productivity possible with feasible and reasonable levels of inputs. Key questions were: what is the optimal plant spacing, temperature, and photoperiod for growing a baby spinach crop, and when must it be harvested? Can night-rate electricity be used in supplementary lighting? It was determined that when growing methods were optimized, the baby spinach crop closely matched the productivity achievable in lettuce, and light use efficiency in achieving this productivity also was very similar between the two crops. Thus the plan to analyze production costs for spinach in terms of production cost analyses for lettuce that have already been done, appears to be well founded. With some modifications in growing technique, the same sized facility can be adapted to produce the same fresh biomass of either crop.

HISTORICAL PRODUCTION COST ANALYSES FOR A LETTUCE MODULE

Two major production-cost analyses of commercial lettuce production have been conducted recently, making use of data produced in a demonstration lettuce greenhouse that has been operating near Cornell University over the past five years. The first of these is incorporated in the dissertation of Gunes Ilaslan (Ilaslan, 2000), and a subsequent paper (Ilaslan et al, 2001). It also presents a model for cost of greenhouse lettuce production using the CEA system in other locations in the USA widely differing in climate and land, labor, and energy cost. The second analysis was produced in 2002 in independent studies by Business School students at Cornell University (Hittle et al, 2002). It reviews the Ilaslan analysis and updates cost estimates in light of some modifications in production practices, and a lengthier track record of costs. A detailed energy audit of the lettuce module was also made covering the years 1999-2002 (DLTech Inc, 2003), which is employed here for yet further refinements in the production cost estimate. The method used in both the production cost analyses was to determine the cost of producing the product that is to be sold in terms of labor and inputs and overhead, exclusive of the original facility cost, and then compare this figure to revenue expected from sales. Revenue needs to be a certain percentage greater than production cost to provide sufficient profit to motivate investment and to pay rental or mortgage on the capital investment in the facility. One justification for separating out cost of the facility from cost of production is that in practice the initial facility cost is likely to be highly variable. The greenhouse may be purchased second hand at a fraction of original cost. Existing greenhouse structures may well be converted to hydroponic production from alternate uses such as, for instance, flower production; a double-wall poly structure may be used rather than a glass house; varying amounts of requisite equipment may already be available, etc.

The results of the two production cost analyses are presented in Table 6-1 below for the Ithaca case; fortuitously, the final figures were much the same despite some considerable differences in the detailed breakdowns. A "best current estimate" for the Ithaca module is also included, taking advantage of the recent energy audit of the facility.

Production Cost Estimates for Lettuce in the Ithaca Module (Dollars month)	**Study 1 Ilaslan** 2000	**Study 2 Hittle** 2002	**Energy Audit 2001-data** 2003	**Best Current Estimate** 2004
Labor				
Allowance for vacations and holidays				428
Part time employees	xxxxxxxx	960		2969
Full-time grower/s	4451	2083		4451
Manager	1250	xxxxxxxx		1549
Subtotal	**5701**	**3043**		**9397**
Direct Materials (Variable with level of production)				
Medium (rockwool/peatlite)	1688	1877		1877
Seeds	536	552		552
Packaging (boxes and bags)	2730	2943		2943
Nutrients (fertilizer)	219	250		250
Oxygen	165	65		65
Subtotal	**5338**	**5687**		**5687**
Utilites and Other Overhead				
Electricity	3927	5449	4167	3095
Gas	1399	2627	2500	2500
Phone/office supplies	200	200		200
Other supplies/misc.	168	200		200
System Maintenance	447	500		500
Water and sewer	105	200		200
Trash	xxxxxxxx	300		300
Nutrient analyses	xxxxxxxx	100		100
IPM	xxxxxxxx	100		100
Lamp replacement	xxxxxxxx	433		30
Snow ploughing	xxxxxxxx	83		83
Freight (local shipping)	1200	xxxxxxxx		
Subtotal	**7446**	**10192**		**7308**
Overhead				
Insurance	456	456		456
Property Tax	752	752		752
Rent/ mortgage	xxxxxxxx	xxxxxxxx		xxxxxxxx
Subtotal	**1208**	**1208**		**1208**
Grand Total	**19693**	**20130**		**23600**

Table 6-1. Compilation of Historical Production Cost Estimates for Lettuce

Figures given are monthly averages in dollars, assuming 12 equal months.

