EVALUATION OF A MINI-CONTAINER, ACCELERATED TRANSPLANT SYSTEM: THE BLACK SPRUCE SUMMER CROP

by

© Hector Eduardo Gonda

A Thesis submitted in partial
fulfillment of the requirements for the Degree
of Master of Science in Forestry

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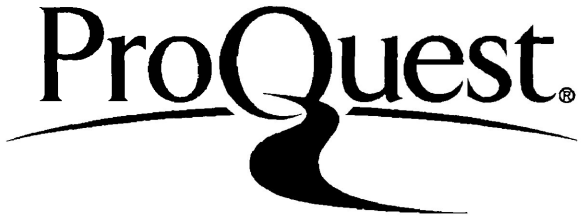

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ABSTRACT

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Key Words: bare root nursery stock, bud development, Castle and Cooke, container stock, Picea mariana, pregermination techniques.

The production of black spruce (Picea mariana (Mill.) B.S.P.) bare root stock in Ontario nurseries presents two main problems. First, seedlings at the end of the first growing season are small, and thus susceptible to frost heaving. Second, it takes a long time, 3 years, to produce shippable seedlings. Trying to solve these problems the Ministry of Natural Resources Thunder Bay Nursery is testing an accelerated transplant system. Seedlings are sown in a greenhouse in a mini-container and after 10 weeks are transplanted outdoors for two growing seasons. There is a winter and a summer crop from the greenhouse each year. The objective of this paper was to evaluate the effect of 3 factors on summer crop seedlings. The 3 factors were SOWING DATE (levels: July 5, 15, 25, and August 4), the duration of an initial 18-h LONG DAY treatment (levels: 7, 10, or 13 weeks), and the duration of a subsequent 8-h SHORT DAY treatment (levels 0, 6, or 12 days). Finally, all seedlings were held under natural photoperiod until the total length of the SHORT DAY and natural photoperiod treatments was 11 weeks. Bud initiation was monitored during this 11 week period. Bud diameter, number of primordia, basal caliper, and root dry weight were measured immediately prior to placing the seedlings in cold storage for the winter. LONG DAY was the most important factor. Seedlings that received the ten-week LONG DAY treatment gave the best response. Even though 13-week LONG DAY seedlings were significantly taller, 10-week specimens showed a similar bud diameter and basal caliper, as well as a significantly heavier root dry weight, and more primordia. Eventually, the containers were too small for 13-week LONG DAY seedlings that showed a potbound situation. Although there were some significant differences, the various levels of the factors SOWING DATE and SHORT DAY did not produce any considerable effect on the growing regime. At the end of the first growing season in nursery beds, seedlings from the best treatment combinations of the summer crop reached almost a shippable size. This confirms the feasibility of the studied system to produce bare root stock in two years or less.

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ACKNOWLEDGEMENTS

This section of my thesis is particularly meaningful to me because not many professionals in my country have the opportunity of studying overseas.

Upon completion of my bachelors Degree in Forestry at home in Argentina, in 1980, I wanted to undertake graduate studies in forestry in Canada. In 1983, my wish came true when I obtained a scholarship from the Rotary Foundation of Rotary International for all the expenses of the first year of the Masters Program at Lakehead University. I am deeply indebted to Rotary. Not only did the scholarship allow me to further my education, but it also opened the doors to a different culture, where I tried to be a goodwill ambassador of Argentina. I am eternally grateful to Dr. James Angus my Rotarian counselor in Canada. His conviction to the goals of the Rotary Foundation of promoting international understanding, led him to help me beyond the call of duty in all concerns, even when he did not know me.

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INTRODUCTION

In Ontario, the production of black spruce (Picea mariana (Mill.) B.S.P.) bare root stock presents two main problems. First, seedlings tend to be small with inadequately developed root systems at the end of the first growing season. As a result, seedlings are susceptible to frost heaving in the fall and following spring. The cause of this problem is the low seed efficiency determined by low germination rate occurring over a long time span, and a low initial vigor (Skeates and Williamson 1979). Second, it takes a long time, usually 3 years, to produce shippable seedlings (Wynia 1974).

One possible solution to these problems is being studied on an operational basis at the Ontario Ministry of Natural Resources nursery in Thunder Bay, Canada. The proposed solution is based on a pregermination technique that uses a mini-container with an elastic medium as a first stage for the production of black spruce bare root stock. It is hoped that the new method will solve the low seed efficiency problem and produce shippable bare root stock in only two years.

Many accelerated transplant techniques have been tried in different places, and their potential advantages are well known to most nurserymen. Thus, an immediate question

arises: what is special or new about this experiment? Are the containers any better; is the rubbery medium the key to possible success?

There is no doubt about the importance of the small size of the containers, since many seedlings per unit area can be held in the greenhouse. The elastic medium helps to obtain a maximum survival ratio by remaining attached to the roots following the transplanting time. However, the key factor for the possible success of this trial is the availability of complete mechanization. The Thunder Bay nursery already has a sowing machine for the Castle and Cooke containers and, more importantly, is developing a transplanting machine.

Current plans for the use of the accelerated transplant system at the Thunder Bay nursery calls for two greenhouse crops a year. The winter crop is sown in late winter. After approximately 10 weeks, seedlings are transplanted to outdoor beds where they will remain for two more growing seasons. The summer crop is sown in late June or July. After approximately 10 weeks of free growth the seedlings will be kept in the greenhouse for eight to 10 additional weeks to promote bud development and frost hardiness. This extended greenhouse culture treatment is used in Ontario for overwintered container stock (Colombo et al. 1984). The following spring the seedlings will be transplanted to outdoor beds where they will remain for two

growing seasons. In the case of both crops, seedlings will be handled as conventional bare root stock upon harvest.

In 1983, Mr. Robert Klapprat, Superintendent of the Thunder Bay nursery, informed me of the need to study the growing regime of the winter and summer crops in order to optimize the production. Consequently, I decided to initiate an experiment on the summer crop. Another graduate student, Mr. Wang Zhangming, elected to undertake a study of the initial stages of the winter crop. The objective of my research was to study the effects of three cultural factors on several attributes of summer crop seedlings at the end of the greenhouse period. The three factors were SOWING DATE, the length of an initial, 18-h, LONG DAY treatment, and the length of a subsequent, 8-h, SHORT DAY treatment.

LONG DAY was the most important factor. Seedlings that received 10 weeks of the LONG DAY treatment reached the largest size. The mini-containers were too small for seedlings from the 13-week LONG DAY treatment. Although significant for some response variables, the different levels of factors SOWING DAY and SHORT DAY did not have any important effects to be considered in the growing regime. These results, and others discussed below, have already been incorporated in what has become a major committment to the Castle and Cooke accelerated transplant production system at the Thunder Bay Nursery.

LITERATURE REVIEW

This chapter is organized in two parts. The first section reviews the literature on the use of accelerated transplant systems for black spruce bare root transplant production. In my experiment, seedlings from the greenhouse summer crop were stored for the winter in the containers. Thus the literature about how to promote frost hardiness and bud development on overwintered container stock is reviewed in the second section.

ACCELERATED TRANSPLANT SYSTEMS

Seed efficiency in the production of bare root stock from Ontario provincial nurseries is low (Table 1). Seed efficiency is defined as the proportion of trees shipped relative to viable seed sown (Skeates and Williamson 1979). Losses are due to many factors. Germination in seed beds is low, and it occurs over a long time span. The result is variable germination time.

Emergence is also a serious problem, especially for a small seeded species like black spruce, because of low initial vigor. The resultant small seedlings often have inadequately developed root systems and are therefore susceptible to frost heaving in the fall and following

TABLE 1 Seed efficiency in Ontario provincial nurseries expressed as percentage of bare root nursery stock shipped relative to viable seed sown, based on germination sand tests (from Skeates and Williamson 1979)

Species	Red pine		White pine			Jack pine		White spruce				Black spruce			
Age class	2+0	3+0	3+0	1+2	2+2	2+0	3+0	3+0	1+2	1½+1½	2+2	3+0	1+2	1½+1½	2+2
Southern Ontario															
Kemptville	25	27	24			42		23	28			22			
Midhurst		34			24	30		28							
Orono		25	28		21	31		28			25				
St. Williams		25	25			27		14		17					
Northern Ontario															
Dryden		27				42				21	18	13		14	13
Thunder Bay		38			25	45		16		20	20	9		12	
Swastika				28		50					30	29	27		
Chapleau			33				51								
Thessalon		43	42			37		28				29			

spring (Skeates and Williamson 1979). This is important since there is a strong relationship between early vigor and subsequent growth (Skeates 1982). Irregular survival results in uneven spacing in the bed, and this in turn makes it difficult to separate seedlings without root damage (Skeates and Williamson 1979).

In Ontario, provincial policy is to improve the quality of seed collected (Armson 1976). It is important, therefore, to develop stock production methodologies which optimize seed efficiency.

Another important problem is that it currently takes three years to produce shippable black spruce bare root stock. Thus, planning for the use of planting stock becomes a long term proposition (Wynia 1974).

One approach to the solution of the seed efficiency and long term investment problems is through pregermination techniques and accelerated transplants systems. The word pregermination is used in a broad sense to include a range of techniques from germinants with emergent radicles to cotyledonous seedlings and mini-container seedlings up to 8-10 weeks of age (Skeates 1982).

Hand planting of germinants has long been practiced in areas of the developing world where labor costs are low (Skeates and Williamson 1979). Hagner (personal

communication with Skeates) developed a system of germinating and holding seed until sowing in an effort to achieve initiated stocking of 100 percent in a thirty-five-million-tree, multipot, greenhouse operation in Sundsval, Sweden. Although their seed efficiency was 90 percent, the estimated loss from the 10 percent of empty containers was approximately $140,000 (Skeates and Williamson 1979).

Scientists in Britain have worked with ethylene glycol as a pregermination medium allowing initial imbibition of moisture to a state of germination readiness. Fluid drilling of pregerminated seed is now an operational technique marketed in Britain (Anon., undated).

Todd (1982) stated that the first "speedling" (accelerated transplant) system in North America was initiated in 1967 in the patented Todd Planter Flat. He also developed high-speed seeding equipment that had a vacuum-equipped planting drum to hold the seed during the planting process. Although the Todd system was initially limited to vegetable crops, it expanded to include ornamentals as well as tree seedlings.

An accelerated transplant system called plug + 1 is being used in the northwestern United States. The recently developed plug + 1 seedling begins life as a typical plug in a containerized nursery. At the end of its first year,

it is transplanted to a bareroot nursery, where it continues to grow for another year before outplanting. This new stock type, has been used mainly for Douglas-fir (Pseudotsuga menziessi (Mirb.) Franco). It has shown good survival (90% on moist sites and 70% on dry sites) and height growth on most sites (Hahn 1984).

In Ontario, the pregermination systems tested to date have been for the production of accelerated transplants (Skeates 1982). Smith (1982) reviewed the development of the accelerated transplant program in Ontario. The program began in Ontario in 1979 at the Swastika Nursery where black spruce seed was simply broadcast on prepared beds inside a greenhouse. This technique produced acceptable seedlings, and successfully cut one year from the conventional rotation period. The Orono and Swastika Nurseries are still using a modification of this system today for black and white spruce (Picea glauca (Moench) Voss), that utilizes trays in the greenhouse.

At Dryden Nursery, the "cigarette system" was initially developed for black spruce accelerated transplants. The "cigarettes" were made using an old cigarette-making machine that had been modified to make larger diameter containers which were filled with sphagnum moss (Smith 1982).

Other systems being tested for their potential with black spruce accelerated transplants include paper pots, the container stock system used at Swastika and Thessalon Nurseries, and Spencer-Lemaire booklets, the system favoured from Northwestern Ontario to Alberta (Smith 1982). Kemptville Nursery is using multipots for their accelerated transplants of cedar, tamarack, and hardwoods (Smith 1982).

Finally, a new concept for growing accelerated transplants is presently undergoing preliminary testing at nurseries in Thunder Bay, Swastika, Kemptville, and Maple, Ontario. These nurseries are using the so called mini-transplant or mini-container systems that dramatically increase greenhouse production. The mini-container system may be used as a pregermination technique system for another container. This possibility is being studied by D. Skeates, at Maple for black spruce because seed efficiency is lowest for this species.

Skeates et al. (1979) developed a technique whereby seed was pregerminated in sphagnum moss cigarette plugs. These were then planted into peat cubes in the greenhouse prior to transplanting in nursery beds. Two-year-old bareroot transplants from this system met OMNR 3+0 standards for height and root collar diameter, provided seed was sown prior to May 1. These results show that it is possible to grow shippable black spruce transplant stock in

two years in southern Ontario.

Hallet et al. (1983) presented the only published work on the use of Castle and Cooke containers with spruce seedlings. He sowed a few flats of black and white spruce and grew them for five to nine weeks in the greenhouse before being transplanted into the bareroot nursery to produce germinant transplants. He only recorded height and survival. Nine-week-old seedlings had a much higher survival.

An accelerated transplant program has several potential advantages over conventional transplant operations. The use of seed pregerminated under optimum conditions results in high seed efficiency rates. As a result, greenhouses can be operated at close to full capacity. Accelerated transplant stock also exhibits a high degree of uniformity at the time of transplanting (Skeates and Williamson (1979). Bunting (1973) stated that, with even spacing in the beds, plants develop at uniform rates. The result is that bed-run, shippable trees are produced without significant culling costs. The reduction in growing time, perhaps from three to two years with black spruce, should give the nursery staff more flexibility to meet District targets (Skeates and Williamson 1979, Smith 1982). Hallett et al.(1983) stated: "If a mini-container system could be used to produce small seedlings for transplanting in the nursery, the seedbed problems of poor

germination, weeds, overwinter frost heaving, and multiple leaders in the first year of growth could be things of the past!".

The main disadvantage of an accelerated transplant system is that a large initial capital investment is required to mechanize nursery operations. This economic disadvantage might be offset if in fact it is possible to produce higher quality planting stock (Smith 1982).

OVERWINTERED BLACK SPRUCE CONTAINER STOCK

Frost Hardiness

In Ontario, over 25 million container tree seedlings were produced in 1983. These account for approximately 25 percent of all nursery stock planted on crown lands (Colombo et al. 1983). Almost half of this stock is grown in greenhouses during the summer and overwintered outdoors for planting the following spring. It is during the winter that container seedlings are most likely to suffer damage. Overwinter losses of up to 50 percent have been observed in some black spruce crops since monitoring began in 1981. This problem has the potential to threaten the Province's ability to meet its regeneration targets (Colombo 1982).

Freezing is a principal cause of winter damage. Container seedlings are hardened outside under prevailing

weather conditions. Thus seedlings are susceptible to freezing damage until sufficient cold hardiness has developed.