Between the time the two analyses were done, the lettuce plant spacing was changed to increase productivity by approximately 25%. Direct costs were somewhat increased as a result. This change is reflected in the second analysis. Subsequent to the second analysis, use of the seedling growth room was discontinued, and seedling production was transferred to a small separate greenhouse. Savings were achieved both in energy use and in specialized lamp costs, since the growth room incurred a considerable expense in energy, comprising 35% of total greenhouse electric energy use, and the water-jacketed lamps needed frequent replacement.

The energy audit showed that the first study underestimated electricity and gas use, and the second study overestimated it, because it relied heavily on data from an atypical year, and improvements in lighting control were subsequently incorporated.

The second study omitted a large component of labor by excluding the salary of the manager. Fringe benefits also appear to have been omitted for employees other than the manager. Both studies seem to neglect the fact of weekends, vacations, and public holidays. Thus a large part of the hours of labor needed are missing.

The first study included a large local shipping cost, which subsequently ceased to be applicable, but this made up for several omissions of costs such as trash removal, snow plowing, nutrient testing etc. In the current "best estimate", all these cost issues are addressed. The estimate is $23,600 per calendar month for maintaining full production of lettuce in the Ithaca facility including in-house seedling production. Three full-time workers are made available for every day of the year, with appropriate fringe benefits, in this estimate, and a third of the salary of a manager is included. Labor costs account for $9,400 of the operating cost.

We believe the monthly figure is realistic for the Ithaca area, but unfortunately, the Ithaca area is unrealistic for modeling production cost in general, if a grower has some discretion about where to locate a facility, because almost everything is unusually expensive in the Ithaca area, from labor to energy costs to property taxes. The average cost of electricity at the Ithaca module is $0.10 kwh^{-1} (Dltech Inc. 2003), whereas there are numerous municipalities and industrial park locations in NY where the cost is lower than $.05 kwh^{-1}. Labor costs are higher than in any of the cities Ilaslan surveyed. Property taxes are more than twice as high as the median for the ten cities surveyed. In production cost analysis for spinach, first we made an estimate of what it would cost to produce spinach in the Ithaca area facility, then we made adjustments to represent more favorably located facilities within the northeast, in particular where the cost of electricity and property taxes are more reasonable.

COMPARISON OF SPINACH AND LETTUCE OF PRODUCTION SYSTEMS

In using lettuce production cost estimates for baby spinach production, one must consider in what ways spinach production differs from lettuce production, and where costs differ. For the purpose of these calculations we imagine how it would be if the whole lettuce module were turned over to spinach production. (It is much more likely that that just one pond will be used for trial purposes in the first simulation of commercial practice for spinach in this facility, but calculations are easier for the whole module.) The expected crop cycle for baby spinach from seeding to harvest is 16 days. If seeding and harvesting are on a daily basis, 184 Speedling-type polystyrene flats (a.k.a. floats) of area 0.2308 m^2 and containing ~300 cells need to be seeded and harvested every day to keep the pond area available at full production capacity. (Purchase cost of 3120 Speedling flats, delivered to Ithaca, with tax is $11000.) Considering the production protocol chronologically, baby spinach will be seeded to final density, and a 48-hour period of germination in a warm place is then required before flats are entered into the floating hydroponics pond system (compared to 11 to 12 days in a germination area for lettuce). Flat filling and seeding will be done using a flat filler and vacuum seeder set up in assembly line fashion. The seeding and germination will be accomplished in the head house of the greenhouse, and germination will take place with flats stacked in cabinets held at 26°C temperature, in the dark. When it is time to place the polystyrene flats in the ponds, labor is required only to transport and float the flats since there is no transplant operation. No re-spacing step half way through the crop cycle will be required for spinach, as is the case for lettuce. We are estimating it will take two people 3 hours each day to take care of the seeding and floating operations for 184 flats.

In large production facilities, cutting and packaging of the spinach with be fully automated, with the work requirement being that of feeding a harvester machine and monitoring its functions. The cost of the equipment will be part of capital costs. We expect emptying and cleaning the flats will also be partially automated. We expect it will take two people three hours each to manage the harvest each day from pulling flats out of the pond to placing packaged spinach in storage and stacking empty flats. In small production facilities, harvest of the crop will entail placing the flats on easel-like stands, steeply angled, and using an electric knife to cut away the desired part of the shoot. Leaves will fall into a collection trough after which they will be packaged for final sale.

After harvest, floats will either be returned to the ponds for re-growth and a second harvest if that is the strategy in use, or cleaned for immediate re-seeding. The area of spinach to be harvested each day and number of ultimate sales units if 7 oz. packages are used is very similar to the area of lettuce harvested and number of heads sold. Rather more lettuce heads must be processed than bags of spinach since lettuce heads weigh less than typical spinach packages. If the harvest is manual, a considerable amount of extra labor will be required.

Several other features of baby spinach production are different than lettuce. Since the crop cycle is only 16 days and seeding is to final density, roughly 15 times more flats must be planted every day than in the lettuce case. For this reason it becomes essential to automate the procedure as much as possible. The cost of this equipment adds to the overall capital cost, as does the harvest equipment. The planting density for spinach will need to be approximately 1500 plant m^{-2}, nearly 40 times denser than for lettuce at final spacing. Considerably more seed will be required than for lettuce as a consequence; however, unless specially treated seed is used, spinach seed is relatively cheap. Unlike lettuce, spinach roots are prone to attack by a water-borne pathogen, Pythium; ponds require a form of continuous treatment to keep levels of Pythium zoospores low. The treatment will incur some initial expense and continuing maintenance costs and energy use. On the positive side in terms of energy use, spinach is not prone to tip burn; use of shade-cloth to avoid tip burn will not be required, and a greater proportion of available natural light can be safely used than with lettuce. Cost of light supplementation in transition seasons and the summer time will be reduced.

SOURCES OF SAVINGS AND EXTRA EXPENSES IN SPINACH PRODUCTION

A development is on the near horizon that will lead to considerable savings and improvement in baby spinach production; it is use of a seedling plate that eliminates the need for particulate mediums such as rockwool and peatlite, saving not only expense in materials, but also keeping the crop cleaner. When this plate is made operational, \$900 $month^{-1}$ will be saved on media cost for seeding spinach in peatlite. However, we estimate seed costs will double because de-hulled seed is a requirement of the plates. Net savings will be approximately \$350 per month.

Transplant and re-spacing operations comprise approximately half of the daily labor time of the module staff. Eliminating these steps coupled with automation of seeding and harvesting is the equivalent of eliminating one full time staff person, or saving \$2226 a month. Labor time required in seeding and harvest of spinach will be dependent on degree of automation, which in turn is governed by the size of the facility. If harvest is largely manual one suspects no time savings will be possible in the harvest work; if seeding or flat filling is also largely manual, additional time will be required at this stage, offsetting time saved in transplant and re-spacing operations. In figuring labor costs, we consider one case where there is little automation, in which case we use the lettuce figure for labor, and another in which 1 full time skilled worker less is required.

In spinach production there is a two-day germination period but no seedling production phase, so <u>all</u> the energy spent in the growth room can be counted as saved. Thus 35% of the total energy cost may be removed from the monthly electricity bill presented in the energy audit for lettuce since this is what the seedling growth chamber cost. An additional 5% is removed to reflect savings through less use of the shade

cloth and more use of natural light. In considering costs in a more favorable location than Ithaca, we can realistically expect to cut electricity costs in half simply through the reduction in kilowatt-hour rate.