Winter hardiness is a characteristic of temperate climate perennial species (D'Aoust 1981). Interest in cold hardiness is not new in Canada. Early work by Scarth (1936), Siminovitch and Briggs (1949) and more recently Glerum (1976) has indicated some fundamental changes in the plant during its annual cycle. The process of cold acclimatization that results in winter hardiness is the result of interactions between the plant genome and the environment. Winter hardiness is a broad term. It has been defined by D'Aoust and Cameron (1982) as the capacity to avoid or tolerate the stresses imposed by winter conditions (low temperature, dry air, frozen ground, frost heaving, sunscald, etc.). The cold hardiness component is of major importance, and is defined by D'Aoust and Cameron (1982) as: "the ability to withstand freezing temperatures".

The Induction of Cold Hardiness

The literature indicates that a number of factors influence the induction of cold hardiness in northern conifers. Cessation of rapid vegetative growth appears to be a prerequisite event (van den Driessche 1970, Weiser 1970, Levitt 1972, Aronson 1975, Sandvick 1976,

Christersson 1978). A minimum light intensity, provided in a short-day regime, is essential (van den Driessche 1970, Timmis and Worral 1975, Sandvick 1976). Cold temperature in some cases can replace the short day requirement (van den Driessche 1970, Sandvick 1976, Christersson 1978). Light frost can also increase the degree of cold hardening (Weiser 1970, Levitt 1972, Timmis and Worral 1975). Finally, moisture and nutrient regimes have occasionally been shown to influence the hardening off process (Levitt 1972, Christersson 1973, Tanaka and Timmis 1974, Timmis 1974).

Species differ in their response to cold acclimatization factors. Norway spruce (Picea abies [L.] Karst) is less sensitive than Scots pine (Pinus sylvestris L.) to lowering of temperature during hardening off (Aronson 1975). In the case of some conifers, the photoperiodic control seems to be a less dominant factor than the amount of light (Mc Guire and Flint 1962).

In the case of black spruce, a reduction of photoperiod with low temperatures at the end of the production period is the best acclimatization treatment, but short days or low temperatures alone can also stimulate cold hardening (D'Aoust and Cameron 1982). As with other species, neither moisture stress nor nitrogen deprivation at the end of the production period appear to affect the cold hardening process in black spruce (Tanaka and Timmis

1974, Timmis 1974, D'Aoust and Cameron 1982).

Bud Development

The process of hardening greenhouse-reared conifer seedlings is also a period in which the seedlings assemble leaf primordia for the next growing season (Pollard et al. 1977). Van den Berg and Lamer (1971) have emphasized that the morphogenetic phase of bud development is probably the most important factor controlling shoot growth in northern conifers.

Northern conifer species display a mode of shoot growth in which an annual complement of new foliage is preformed. That is, all leaf primordia are formed in the bud in the summer prior to bud flush. This pattern may be modified through precocious expansion of new buds (lammas growth and prolepsis), or through neoformed growth (Pollard and Logan 1974). In most northern species, however, almost all foliage is preformed.

Since each primordium represents a unit of stem tissue (Doak 1935), the extension growth of branches and the height growth of the tree are similarly predetermined. Cannel et al.(1976) presented evidence for the importance of internode number rather than internode dimension in the establishment of shoot length of lodgepole pine (Pinus

contorta Dougl.) and Sitka spruce (Picea sitchensis (Bong.) Carr.). Experiments reported by Duff and Nolan (1953), Kozlowski (1971) and Pollard and Logan (1977) show that environmental factors, especially those associated with weather, show their greatest influence on growth during the subsequent year rather than in the current year because they influence the morphogenetic stage of shoot growth, i.e., bud development.

Pollard and Logan (1977) investigated the effect of some environmental factors on bud morphogenesis of black spruce. Treatment effects were assessed by means of the number of needle primordia accumulated within the terminal buds of seedlings by the end of each of a series of controlled experiments. Of the environmental factors examined, temperature had the greatest effect on morphogenesis. Temperature affects all growth processes, of course, including net photosynthesis. Optimum temperature for photosynthesis in seedlings of black spruce is about 19° C at 22000 lx (Logan, unpublished, cited by Pollard and Logan, 1976) In contrast, the optimum temperature for needle initiation appears to be 25° C or above, and initiation was much slower at 20° and 15° C. A temperature of 25° C has been reported as optimum for dry matter production in both black and white spruce seedlings (Pollard and Logan 1976).

Concerning the other factors studied by Pollard and Logan (1977), light intensities between 3350 and 22000 lx had little effect on bud initiation. There was no response to photoperiod between 6 and 15 h., although bud initiation was markedly reduced at less than 6 h. Responses to soil moisture potential were also weak (Pollard and Logan 1977).

The experiments of Pollard and Logan have several implications for reforestation programs. Gains in establishment success of seedlings after the winter might require more than simply inducing dormancy and acclimatizing plants to outdoor conditions. For example, optimizing the environmental requirements for bud morphogenesis might also result in an increase establishment success, whereas if low temperatures persist throughout the fall hardening period, the shoot growth potential of the seedlings might be limited. Favorable conditions of warm temperatures for bud morphogenesis, need only be maintained for five weeks in the case of white spruce. This specification could be adapted to suit other species once their pattern of morphogenesis is established (Pollard and Logan 1977).

In 1982 Colombo published his first papers on bud morphogenesis of black spruce. His work is important because it represents a major step towards solving a main problem in container stock production in Ontario, namely

freezing damage. Colombo found that the damage occurred when seedlings were moved outside in the fall before sufficient cold hardiness had been attained in the greenhouse. The high incidence of overwinter damage was attributed to failure of the hardening regime (Colombo et al. 1982b). The regime relied upon a two to four week period of reduced nitrogen fertilization accompanied by lowered temperatures achieved by ventilating the greenhouses (Carlson 1983, Tinus and McDonald 1979).

Colombo et al. (1982b) found that cold hardiness in first-year seedlings of black and white spruce was increased by exposure to 8 h at 20° C . Shoot elongation ceased and bud development began after exposure to short days. After five weeks of short days a temperature of -9° C did not cause damage in either species. After eight weeks of short days bud development in both species was virtually complete. Seedlings of both species exposed to eight weeks of short days in growth cabinets produced large shoot tips with an average of 209 needle primordia for black spruce and 182 for white spruce. In comparison only 95 primordia formed in buds of production-run black spruce container seedlings (Colombo et al. 1982b).

Container seedlings with increased bud development possess a significant advantage in field performance, especially where competition is a problem. There is, however, another advantage to be gained from the use of

seedlings that have been properly preconditioned for overwintering. Not only can bud development be tremendously improved, but during the time bud development is taking place there is increased root activity, so that the shoot/root ratio is more favorable. Greater height growth following outplanting will be accompanied by greater root growth resulting in a well-balanced seedling with improved chances for survival (Colombo 1982).

Once the bud morphogenesis of black spruce was understood, modifications were made to the production schedule in order to provide short day length and warm temperature in the greenhouse. This combination favours both cold hardening and bud development while preventing exposure to potentially damaging temperatures. Two modifications were tried (Colombo 1982). In the first, seedlings were given an artificial, 8-h, short-day treatment beginning at the end of July when greenhouse temperatures were still warm. Seedlings were moved outside with the regular production run stock on August 14th, at which time, the short day treatment was also stopped. In the second modification, a group of seedlings was given a period of extended culture inside a heated greenhouse until October 28th, allowing the naturally declining day lengths to induce budset and cold hardiness (Colombo 1982). It was found that cold hardiness was low throughout August in all stock types. But in the first two weeks of September there was a dramatic increase in the cold hardiness of 8-h day

seedlings. There were comparable increases in cold hardiness in the production run and extended greenhouse seedlings, but these increases were not evident until two weeks later, on September 29th (Colombo 1982). Extended greenhouse stock produced needle primordia over a longer period of time. In comparison, production run seedlings produced few needle primordia because of the shorter time and lower temperature available for bud growth that by November 11th there were a total of only 68 needle primordia per seedling. In 8-h day treated seedlings there were a total of 140 primordia, while extended greenhouse seedlings produced 270 primordia (Colombo 1982, Colombo et al. 1983).

The two methods of inducing cold hardiness in black spruce have their own advantages and disadvantages. The short day treatment provides greater flexibility in production schedules than does extended greenhouse culture (Colombo 1982). By using a short-day treatment, bud development may be induced at any time of the year when seedlings have reached the size requirements specified by field staff. However, establishing a production system using an artificial short-day treatment requires capital expenditures to install lighting for day length extension in the spring to allow early seeding, and to install a system of shades for shortening the day length in the fall (Colombo 1982).

The use of extended greenhouse culture to induce cold hardiness in black spruce may be an attractive option in many cases as it requires no additional equipment, with the possible exception of heaters, although an unheated greenhouse could be used (Colombo 1982). Extended greenhouse culture may be a much more practical technique to use. In 1982-83 trials were performed in different provincial tree nurseries in Ontario to determine the effects of varying the bud development time. Accordingly, seedlings were moved outside at regular intervals from late July through to late October/early November (Colombo 1982). The results showed that the provincial average for bud development in black spruce container stock grown in 1982 and overwintered until the spring of 1983 was 148 needle primordia. That was more than twice the 68 needle primordia obtained from production-run seedlings (Colombo and Odlum 1984, Colombo 1982, Colombo et al. 1983). The extended greenhouse culture has not been tested long enough to date. However, overwinter damage on container stock is so severe that the OMNR has decided to adopt this technique since much better survival and quality already have been achieved in northern Ontario.

Colombo et al. (1984) recommended the following hardening regime for operational use in Ontario for fall hardening of spruce container stock:

1) Stop fertilization and leach the growing medium when seedlings are within 3 cm of the desired height.

2) Water without fertilizer and maintain optimum growing temperatures (20-30° C) until bud initiation reaches 100 percent.

3) Following 100 percent bud initiation, resume fertilization at normal rates for optimum growing temperatures until bud development (as shown by number of needle primordia) is complete.

4) Following the completion of bud development, commence cooling the greenhouse to between -2° C and +5° C where possible.

5) As soon as frost hardiness monitoring indicates that it is safe to do so, move the crop outside.

It is important to know if snow can be expected soon to protect the crop. However if the containers are moved outside before December a snow cover is not critical. Through the application of the freezing test methods, improved hardening regimes are expected to significantly increase the quality of overwintered container seedlings produced in this Province (Colombo et al. 1984).

To date extended greenhouse culture has been used extensively for black and white spruce container seedlings. Preliminary results using jack pine (Pinus banksiana Lamb) have also been favorable. In 1983-1984 in Ontario approximately 25 million seedlings were prepared for overwintering using extended greenhouse culture.

MATERIALS AND METHODS

The objective of this research was to study the effect of three cultural factors on several growth and developmental attributes of black spruce seedlings grown in Castle and Cooke mini-containers. The experiment examined only the greenhouse phase of an accelerated transplant stock production system based on these mini-containers.

The experimental work was conducted at the OMNR Thunder Bay Nursery 20 km west of the city of Thunder Bay (48 22' N Lat., 89 22' W Long.), and on the campus of Lakehead University in the city of Thunder Bay (48 25' N Lat., 89 15' W Long.), Ontario, Canada.

EXPERIMENTAL DESIGN

The three factors studied, and their levels, were:

1. SOWING DATE (levels: July 5, July 15, July 25, and August 4)
2. the duration of an initial, 18-h, LONG DAY treatment (levels: 7, 10, and 13 weeks)
3. the duration of a subsequent, 8-h, SHORT DAY treatment (levels: 0, 6, and 12 days).

I chose these factors and levels so that the experiment would be more-or-less centred on the guessed-at optimum growing regime specified by the staff of the Thunder Bay Nursery.

The experiment was originally planned as a completely randomized, 4 x 3 x 2 factorial design, with two replicates. Individual Castle and Cooke flats were the experimental units. The design, unfortunately, had to be revised after all the flats had been sown. Under the revised design, the number of treatment combinations was increased. Hence, the number of replicates had to be reduced from two to one. However, six extra flats were available and these were used to replicate six treatment combinations to give an estimate of pure experimental error (Appendix II).

The linear model is the following:

$$Y_{ijkl} = u + LD_i + SHD_j + LD\,SHD_{ij} + SOD_k + LD\,SOD_{ik} + SHD\,SOD_{jk} + LD\,SHD\,SOD_{ijk} + e_{(ijk)l}$$

where: $i = 1,2,3$

$j = 1,2,3$

$k = 1,2,3,4$ or $1,2,3$

$l = 1$ or $1,2$

and Y_{ijkl} = the response associated with the $ijkl^{th}$ treatment combination

u = the overall mean

LD_i = the effect of the i^{th} LONG DAY

SHD_j = the effect of the j^{th} SHORT DAY

$LD\ SHD_{ij}$ = the effect of the interaction of the i^{th} LONG DAY with the j^{th} SHORT DAY

SOD_k = the effect of the k^{th} SOWING DATE

$LD\ SOD_{ik}$ = the effect of the interaction of the i^{th} LONG DAY with the k^{th} SOWING DATE

$SHD\ SOD_{jk}$ = the effect of the interaction of the j^{th} SHORT DAY with the k^{th} SOWING DATE

$LD\ SHD\ SOD_{ijk}$ = the effect of the interaction of the i^{th} LONG DAY with the j^{th} SHORT DAY and the k^{th} SOWING DATE

$e_{(ijk)l}$ = the random error associated with the l^{th} experimental unit in the ijk^{th} treatment combination.

GREENHOUSES

All containers were sown in a greenhouse at the Thunder Bay Nursery. However, since in September the lights as well as the heating system were going to be turned off for economical reasons, on August 28, 1984 all flats were moved to a greenhouse at Lakehead University.

Two differences between these greenhouses were the light (quality and intensity), and the efficiency of the ventilation systems. At the Thunder Bay nursery the greenhouse cover was polyethylene, and the ventilation system was not very efficient. Although at night and in cloudy days the temperature was around 20° C, the high temperatures during July and August went up to 48° C on two occasions, and above 30° C almost every sunny day. The greenhouse at the University was a glasshouse. However, it had been lightly sprayed with white paint to reduce the brightness. The ventilation system kept a constant day/night temperature of 20° C.

Since no difference in bud initiation has been registered in growing black spruce seedlings within the wide range of 3,350 and 22,000 lx (Pollard and Logan 1976) it is assumed the different covers did not affect bud development.

The difference in temperature could have an effect on bud development of black spruce because temperature does greatly affect the rate of needle initiation in white spruce. For white spruce the optimum is at least 25° C, and initiation is much slower at 20° and 15° C (Pollard and Logan 1977).

DETAILS OF CULTURE

The seed used in this experiment came from Site Region 3 W (OMNR code), near Thunder Bay.

The mini-container used in the experiment was developed a few years ago by Castle and Cooke Techniculture Inc. of Watsonville, California. It consists of a square styrofoam flat (30 x 30 x 4.5 cm) with 400 small cavities that measure 1.3 x 4.5 cm. The cavities are filled with a special, patented, medium (8 ml) of peat moss bonded with an inert adhesive that allows the seedlings to be transplanted with a minimum of disruption of their root system.

Individual flats were sown by hand on its assigned date. One seed was sown per cavity for a total of 400 seeds per flat. The growing schedule is presented in Figure 1. The seedlings were initially grown for 7, 10, or 13 weeks under, 18-h, long days and warm temperatures (minimum 20° C). The specimes grew freely during this portion of the cultural regime. At the end of the LONG DAY treatment, individual flats were held for 0, 6, or 12 days (depending on the treatment assignment) under, 8-h, short days. The purpose of this treatment was to evaluate the effect of an 8-h photoperiod on bud development. Finally, each flat was held under natural photoperiod and warm temperatures (minimum 20° C) until the total length of the SHORT DAY and

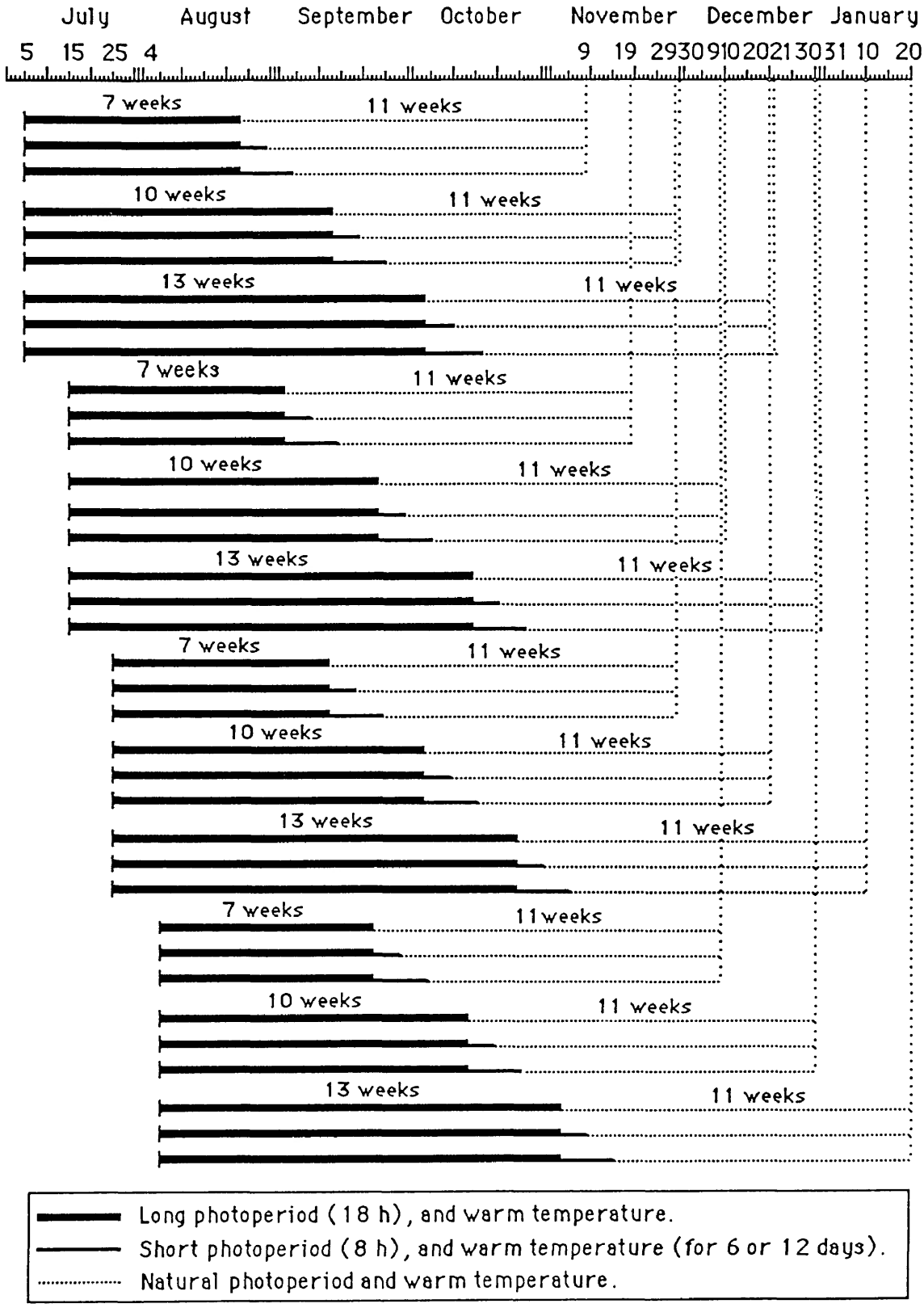

Figure 1. Growing schedule for the 36 treatment combinations. From sowing date to the time seedlings were placed in cold storage.

natural photoperiod treatments was 11 weeks. This is the extended greehouse culture period.

The, 8-h, SHORT DAY treatment was provided by means of black out covers. Special care was taken in blacking the flats completely out, since a very low light intensity can affect bud development (Colombo and Smith, 1984).

Since there are no nutrients in the Castle and Cooke medium, all flats were fertilized through the watering system. The fertilizer regime was applied as follows:

- Weeks 1 and 2 following the sowing date: no fertilizer was applied.

- Weeks 3 and 4: 10 - 52 - 10 NPK at 75 ppm of N was applied.

- Week 5 until the flats were placed in cold storage: 20 - 20 - 20 NPK at 100 ppm of N was applied.

Once the seedlings finished their extended greenhouse culture period, the flats were placed in cold storage for the winter. Some flats were kept in a cooler at the University at 2° to 4° C. The rest of the flats were placed in a cooler at the Thunder Bay nursery at -4° C (Appendix 1). The seedlings in the University cooler dried out quickly, and had to be watered often. The seedlings at the

nursery were kept frozen so desiccation was not a problem.

The seedlings from the nursery and University coolers were transplanted by hand to an outdoor bed at the Thunder Bay nursery on May 3, 1985. The different treatment combinations were assigned to individual plots in a completely randomized design. At the time of outplanting the crops from the two coolers differed noticeably in foliage color. The University seedlings were light green yellowish, whereas the nursery cooler seedlings showed a very healthy dark green colour.

MONITORING SCHEDULE AND SAMPLING

Measurements Taken During Bud Development

Measurements began once the seedlings finished their free growth period under an 18-h, long photoperiod. Due to mortality and a low germination rate there were between 150 and 350 seedlings left per flat of the 400 sown.

Total height (cm) was monitored on a permanent sample of 15 seedlings from every flat. Bud initiation was monitored on a permanent sample of 30 seedlings using a low power microscope. Both measurements were taken every six days for a month, and then every 12 days until height growth ceased.

To monitor bud initiation, seedlings were classified (following Steve Colombo's* advice) as follows:

- Active, characterized by the lack of bud scales.

- Seedlings in the first state of bud initiation, recognized by the presence of white bud scales on terminal buds.

- Seedlings in the advance state of bud initiation, characterized by the presence of brown scales on terminal buds.

Stein's two-stage sample method was used to determine the sample size for height measurements (Steel and Torrie 1980). Sampling 15 seedlings from every flat (experimental unit) resulted in a confidence interval of +/- 0.4 cm, at a probability level of 0.05.

The sample size for bud initiation was determined by following the advice of Colombo (personal communication). This is a sufficiently large sample size since 30 seedlings represent approximately 10 percent of the whole population.

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Every time height and bud initiation activity were measured on the permanent sample seedlings, shoot tips were collected from 10 additional seedlings in every flat and placed in McClintock's fixative (75% ethanol and 25% glacial acetic acid, V/V). Some of the terminal buds on the preserved shoots were later examined to determine the number of needle primordia and the level of mitotic activity.

The sample size of 10 for the destructive samples was limited by the following constraints:

a. The number of seedlings per flat was limited. Since high mortality took place in some flats, it was not possible to take more than 10 seedlings per sample.

b. The time and funds available for measurements were limited. To determine the number of primordia and mitotic activity only on the last sample taken from every flat at storage time, required an expenditure of $ 1,500, and 160 hours of technical work. The fact that many seedlings presented disorganized primordia, dramatically slowed down the process of counting.

The shoots kept in McClintock's fixative were placed in 70 percent alcohol at least 24 hours before dissection to avoid any skin irritation on the hands due to the presence of acetic acid.

The number of needle primordia on each shoot was estimated under a dissecting microscope after bud scales had been removed (Figure 2). The shoot tips have a phyllotaxis of 13:21 parastichies. Therefore, the primordia in only two rows were counted and the average count was then multiplied by the total number of rows (parastichies) as described by Pollard (1974 b). In many cases the primordia were not organized in rows and had to be counted one by one due to a complete lack of a regular phyllotaxis.

Mitotic activity was determined using a modification of the procedures of Johansen (1940), Carlson et al. (1980), and Colombo et al. (1983). The apical meristem, while still attached to the shoot, was placed in Warmke's solution (1:1 by volume 95 % ethanol and concentrated HCL) for 20 minutes. This treatment dissolves the pectin in the middle lamella (Johansen 1940). The buds were then transferred to Carnoy's solution (60% absolute ethanol, 30% cloroform, and 10% glacial acetic acid) for 15 minutes (Johansen 1940) in order to counteract the softening of the cell caused by Warmke's solution. Following these treatments, the apical meristem was then dissected from the shoot, placed on a slide, and bathed in a drop of acetocarmine stain for 20 - 30 minutes (Colombo et al. 1983). Half way through the staining period a cover slip was placed gently on top of the bud. The weight of the cover slip flattened the bud slightly. At the end of the

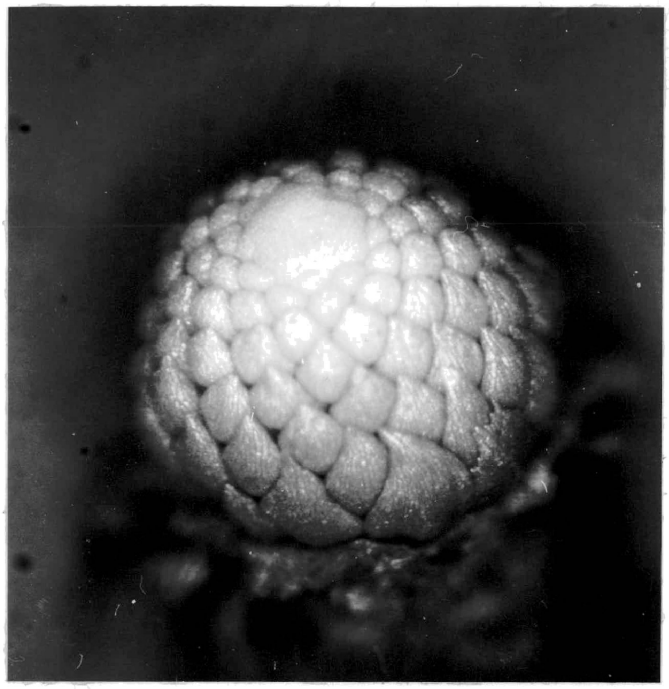

Figure 2. Leaf primordia of a black spruce seedling after bud scales removal. This seedling, held under a LONG DAY treatment for 7 weeks, had 91 primordia.

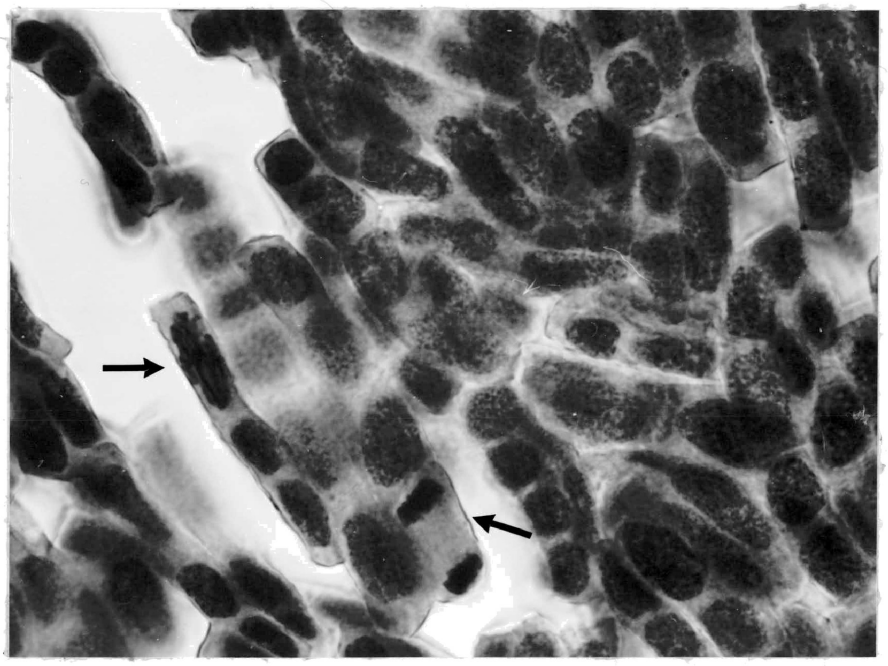

Figure 3. Partial view of a bud squash of a black spruce seedling (1440 x). The arrows point at the two cells undergoing karyokinesis.

staining time the bud was crushed by applying pressure to the cover slip directly above the bud.

Immediately after staining, the bud squash was viewed under a Zeiss microscope equipped with a 10 x ocular, and a 40 x objective. All the cells in the field were observed to determine the onset of dormancy. All the stages of karyokinesis were counted as dividing (Figure 3). Carlson et al. (1980) and Colombo et al. (1983) determined the proportion of mitotically active cells (mitotic index) just by looking at the least dormant area of the squash. In this study the whole area of the squash was scanned, and the total number of cells undergoing division was counted. Since the buds were very small, and because it was desirable to determine the dormant state accurately, the problem of differences in mitotic activity in different areas of the squash had to be taken into account. Buds were considered dormant if no cells undergoing division were found in the apical meristem (Owens and Molder 1973).

Measurements Taken Prior to Storage

Several physical attributes were measured immediately prior to placing seedlings into cold storage. Top dry weight (g) and root dry weight (g) were taken on a sample of six and 22 seedlings per flat respectively. Basal caliper (mm) was determined to 0.01 mm with a dial

thickness gauge. Eleven seedlings were sampled from every flat for this purpose. Bud diameter (mm) was determined on six seedlings per flat with callipers after separating the leaves which cover the buds.