	Cost of Production in Ithaca area module					Costs in a Commercial Park		
	Lettuce		Estimates for Spinach			Estimates for Spinach		
Costs are given in Dollars/month	Best Current Estimate 2004		Little Change from lettuce	Full Automation Lowered Labor	Media not reqd. plus Low Labor	Little Change from lettuce	Full Automation Lowered Labor	Media not reqd. plus Low Labor
Labor								
Allowance for vacations a	428		428	325	325	428	325	325
Part time employees	2969		2969	1188	1188	2969	1188	1188
Full-time grower/s	4451		4451	4451	4451	4451	4451	4451
Manager	1549		1549	1549	1549	1549	1549	1549
Subtotal	**9397**		**9397**	**7188**	**7188**	**9397**	**7187.5268**	**7187.5268**
Direct Materials (Variable with level of production)								
Medium (rockwool/peatli	1877		890	890	0	890	890	0
Seeds	552		536	536	1072	536	536	1072
Packaging (boxes and ba	2943		2943	2943	2943	2943	2943	2943
Nutrients (fertilizer)	250		250	250	250	250	250	250
Oxygen	65		65	65	65	65	65	65
Subtotal	**5687**		**4684**	**4684**	**4330**	**4684**	**4684**	**4330**
Utilites and Other Overhead								
Electricity	3095		2500	2500	2500	1250	1250	1250
Gas	2500		2500	2500	2500	2000	2000	2000
Phone/office supplies	200		200	200	200	200	200	200
Other supplies/misc.	200		200	200	200	200	200	200
System Maintenance	500		500	500	500	500	500	500
Water and sewer	200		200	200	200	170	170	170
Trash	300		300	300	300	300	300	300
Nutrient analyses	100		100	100	100	100	100	100
IPM	100		200	200	200	200	200	200
Lamp replacement	30		30	30	30	30	30	30
Snow ploughing	83		83	83	83	83	83	83
Freight (local shipping)								
Subtotal	**7308**		**6813**	**6813**	**6813**	**5033**	**5033**	**5033**
Overhead								
Insurance	456		456	456	456	438	438	438
Property Tax	752		752	752	752	316	316	316
Rent/ mortgage	xxxxxxxx		xxxxxxxx	xxxxxxxx	xxxxxxxx	xxxxxxxx	xxxxxxxx	xxxxxxxx
Subtotal	**1208**		**1208**	**1208**	**1208**	**754**	**754**	**754**
Grand Total	**23600**		**22102**	**19893**	**19539**	**19868**	**17659**	**17305**
Production (lb) @ 330 g m^{-2} d^{-1}			13124	13124	13124	13124	13124	13124
Unit cost Whole shoot ($ / lb)			**1.68**	**1.52**	**1.49**	**1.51**	**1.35**	**1.32**
Unit cost Baby spinach($ / lb)			**3.37**	**3.03**	**2.98**	**3.03**	**2.69**	**2.64**
Production (lb) @ 400 g m^{-2} d^{-1}			15908	15908	15908	15908	15908	15908
Unit cost Whole shoot ($ / lb)			**1.39**	**1.25**	**1.23**	**1.25**	**1.11**	**1.09**

Table 6-2. Production Cost Estimates for Spinach Production in Different Locations
Figures given are monthly averages in dollars, assuming 12 equal months.

The nutrient solution treatment process, once installed, will require routine replacement of UV bulbs, for which \$ 100 month $^{-1}$ has been added to all spinach cost estimates.
Cost of production of spinach under the different conditions discussed is represented in Table 6-2 above. If all the savings and extra costs discussed are implemented, the average monthly cost of spinach production under full capacity of the Ithaca module is \$19539, a 17% reduction over that for lettuce currently, which is \$23,600. This figure assumes automation; however, the added capital cost of seeding and harvesting equipment is not reflected in this figure, and needs to be borne in mind.