All measurements were taken on different seedlings, and the size of each sample was determined by using Stein's two stage method (Steel and Torrie 1980). The confidence interval was 15 percent of the mean, and the confidence level was 0.95 in all cases. Fifteen percent of the mean is a reasonable confidence interval from a nurseryman's point of view.

The number of primordia at the end of the greenhouse phase was calculated as explained above.

DATA ANALYSIS

A total of 36 (=4x3x3) treatment combinations were measured for the responses height and number of primordia. With the six duplicate treatments, there were 42 experimental units altogether. All seedlings from three experimental units (flats) sown on August 4 died shortly before storage time. This did not affect the measurements of height and number of primordia, however, because these measurements were taken periodically before storage time. Under these circumstances all treatment combinations sown

on August 4 were left out of the design of the response variables basal caliper, bud diameter, and root dry weight. Thus the total number of treatment combinations for these three variables was 27 (=3x3x3). The alternative would have been to analyze all the data even though there were missing treatments. Although possible, the analysis would have been complicated because the design is unbalanced with respect to these variables.

Since all factors are fixed and the design completely randomized, the error mean square was used as the denominator for all tests (Tables 2 and 3). This gives a weak test with only six degrees of freedom. Nevertheless, whenever the 3-way interaction term was negligible, it was pooled with the experimental error, increasing to 18 or 14 the degrees of freedom available for the F test.

Table 2. Expected mean squares for the ANOVA of height and number of primordia.

Source	df	EMS
LD	2	$\sigma^2 + 12\phi$ (LD)
SHD	2	$\sigma^2 + 12\phi$ (SHD)
LD * SHD	4	$\sigma^2 + 4\phi$ (LD SHD)
SOD	3	$\sigma^2 + 9\phi$ (SOD)
LD * SOD	6	$\sigma^2 + 3\phi$ (LD SOD)
SHD * SOD	6	$\sigma^2 + 3\phi$ (SHD SOD)
LD * SHD * SOD	12	$\sigma^2 + \phi$ (LD SHD SOD)
Exp. error	6	σ^2
Total	41	

Table 3. Expected mean squares for the ANOVA of basal caliper, bud diameter, and root dry weight.

Source	df	EMS
LD	2	$\sigma^2 + 9\phi$ (LD)
SHD	2	$\sigma^2 + 9\phi$ (SHD)
LD * SHD	4	$\sigma^2 + 3\phi$ (LD SHD)
SOD	2	$\sigma^2 + 9\phi$ (SOD)
LD * SOD	4	$\sigma^2 + 3\phi$ (LD SOD)
SHD * SOD	4	$\sigma^2 + 3\phi$ (SHD SOD)
LD * SHD * SOD	8	$\sigma^2 + \phi$ (LD SHD SOD)
Exp. error	6	σ^2
Total	32	

Since only six treatment combinations were duplicated, special care was taken to choose an appropriate analysis for unequal cell frequencies. The classical method of partitioning the total sum of squares is appropriate only with balanced, or equal-subclass-numbers, designs. In the case of unbalanced designs the hypotheses tested by the classsical analysis may not be very interesting, and the rejection or acceptance of these hypotheses may not be easy to interprete (Milliken and Johnson 1984). Thus, I chose to use Yates' weighted squares of means technique. This is the "Type III" analysis discussed by Milliken and Johnson (1984). Type III hypotheses are developed so that they do not depend on the cell sizes, but only on which cells are observed. These hypotheses are equivalent to the hypotheses tested in the balanced or equal-subclass-number case (Milliken and Johnson 1984).

Cell means associated with significant 2-way interactions were contrasted by means of Tukey's Honest Significant Difference test. These tests were run by considering the 2-way interaction as a 1-way treatment structure (Milliken and Johnson 1984, Anderson and McLean 1974).

The Tukey's HSD was chosen because it is the most similar test to Bonferroni's, which is the one recommended by Milliken and Johnson (1984) in the case of unplanned comparisons. Bonferroni's was not available in the SPSS

statistical computer package that I used. For nearly equal sample sizes, Tukey's HSD critical value is usually smaller than Bonferroni's, but for more unequal sample sizes Bonferroni's critical value is usually smaller (Milliken and Johnson 1984). In this case, where the sample sizes are nearly equal (only six treatments, of 36 or 27, were duplicated) the critical values are very close. For unplanned comparisons Scheffe's test also is recommended. However, it is mainly used for infinite number of comparisons, or in the case of "data snooping" (Milliken and Johnson 1984).

RESULTS

PEARSON CORRELATION

Pearson correlation coefficients for all response variables measured in the experiment are presented in Table 4. I considered two variables to be highly correlated if their Pearson correlation coefficient was 0.7 or higher.

Of the three quantitative variables monitored during bud development, the length of the bud initiation period and the number of days to reach an advanced state of bud initiation showed a correlation coefficient greater than 0.7 (0.93). Of the six response variables measured immediately before placing the seedlings in cold storage, only height and top dry weight showed a correlation coefficient higher than 0.7 (0.95).

Since the correlation coefficients were generally low, I elected to use a univariate analysis of variance for every response variable except top dry weight. The top dry weight response was explained in terms of height. If the reader is interested in running a multivariate analysis on these data, the measurements are presented in Appendix 2.

Table 4. Pearson correlation coefficients for all response variables.

	HT	BAS. CAL.	BUD DIA.	RDW	TDW	N.OF PRIM.	CESS. GROW.	BUD INI.
BAS. CAL.	.277							
BUD DIA.	.538	.107						
RDW	-.225	.622	-.059					
TDW	.950	.342	.609	-.104				
N.OF PRIM.	-.423	.082	-.016	.420	-.388			
CESS. GROW.	-.445	-.180	-.378	-.072	-.404	.276		
BUD INI.	-.893	-.188	-.600	.291	-.846	.290	.457	
ADV. INI.	-.878	-.144	-.584	.285	-.837	.316	.493	.928

Response variables code: HT= height, BAS. CAL.= basal caliper, BUD DIA.= bud diameter, RDW= root dry weight, TDW= top dry weight, N.OF PRIM.= number of primordia, CESS. GROW.= number of days to cessation of height growth, BUD INI.= length of the bud initiation period, ADV. INI.= number of days to reach an advanced state of bud initiation.

RESPONSE VARIABLES MONITORED DURING BUD DEVELOPMENT

Bud Initiation and Height Growth Under Natural Day Length

During the 11 weeks of extended greenhouse culture I monitored four developmental events: the number of days to cessation of height growth, the number of days to reach early bud initiation, the length of the bud initiation period (days), and the number of days to reach an advanced state of bud initiation.

All 36 treatments took the same time to reach the early stage of bud initiation. Therefore, ANOVA tables are given only for the other three developmental responses.

Table 5. Analysis of variance of the number of days to cessation of height growth.

Source	df	MS	F	Sig. of F
LD	2	150.13	25.0	.001
SHD	2	117.04	19.0	.002
SOD	3	15.95	2.6	.142
LD * SHD	4	10.31	1.7	.263
LD * SOD	6	14.90	2.5	.146
SHD * SOD	6	17.66	2.9	.107
LD * SHD * SOD	12	13.12	2.2	.173
Experimental error	6	6.00		
Total	41			

Table 6. Analysis of variance of the length of the bud initiation period (days).

Source	df	MS	F	Sig. of F
LD	2	1770.67	295.1	.000
SHD	2	23.02	3.8	.084
SOD	3	470.19	78.4	.000
LD * SHD	4	7.04	1.2	.409
LD * SOD	6	88.12	14.7	.002
SHD * SOD	6	14.34	2.4	.157
LD * SHD * SOD	12	12.63	2.1	.185
Exp. error	6	6.00		
Total	41			

Table 7. Analysis of variance of the number of days to reach an advanced state of bud initiation.

Source	df	MS	F	Sig. of F
LD	2	1230.13		
SHD	2	39.26		
SOD	3	292.18		
LD * SHD	4	9.13		
LD * SOD	6	107.40		
SHD * SOD	6	8.27		
LD * SHD * SOD	12	10.00		
Exp. error	6	0.00		
Total	41			

Seedlings were monitored every six days at the beginning, and every 12 days towards the end of the bud initiation period. Hence, it was not likely to record differences between double replicates when seedlings were reaching the advanced state of bud initiation. This resulted in an experimental error equal to zero (Table 7). It is not wise to accept this nil experimental error blindly, since it most likely would be differences between

repeated treatment combinations had the samples been taken more often. Then, I ran a new ANOVA using the mean square for the 3-way interaction as the denominator for testing all other main effects and interactions (Table 8). Since the true experimental error should be less than or equal to the 3-way interaction mean square, the tests given in Table 8 are conservative (Anderson and MacLean 1974).

Table 8. Analysis of variance of the number of days to reach an advanced state of bud initiation using the 3-way interaction mean square as an estimate of experimental error.

Source	df	MS	F	Sig. of F
LD	2	1230.13	122.95	.000
SHD	2	39.26	3.92	.049
SOD	3	292.18	29.20	.000
LD * SHD	4	9.13	.91	.484
LD * SOD	6	107.40	10.73	.000
SHD * SOD	6	8.27	.83	.569
LD * SHD * SOD (Error)	12	10.00		
Residual	6	.00		
Total	41			

The main effect cell means by factor and developmental event are presented in Table 9.

Table 9. Average number of days during the extended greenhouse culture period for 3 developmental stages to be achieved by main effect and level.

Developmental event	Main effect									
	LONG DAY Duration of free growth period (weeks)			SOWING DATE				SHORT DAY Number of days under 8h photop		
				July			Aug			
	7	10	13	5	15	25	4	0	6	12
CESS. GROW.	27	15	15	24	22	22	21 1/	24	24	19
BUD INI.	38	27	16	39	24	24	24	26	30	26 1/
ADV. INI.	54	44	36	54	42	42	42	43	48	43

1/ main effect not significant at a probability level of five percent.

The cell means for the LONG DAY * SOWING DATE interaction for the variables length of the bud initiation period and time to reach an advanced state of bud initiation are presented in Table 10.

Table 10. LD * SOD means for the number of days to achieve two developmental stages during the extended greenhouse culture period.

LONG DAY and RESPONSE VARIABLES	SOWING DATE			
	July 5	July 15	July 25	August 4
7 weeks				
BUD INI.	52	34	36	28
ADV.INI.	63	54	54	42
10 weeks				
BUD INI.	36	27	24	24
ADV.INI.	54	42	42	42
13 weeks				
BUD INI.	24	12	10	22
ADV.INI.	42	32	28	42

Primordia Development

The two treatment combinations that developed the greatest number of primordia were monitored during bud development (Figure 4). The main difference between these two treatments was the marked increase in primordia production at the end of the period of extended greenhouse culture that occurred in the seedlings that received the LONG DAY treatment for seven weeks.

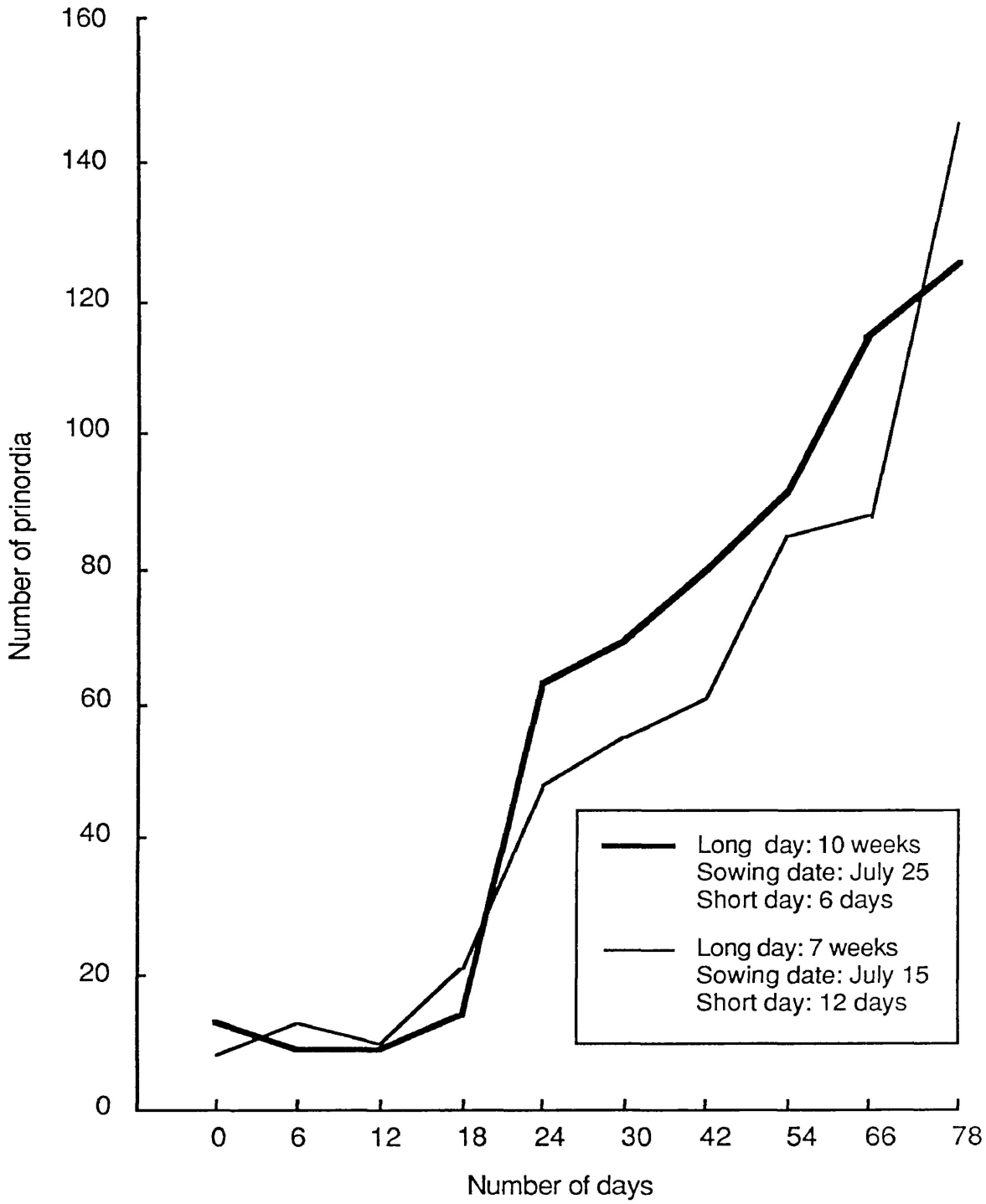

Figure 4. Needle primordia initiation in two treatments during the 11-week period of extended greenhouse culture. Day zero marks the beginning of this period.

RESPONSE VARIABLES MEASURED IMMEDIATELY PRIOR TO PLACING THE SEEDLINGS IN COLD STORAGE

Height

The ANOVA table for height is presented in Table 11.

Table 11. Analysis of variance table for height.

Source	df	MS	F	Sig. of F
LD	2	35.60	1056.69	.000
SHD	2	.18	5.22	.049
SOD	3	7.76	230.29	.000
LD * SHD	4	.19	5.81	.029
LD * SOD	6	.67	20.05	.001
SHD * SOD	6	.26	7.78	.012
LD * SHD * SOD	12	.25	7.36	.011
Exp. error	6	.03		
Total	41			

These results are suspicious since all the terms including the 3-way interaction are significant. One explanation for such an outcome is that the experimental error mean square seriously underestimates the true error variance.

Examination of the residuals for the six pairs of replicated treatment combinations (Table 12) has two possible explanations:

1. the five similar values are a representative "good" sample that estimates accurately the distribution of the

residuals, and the biggest values (+0.29 and -0.29) are due to an outlier (Figure 5).

2. or the five similar values are a "bad" sample, being very close to the mean just by chance (Figure 6).

Table 12. Observed, predicted, and residuals for the six sets of duplicate treatment combinations.

Treatment	Case	Observed	Predicted	Raw residuals
1	1	1.71	1.75	-.04
1	2	1.79	1.75	.04
2	3	3.01	2.95	.06
2	4	2.89	2.95	-.06
3	5	2.83	2.53	.29
3	6	2.24	2.53	-.29
4	7	5.11	5.06	.04
4	8	5.02	5.06	-.04
5	9	7.15	7.23	-.08
5	10	7.31	7.23	.08
6	11	6.05	6.07	-.02
6	12	6.09	6.07	.02

There is no evidence that the "outlier" is a mistake since it is not an unexpectedly large value. On the other hand, the five similar values appear to be too small. Yet, each pair of treatment combinations gives an individual estimate of mean square error with one degree of freedom (Table 13). The biggest value .17 is pretty close to four of the mean squares associated with main effects of factors and their interactions. They are the mean square values for SHD, LD * SHD, SHD * SOD, and LD * SHD * SOD (Table 11). Hence, it is easy to imagine that none of these four effects are real, and that the four similar values are in

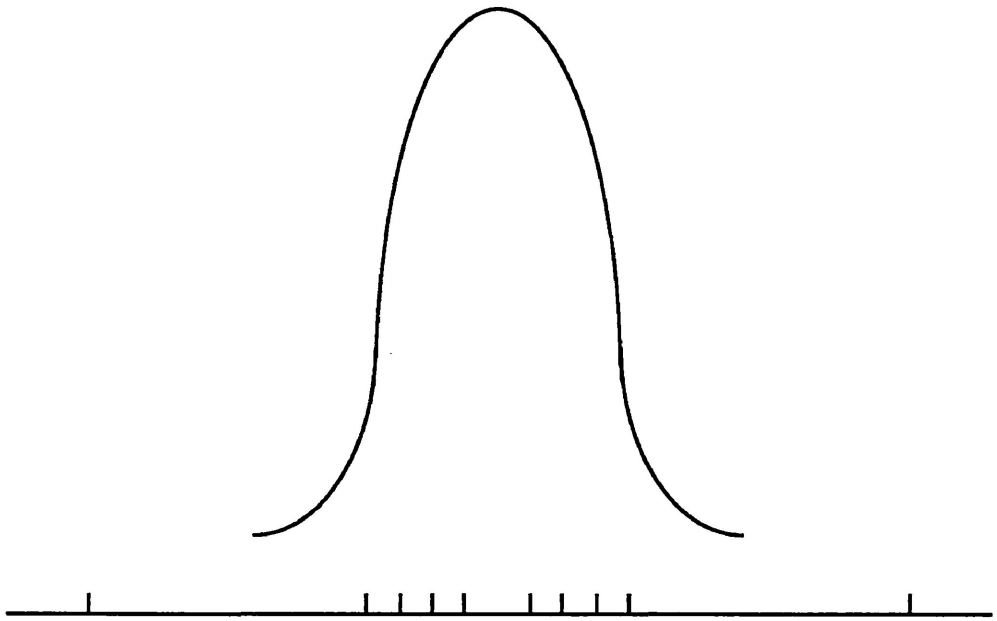

Figure 5. Possible distribution of the height residuals assuming the biggest value is due to an outlier.

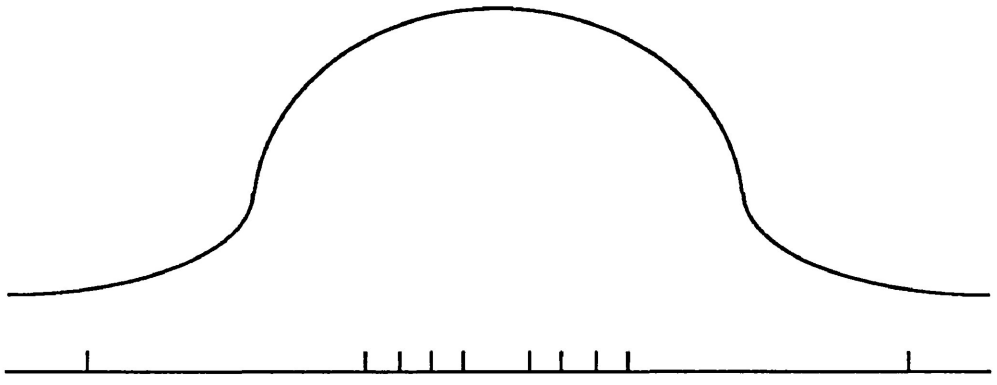

Figure 6. Possible distribution of the height residuals assuming the biggest value is plausible.

fact simply independent measures of experimental error.

Table 13. Individual estimate of mean of square error given by the six replicated treatment combinations.

Treatment	Mean of square
1	.0032
2	.0072
3	.1682
4	.0032
5	.0128
6	.0008

This interpretation seems even more plausible when these effects are examined graphically, since none of them seem to have a biological interpretation (Figures 7, 8, 9 and 10).

I understand that the results from the original ANOVA (Table 11) strongly suggest the three way interaction should not be pooled. Nevertheless, after carefully analyzing all of the evidence, I believe it is highly likely that the true value of the error mean square has been underestimated in this experiment. Based on this conclusion, the four terms that show almost the same mean of square values have been pooled and the F-ratios recalculated (Table 14).

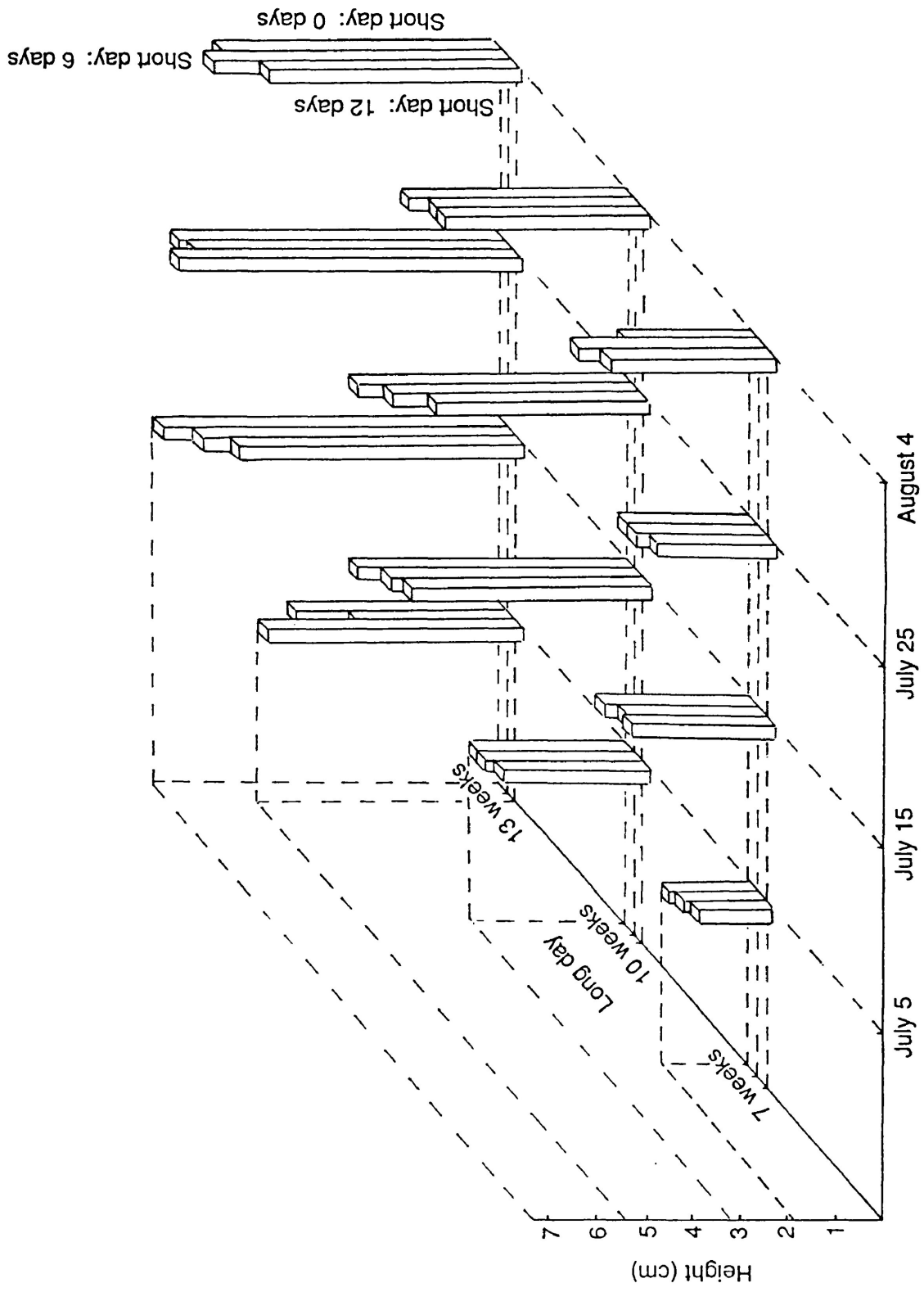

Figure 7. LONG DAY * SHORT DAY * SOWING DATE means for the variable height.

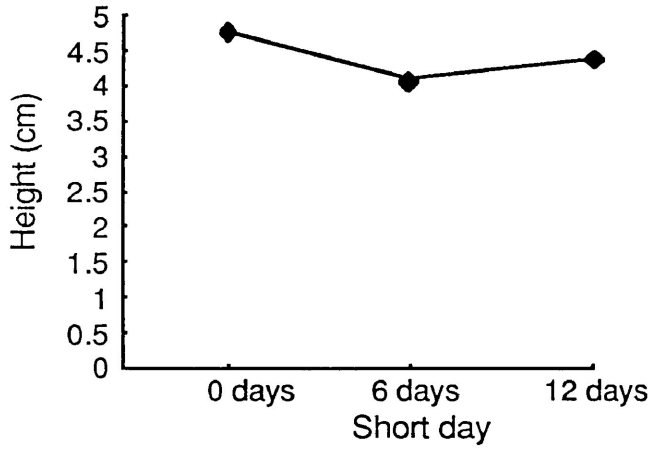

Figure 8. SHORT DAY means for the variable height.

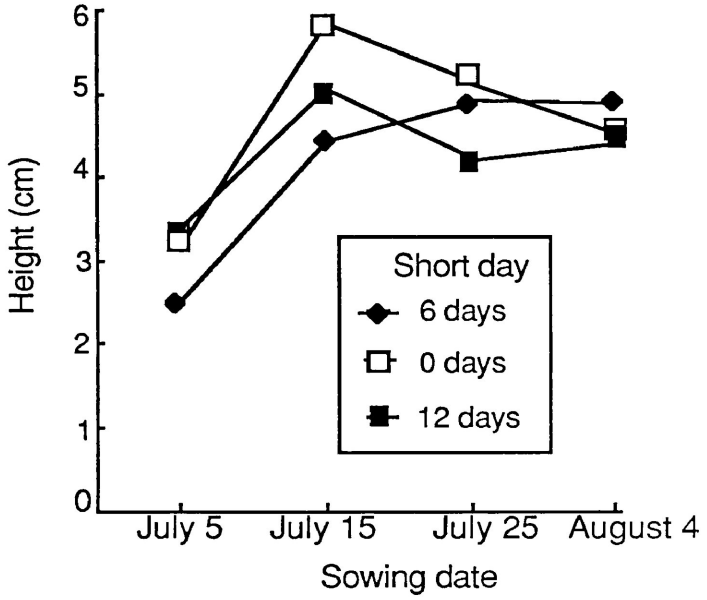

Figure 9. SHORT DAY * SOWING DATE means for the variable height.

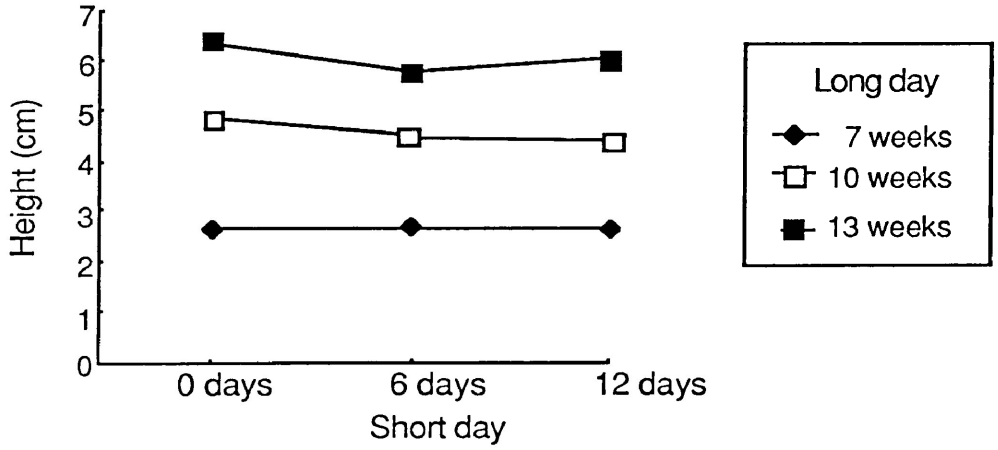

Figure 10. SHORT DAY * LONG DAY means for the variable height.

Table 14. Analysis of variance of height based on pooled estimate of error mean square.