It is not a sure thing the new seed tray/plate will work out as anticipated. If it does, it will also eliminate need for flat-filling equipment, and thus provide a double benefit. An estimate of cost of production without benefit of the plate but with the benefit of automation is provided. It is \$19893 a month, a 15% reduction over that for lettuce. If automation is minimal, no overall labor savings are expected, just a small energy savings, and cost of production will be very much like that of lettuce at \$22,102 a month.

Projecting a location in NY where utility costs and property taxes are lower than in the Ithaca area results in a substantial reduction in cost to \$17,305 a month. It is likely there are locations where the climate is better, in addition, so energy demands are reduced in addition to unit price for energy, such that the utility bills are reduced substantially more than this. Some locations will have slightly cheaper labor, but little lowering of monthly cost is expected in terms of labor.

COST OF PRODUCTION OF SPINACH ON A UNIT BASIS

The area of the greenhouse is 8000 sq. ft, of which 6384 sq. ft is available as water surface area for growing spinach in ponds. Experimentally determined productivity for whole shoot of spinach during the time the crop is in the greenhouse (14 days after two days germination in the headhouse) is assumed for present purposes to be 330g m^{-2} d^{-1}, a middle-of-the-road figure derived experimentally for the cultivar Eagle. (See Tables 3-38 and 3-44 in Chapter 3 on Crop Production Systems.) This is the equivalent of 0.06759 lb $sq.ft^{-1}$ d^{-1}, and will theoretically yield 431 lb d^{-1} for the whole pond area, or 13,124 lb $month^{-1}$. (In a 242-cell flat, average yield per cell after 14 days would be c. 4.4g, which is reasonable.) Light use efficiency matches that of lettuce. At this level of productivity, the unit production cost for matching the highest quality of what is currently sold as baby leaf spinach can be expected to fall in the 2.98 to 3.37 \$/lb range in the Ithaca area, and 2.64 to 3.03 \$/lb in more favorably located modules, with the range depending on how much automation is employed.

However, *only approximately half of the whole shoot can be sold as baby leaf spinach if current marketing practices are followed*, the rest being comprised of stems, cotyledons and petiole pieces, which must be discarded. Should it be possible to sell the whole plant, the production cost would automatically be halved to 1.32 to 1.56 \$/lb. If a larger plant could be sold, say for cooking purposes, productivity might

reasonably be increased to 400g m^{-2} d^{-1}, as was recorded in the day-16 harvest of Alrite in the same experiment in which the figure of 330 g m^{-2} d^{-1} was produced for Eagle. In that case, unit production cost would fall to 1.09 to 1.25 $/lb.

CONCLUSIONS

We have tried to be realistic in this cost of production estimate. For this reason, we have substantially increased the labor requirements over earlier estimates. However, it must be remembered the cost of production estimate as formulated here does not take into account the capital cost of the facility or the cost of paying rent or mortgage on the facility, which could be quite large monthly costs depending on how it was acquired, and would need to be included in the sale price of the spinach. If, for instance, it was necessary to generate $8000/ month to pay a mortgage, this would require adding $0.60/lb to the price when the monthly volume sold is 13,000 lb. On the other hand, the estimate also does not take into account such factors as possible economies of scale, the possibility of CO_2 enrichment to save light energy, improved space use efficiency of the greenhouse, or other cost saving possibilities. We take satisfaction that the unit cost of production is in any case half of the typical retail price of the product. If a grower was able to direct market their product, they would be on a sound financial basis. Within this sea of variables, we are reasonably certain that the productivity figure of 330 g m^{-2} d^{-1} is solid and achievable on an ongoing basis; with proper cultivar selection and fine tuning of cultural technique it probably can be improved upon without diminishment of light use efficiency, which also impacts production cost.

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