Source	df	MS	F	Sig. of F
LD	2	37.75	182.07	.000
SOD	3	7.91	38.16	.000
LD * SOD	6	.72	3.47	.010
Pooled Error	30	.21		
Total	41			

The means for the variable height are presented in Tables 23, 24, and 25 at the end of this chapter.

Number of Primordia

The ANOVA for number of primordia was run in two stages. In the first stage (Table 15) the 3-way interaction was tested at the five percent level of significance. The null hypothesis was accepted and the 3-way interaction was pooled with the experimental error (Winer 1971, Anderson and McLean 1974).

Table 15. Analysis of variance for number of primordia.

Source	df	MS	F	Sig. of F
LD	2	3754.72	10.87	.010
SHD	2	220.80	.63	.560
SOD	3	1093.93	3.17	.107
LD * SHD	4	151.28	.44	.778
LD * SOD	6	1154.07	3.34	.084
SHD * SOD	6	70.31	.20	.963
LD * SHD * SOD	12	176.62	.51	.848
Exp. error	6	345.47		
Total	41			

By pooling the three way interaction with the error term I obtained a new estimate of the error mean square with 18 degrees of freedom (Table 16). Pooling one or both of the not significant 2-way interactions, LD * SHD and SHD * SOD, there is no change in terms of significance for the different sources of variation.

Table 16. Analysis of variance for number of primordia based on pooled estimate of the error mean square.

Source	df	MS	F	Sig. of F
LD	2	3837.32	16.47	.000
SHD	2	226.36	.97	.397
SOD	3	1117.07	4.80	.013
LD * SHD	4	161.72	.69	.606
LD * SOD	6	1157.34	4.96	.004
SHD * SOD	6	59.44	.25	.951
Pooled error	18	232.90		
Total	41			

The means for the variable number of primordia are presented in Tables 23, 24, and 25 at the end of this chapter.

Basal Caliper

Table 17 shows the analysis of variance for basal caliper.

Table 17. Analysis of variance for basal caliper.

Source	df	MS	F	Sig. of F
LD	2	.042	7.90	.021
SHD	2	.000	.06	.944
SOD	2	.046	8.54	.018
LD * SHD	4	.005	.92	.509
LD * SOD	4	.007	1.30	.366
SHD * SOD	4	.004	.71	.609
LD * SHD * SOD	8	.001	.17	.987
Exp. error	6	.005		
Total	32			

Since the 3-way interaction is negligible, it was pooled in order to increase the number of degrees of freedom for the error term. The final ANOVA is presented in Table 18.

Table 18. Analysis of variance for basal caliper pooling the 3-way interaction.

Source	df	MS	F	Sig. of F
LD	2	.043	15.27	.000
SHD	2	.000	.17	.846
SOD	2	.047	16.84	.000
LD * SHD	4	.005	1.76	.193
LD * SOD	4	.007	2.47	.093
SHD * SOD	4	.004	1.41	.179
Pooled error	14	.003		
Total	32			

The means for the variable basal caliper are presented in Tables 23, 24, and 25 at the end of this chapter.

Bud Diameter

The ANOVA for bud diameter is shown in Table 19. The negligible 3-way interaction was pooled with the experimental error. The final ANOVA is presented in Table 20.

Table 19. Analysis of variance for bud diameter.

Source	df	MS	F	Sig. of F
LD	2	.120	13.99	.006
SHD	2	.007	.82	.485
SO D	2	.014	1.59	.280
LD * SHD	4	.026	3.00	.111
LD * SOD	4	.038	4.41	.053
SHD * SOD	4	.012	1.38	.345
LD * SHD * SOD	8	.011	1.27	.397
Exp. error	6	.008		
Total	32			

Table 20. Analysis of variance for bud diameter pooling the 3-way interaction.

Source	df	MS	F	Sig. of F
LD	2	.126	12.71	.001
SHD	2	.009	.91	.423
SOD	2	.017	1.78	.205
LD * SHD	4	.026	2.63	.079
LD * SOD	4	.036	3.64	.031
SHD * SOD	4	.014	1.47	.264
Pooled error	14	.010		
Total	32			

The means for the variable bud diameter are presented in Tables 23, 24, and 25 at the end of this chapter.

Root Dry Weight

The Anova tables for this response variable, before and after pooling the 3-way interaction are as follows:

Table 21. Analysis of variance for root dry weight.

Source	df	MS	F	Sig. of F
LD	2	.026	24.18	.001
SHD	2	.002	2.12	.201
SOD	2	.019	16.79	.003
LD * SHD	4	.001	.72	.610
LD * SOD	4	.007	6.57	.022
SHD * SOD	4	.001	.66	.639
LD * SHD * SOD	8	.001	1.39	.353
Exp. error	6	.001		
Total	32			

Table 22. Analysis of variance for root dry weight pooling the 3-way interaction.

Source	df	MS	F	Sig. of F
LD	2	.026	18.90	.000
SHD	2	.001	1.15	.345
SOD	2	.018	13.53	.001
LD * SHD	4	.001	.67	.621
LD * SOD	4	.006	4.74	.013
SHD * SOD	4	.001	.58	.681
Pooled error	14	.001		
Total	32			

The means for the variable root dry weight are presented in Tables 23, 24, and 25 at the end of this chapter.

Mitotic Activity

Seedlings from all treatments were dormant by the end of the extended greenhouse culture treatment. No cell divisions on their apical meristems were found. Thus, there were no factor effects in this variable to examine.

SEEDLINGS AT THE END OF THE FIRST GROWING SEASON

Most seedlings from the flats which were kept in the University cooler died shortly after being transplanted outdoors. Seedlings stored in the cooler at the Thunder Bay Nursery grew well, and in the case of some treatments achieved close to the minimum shippable height.

Since many treatments were missing, no measurements were taken due to the impossibility of doing any meaningful statistical analysis.

Table 23. LONG DAY means for response variables measured prior to placing the seedlings in cold storage.

LONG DAY	HT	N.OF PRIM.	BAS. CAL.	BUD DIA.	RDW
7 weeks	2.67	114	.76	1.29	.2321
10 weeks	4.58	97	.88	1.43	.2900
13 weeks	6.08	81	.81	1.51	.1838

Table 24. SOWING DATE means for response variables measured prior to placing the seedlings in cold storage.

SOWING DATE	HT	N.OF PRIM.	BAS. CAL.	RDW
July 5	2.98	95	.79	.2341
July 15	5.12	104	.88	.2661
July 25	4.70	107	.75	.1908
August 4	4.61	83		

Table 25. LONG DAY * SOWING DATE means for response variables measured prior to placing the seedlings in cold storage.

LONG DAY and VARIABLES	SOWING DATE			
	July 5	July 15	July 25	August 4
7 weeks				
HT	1.77	3.04	2.65	3.42
N.OF PRIM.	82	138	123	113
BAS. CAL.	.71	.84	.74 1/	
BUD DIA.	1.23	1.37	1.27	
RDW	.1979	.2843	.2142	
10 weeks				
HT	3.19	5.29	5.16	4.44
N.OF PRIM.	109	98	104	78
BAS. CAL.	.92	.93	.77 1/	
BUD DIA.	1.27	1.46	1.55	
RDW	.3087	.3464	.1962	
13 weeks				
HT	4.38	6.65	6.96	5.97
N.OF PRIM.	96	80	8	59
BAS. CAL.	.77	.86	.74 1/	
BUD DIA.	1.57	1.48	1.47	
RDW	.2076	.1872	.1544	

1/ interaction not significant at a probability level of five percent.

DISCUSSION

EFFECTS OF THE CONTROLLED FACTORS ON BUD INITIATION

Main Effects

Long Day

The highly significant influence of LONG DAY on growth cessation and bud initiation may be explained as follows. Young spruce seedlings continue to grow indeterminately after flushing (Jablanczy 1971). However, the tendency is less pronounced in older seedlings (Pollard 1974 a). Thus, it is not surprising that those seedlings that were youngest at the end of the LONG DAY treatment maintained their growth further into the extended greenhouse phase, and reached the different stages of bud initiation later (Table 9).

A second explanation for the relationship between LONG DAY and the chronology of bud development may be due to the small volume of the growing medium. Seedlings that received 13 weeks of the LONG DAY treatment were already potbound going into the extended greenhouse treatment. This is important since indeterminate growth may stop, not only under short days, but also under physiological stress (Pollard 1974 a). Hence, seedlings from the 13-week LONG

DAY treatment were more sensitive to the induction of bud development than 7- or 10-week seedlings.

Short Day

The artificial SHORT DAY treatment (8-h photoperiod) when applied for 12 days (level 3) induced growth cessation five days sooner than when it was applied for six days (level 2), or not applied at all (level 1) (Table 9).

Seedlings under level 1 of the factor SHORT DAY did not receive an artificial 8-h treatment. Thus, they went into bud initiation under natural photoperiod conditions. The natural day length varied progressively during the experiment from approximately 15 h for seedlings sown on the first sowing day (July 5), to close to 8 h for seedlings sown on the fourth sowing day (August 4). Thus, for the older seedlings sown on the last sowing day the difference between the three levels of this factor was nil.

Since the critical daylength for budset of black spruce in Nothwestern Ontario is about 15 h, all three levels of the factor SHORT DAY were able to induce bud initiation. However, the induction of growth cessation was significantly faster, when an 8-h photoperiod was applied for 12 days.

These results support the conclusions of Pollard and Logan (1977) that photoperiod is very important in promoting the onset of bud development in seedlings in the free growth state.

Unexpectedly, the seedlings which stopped growing first because of the exposure to a short photoperiod did not achieve bud initiation any sooner than other treatments (Table 9). This can be explained by the fact that at the time the seedlings stopped growing they were checked every six days, whereas later on during bud initiation they were checked every 12 days. Hence, if some of the treatments initiated buds sooner than others by only a few days, the difference was not detected. However, the 8-h photoperiod applied during six days retarded the achievement of the advanced state of bud initiation (Table 9). This is an unexplainable result since the provision of such a short photoperiod should accelerate the onset of bud development.

Sowing Date

Seedlings from the first SOWING DATE were slower to initiate buds (Table 9). This apparent resistance to initiate buds can be explained by the fact that when some seedlings from the first sowing day were placed under natural day length, the number of hours of natural light was the minimum period required for bud initiation. Thus,

these seedlings could continue to grow slowly instead of starting bud development. However, only 1/3 of the seedlings from the first sowing day, the ones 7 weeks old, were under these conditions. Yet, 1/3 of the 7-week old seedlings were provided with an 8-h photoperiod for 12 days before being placed under natural photoperiod. Since this 12 days treatment favoured bud initiation, only 2/3 of the 7-week old seedlings could extend the bud initiation period because of the 15-h natural day length.

These results support the conclusions of Pollard and Logan (1977) that bud development could not normally be induced in young spruce seedlings in 15-h photoperiod without an initial short-day treatment.

A second, and more likely explanation for the effect of SOWING DATE on bud initiation is the poor physiological condition (weak and slow growing plants),and high mortality (mainly due to damping off) that happened only among seedlings from the first sowing day. These circumstances could easily affect the response of those seedlings to bud initiation conditions (Thompson 1982).

Two Way Interaction

The LD * SOD interaction is significant because seedlings sown on August 4 showed no significant

differences between either the length of the bud initiation periods or the number of days to reach an advanced state of bud initiation, regardless of the LONG DAY treatment received (Figures 11 and 12). This may be due to the fact that for seedlings sown on August 4 the natural photoperiod during the bud initiation period was as short as 8 or 9 h. Apparently such a short photoperiod can induce bud initiation at the same speed on all seedlings regardless of their LONG DAY treatment. On the other sowing days the effect of a longer natural photoperiod, 10 to 15 h, resulted in shorter bud initiation periods for the seedlings that received the longer LONG DAY treatments.

EFFECT OF THE CONTROLLED FACTORS ON THE FINAL SEEDLINGS

Main Effects

Long Day

As expected, factor LONG DAY strongly influenced the final height of seedlings. Seedlings that received the longest duration of the LONG DAY treatment (13 weeks) were tallest at the end of the experiment (Figure 13).

Seedlings held under long days for 10 weeks showed a significantly bigger basal caliper and bud diameter than seedlings in the 7-week LONG DAY treatment (Figures 14, 15). Unexpectedly I did not find any significant difference

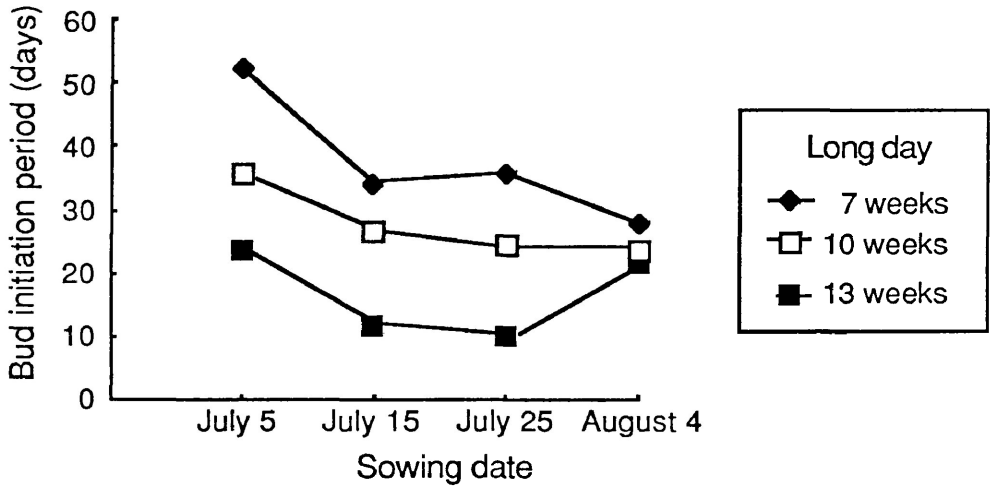

Figure 11. LONG DAY * SOWING DATE means for the bud initiation period.

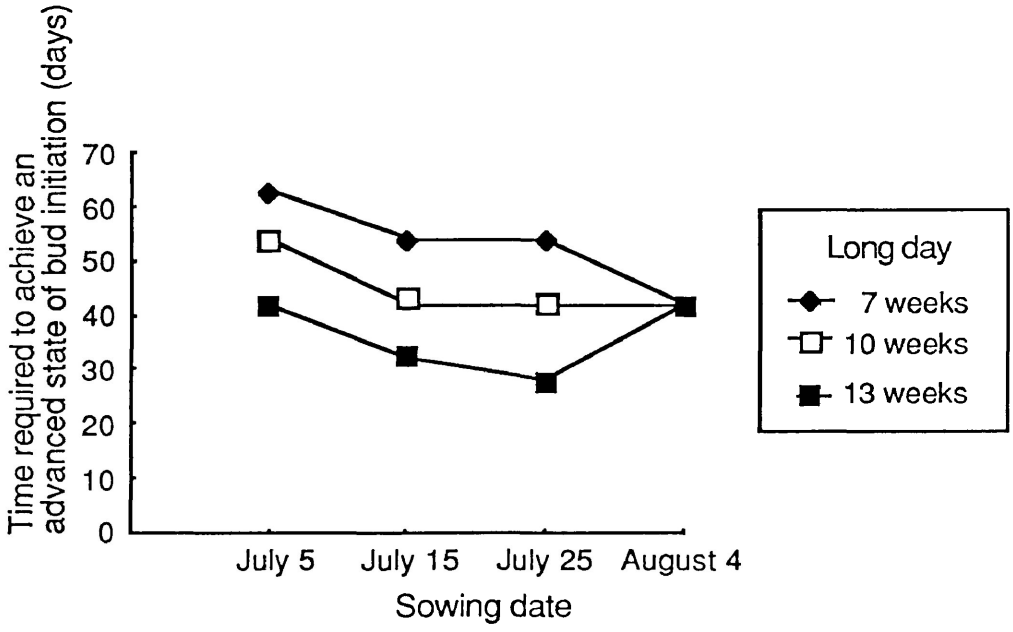

Figure 12. LONG DAY * SOWING DATE means for the advanced state of bud bud initiation.

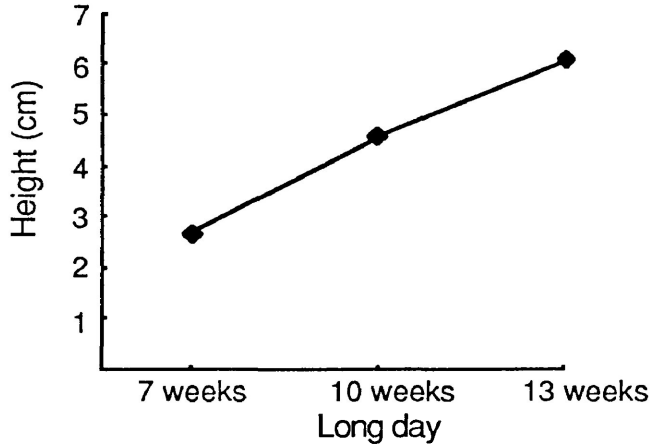

Figure 13. LONG DAY means for the variable height. All means are significantly different from each other (Tukey's HSD).

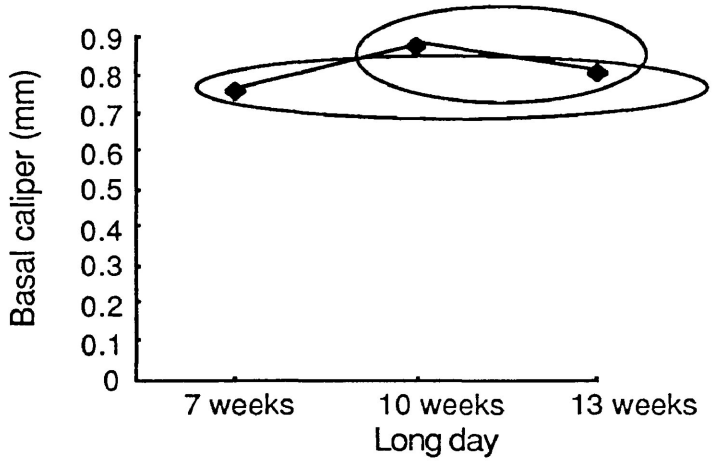

Figure 14. LONG DAY means for the variable basal caliper. Means located within the same circle are not significantly different (Tukey's HSD).

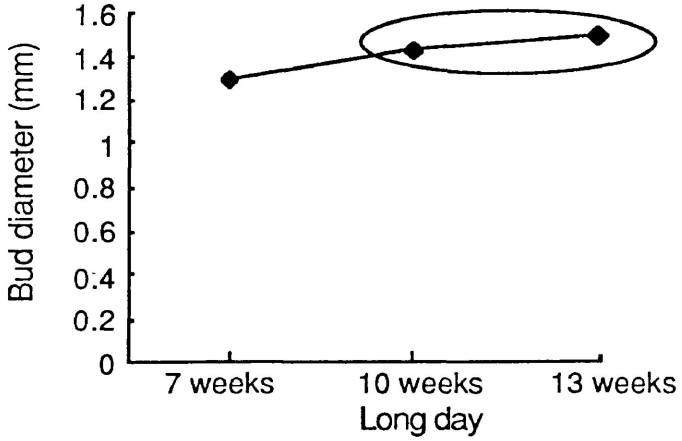

Figure 15. LONG DAY means for the variable bud diameter. Means located within the same circle are not significantly different (Tukey's HSD).

between 10- and 13-week LONG DAY specimens. Since most researches have found significant size differences between 10- and 13-week-old seedlings, the next step was to try to find the main difference between this experiment and the previous ones. This difference was obviously determined by the containers. Castle and Cooke trays hold an 8 cm^3 medium, while containers used by other researchers almost always were over 40 cm^3. Eventually seedlings from the 13-week LONG DAY treatment are too big for this size of Castle and Cooke container.

The potbound situation, however, was not reflected on the variable height. This can be explained by the fact that when the supply of nitrogen is limited, only the most active meristems, usually the terminal bud, gets enough nitrogen for growth (Gregory and Vealy 1957, cited by Kramer and Kozlowski, 1960).

The potbound problem was maximal in the case of root dry weight since, seedlings in the 13-week LONG DAY treatment gave a significantly lower response than 10-week LONG DAY specimens (Figure 16). There was not even any significant difference between the root dry weight of 7- and 13-week LONG DAY seedlings. That is easy to understand because many of the roots of 13-week seedlings occupied the air space between the medium and the inner surface of the cavities, while many others grew through the bottom draw hole. This was also reflected on the top/root ratio, which

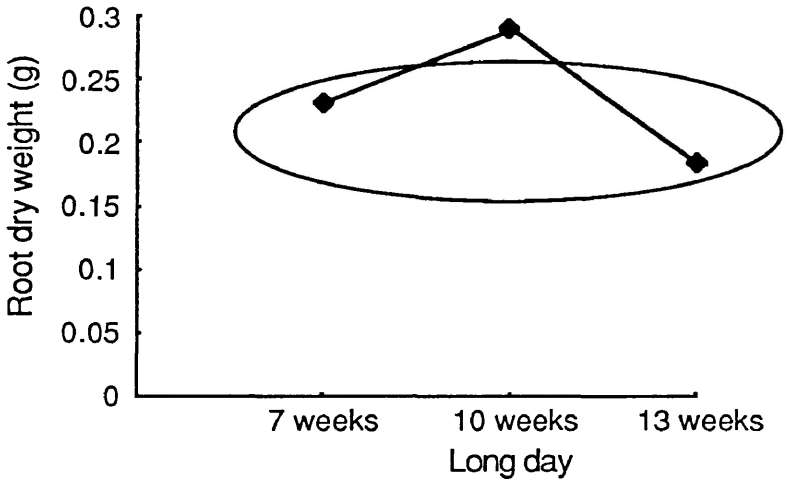

Figure 16. LONG DAY means for the variable root dry weight. Means located within the same circle are not significantly different (Tukey's HSD) .

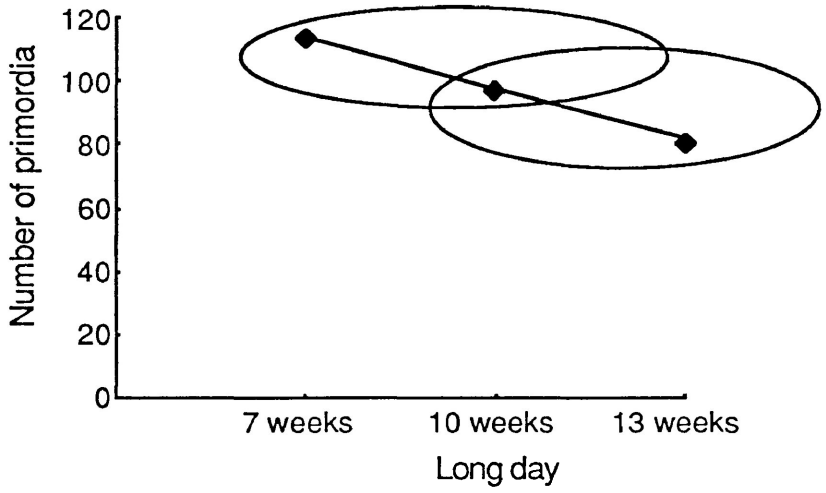

Figure 17. LONG DAY means for the variable number of primordia. Means located within the same circle are not significantly different (Tukey's HSD) .

was 2.18 and 1.04 for 13- and 10-week LONG DAY specimens respectively.

The way factor LONG DAY affected the number of primordia developed by the seedlings is, undoubtedly, the most surprising find of my study (Figure 17).

Larger seedlings develop more primordia than small seedlings (Pollard 1974 a). Considering that increasing age represents a longer phase of indeterminate growth, and consequently a larger plant, I expected the seedlings from the longer LONG DAY treatments to show the highest number of primordia. In this experiment, however, 10-week LONG DAY seedlings initiated more primordia than 13-week specimens.

Pollard (1974 a) stated: "Seedling age appears to be associated with the influence of seedling size in two ways. First, increasing age represents a longer phase of indeterminate growth and consequently results in larger plants. A second effect was indicated where the ranges of sizes within successive age groups overlap: younger seedlings exhibit more vigorous morphogenesis than older seedlings of comparable size." He gave an example by saying that a 10 cm seedling produces more primordia as a large 10-week-old specimen than as a 16-week-old specimen.

This explains why in my experiment (although the difference was not significant) seedlings exposed to a LONG DAY treatment for 10 weeks developed more primordia than 13-week specimens. Despite the difference in age these seedlings were similar in size, and the root dry weight was even lighter for 13-week LONG DAY specimens.

The reason why 7-week LONG DAY seedlings developed more primordia than 10- and 13-week specimens is less obvious. Ten-week LONG DAY seedlings were significantly bigger in all measurements, and they did not show any stress symptoms. Seven-week LONG DAY seedlings could have had a longer bud initiation period, a faster bud initiation rate, or both. The only way to discover this is to analyse all the periodic shoot samples taken during the bud initiation phase. Unfortunately this is not possible as it would require approximately eight months of work.

Sowing Date

One of the two best sowing dates for root dry weight and the best SOWING DATE for basal caliper was July 15 (Figure 18, 19). This may be due to the lack of a good ventilation system in the greenhouse at the Thunder Bay nursery. There the temperature was usually above 25° C during the day. The ventilation system at the University greenhouse was so efficient that a temperature of 20° C was almost always maintained. Hence, seedlings from the first

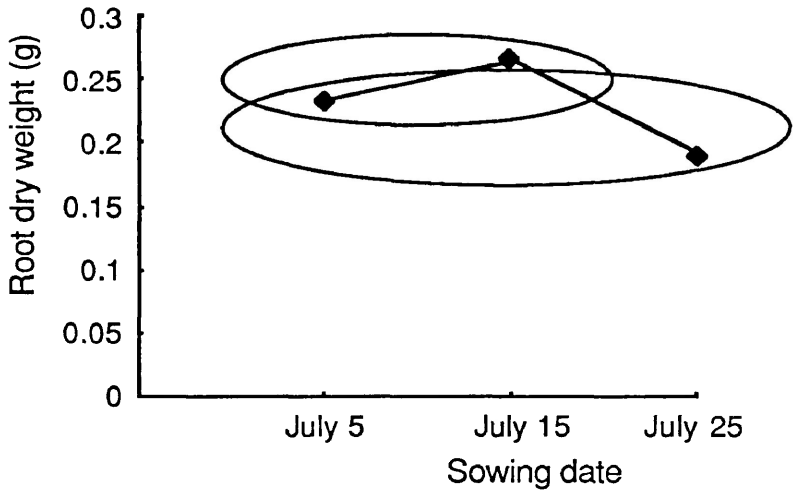

Figure 18. SOWING DATE means for the variable root dry weight. Means located within the same circle are not significantly different (Tukey's HSD).

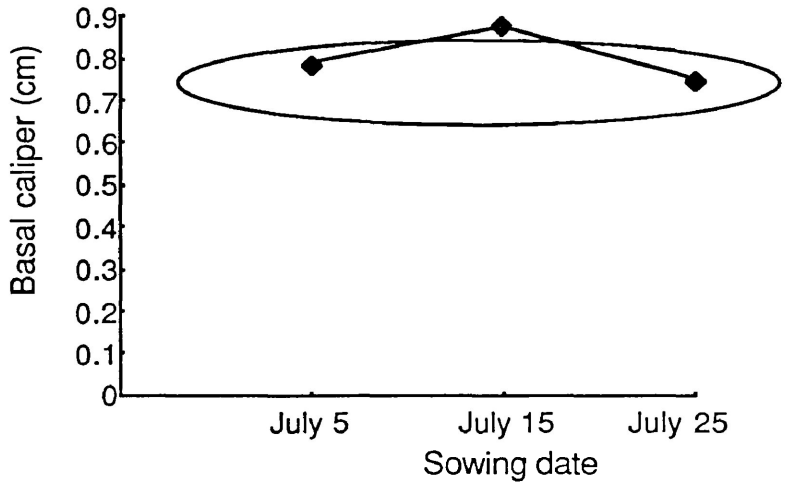

Figure 19. SOWING DATE means for the variable basal caliper. Means located within the same circle are not significantly different (Tukey's HSD).

and second sowing dates grew for a longer period of time at 25° C or more. This is the optimum temperature for dry matter production in black spruce (Pollard and Logan 1976).

However, seedlings sown on July 5 did not grow well because of their poor physiological condition. These specimens, although expected to be the tallest, were significantly shorter than the seedlings sown on July 15 and 25 (Figure 20).

One of the two best responses for root dry weight (Figure 18) was from seedlings sown on the first sowing day. This can be explained by the fact that the health problem was mainly confined to the aerial part of the seedlings.

Although the results indicated that the second SOWING DATE showed the best response, it is evident that the seedlings from the first sowing day might have achieved a superior response had the physiological problem not occurred. Unfortunately this cannot be confirmed without rerunning the experiment.

Although the effect of SOWING DATE on the number of primordia turned out to be significant when the 3-way interaction term was pooled (Table 16), the difference in the number of primordia produced along the four sowing days

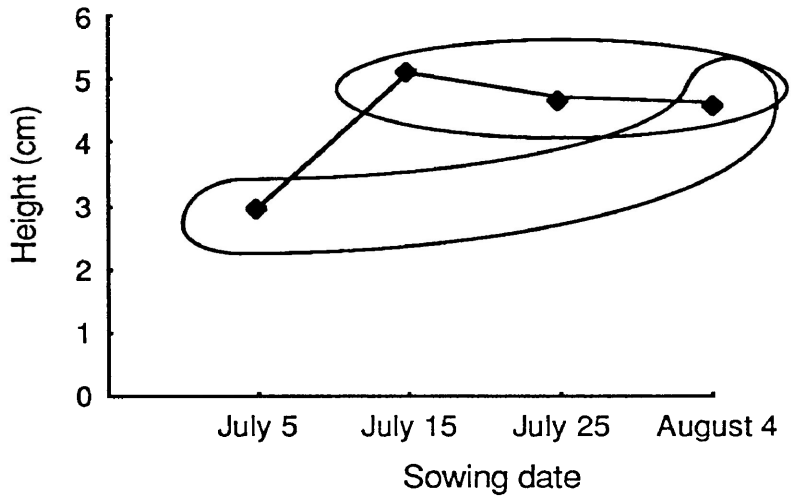

Figure 20. SOWING DATE means for the variable height. Means located within the same circle are not significantly different (Tukey's HSD).

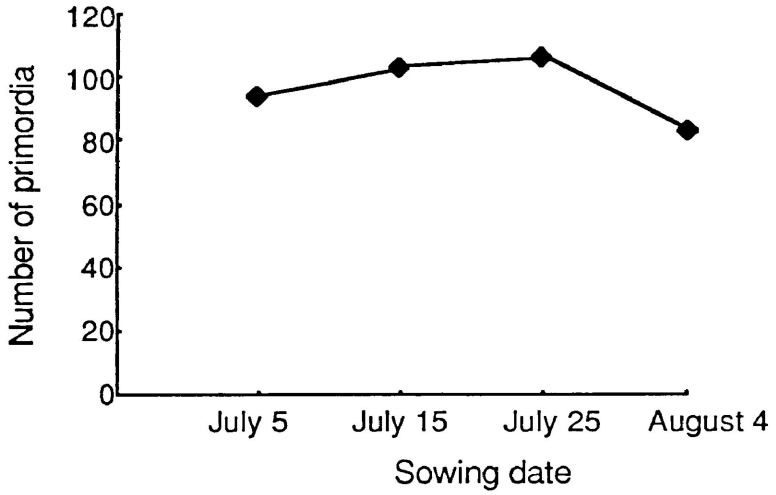

Figure 21. SOWING DATE means for the variable number of primordia. No two means are significantly different (Tukey's HSD).

was so small that it does not deserve attention (Figure 21). This was expected since all seedlings were provided with 11 weeks of extended greenhouse culture which is more than long enough to develop the maximum possible number of primordia. Similarly, there was not any significant difference in terms of bud diameter.

Short day

Over the range of naturally encountered day lengths (8 to 15 h), photoperiod does not strongly influence bud morphogenesis (Pollard and Logan 1977). This explains why SHORT DAY did not produce any significant effect on the final response variables.

It has to be pointed out that even though this experiment provides information only about the greenhouse period of the summer crop, there are several reasons to think that the seedlings from the best greenhouse treatments are going to be the best seedlings two years later at harvest time. In young seedlings such parameters as height, dry weight, basal caliper, bud size, and number of primordia, are highly correlated to subsequent growth for many years. That is a universal phenomenon for all species, as long as the physiological status of the seedlings is equal (Thomson 1985).

Two Way Interactions

Interactions, when significant, should be analyzed before the main effects (Milliken and Johnson 1984). In this experiment, however, none of the explanations for the significant two-factor interactions involve the studied factors. Furthermore, most interactions are a response to accidental situations, and some of them may simply be random effects.

In order to elucidate this situation all significant two way interactions are discussed separately for every response variable.

Height

The LD * SOD interaction is determined by the lack of parallelism between lines in Figure 22. Basically seedlings that received the 7-week LONG DAY treatment do not follow the pattern of 10- and 13-week specimens. However, the difference in height between 7-week LONG DAY seedlings sown on SOWING DATE 2, 3, and 4 is no significant, as it is for 10- and 13-week seedlings sown on the same days. Therefore, although the pattern for 7-week LONG DAY seedlings through the four sowing days is different than the one for 10- and 13-week seedlings, this could not occur if the experiment was rerun.

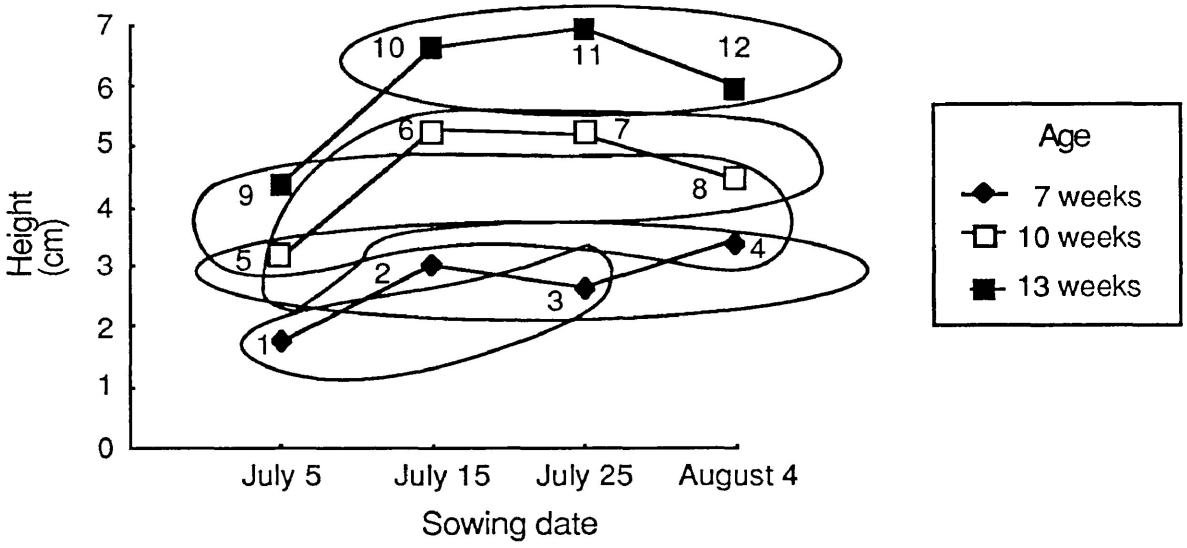

Figure 22. AGE * SOWING DATE means for the variable height. Means located within the same circle are not significantly different (Tukey's HSD).

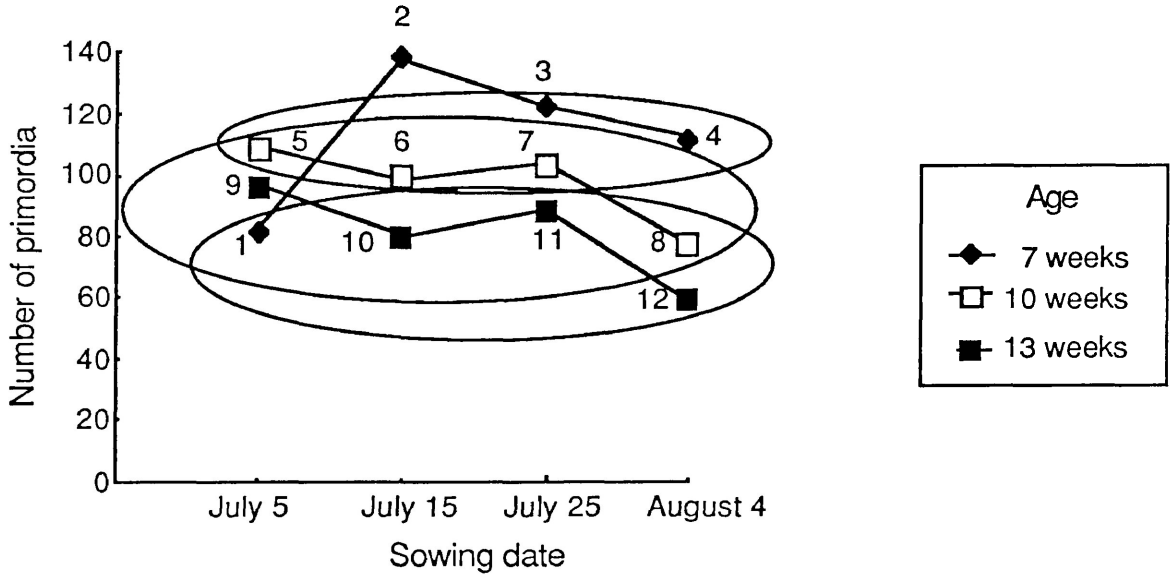

Figure 23. AGE * SOWING DATE means for the variable number of primordia. Means located within the same circle are not significantly different (Tukey's HSD).

The contrast for the means in Figure 22 are presented in Table 26 (The mean group numbers in the table correspond the ones in the figure).

Table 26. Tukey's HSD procedure for LD * SOD interaction for the response height.

Groups	1	3	2	5	4	9	8	7	6	12	10	11
1												
3												
2	*											
5	*											
4	*											
9	*	*	*									
8	*	*	*									
7	*	*	*	*	*							
6	*	*	*	*	*							
12	*	*	*	*	*	*	*					
10	*	*	*	*	*	*	*	*	*			
11	*	*	*	*	*	*	*	*	*			

* Denotes pairs of groups significantly different at the .05 level.

Number of Primordia

The LD * SOD interaction is significant mainly because of the low response obtained from 7-week LONG DAY seedlings sown on July 5, in contrast with the behavior of 10- and 13-week specimens (Figure 23). That can be explained by the fact that seedlings sown on July 5 showed a poor physiological condition that was critical for the specimens exposed to a 7-week LONG DAY treatment. The contrast for the means in figure 23 are shown in table 27.

I did not find any explanation for the second cause of the interaction, which is the outstanding response of 7-week LONG DAY seedlings sown on July 15.

Table 27. Tukey's HSD procedure for LD * SOD interaction for the response number of primordia.

Group	12	8	10	1	11	9	6	7	5	4	3	2
12												
8												
10												
1												
11												
9												
6	*											
7	*											
5	*											
4	*											
3	*	*	*	*								
2	*	*	*	*	*	*	*					

* Denotes pairs of groups significantly different at the .05 level.

Basal Caliper

The interaction LD * SOD is close to being significant because of the lack of parallelism among lines. This is mainly determined by the much higher response obtained from 10-week LONG DAY seedlings sown on July 5, in contrast with 7- and 10-week specimens sown on the same day (Figure 24). The level of significance between all the means presented in Figure 24 are given in Table 28.

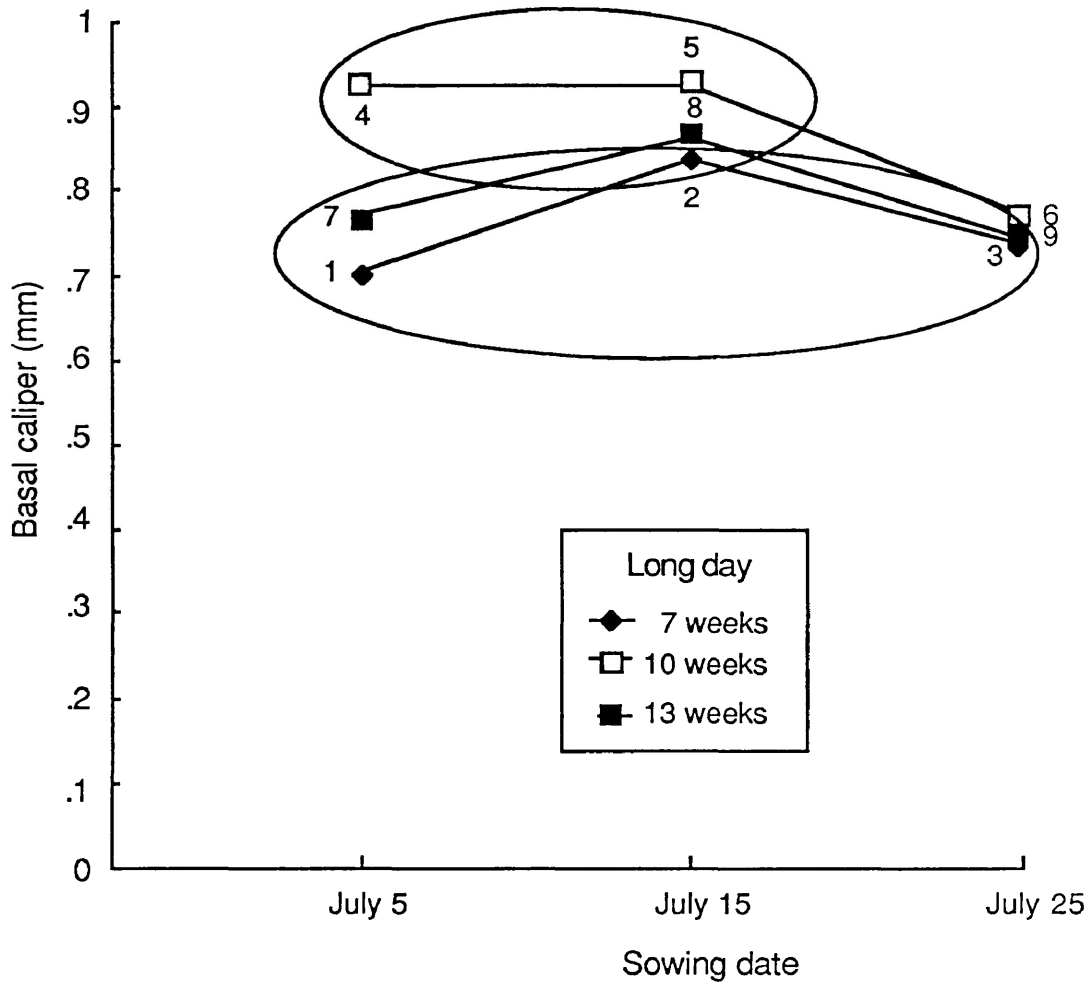

Figure 24. LONG DAY * SOWING DATE means for the variable basal caliper. Means locatedwithin the same circle are not significantly different (Tukey's HSD).

Table 28. Tukey's HSD procedure for LD * SOD interaction for the response basal caliper.

Group	1	3	9	7	6	2	8	4	5
1									
3									
9									
7									
6									
2									
8	*								
4	*	*	*						
5	*	*	*	*	*				

* Denotes pairs of groups significantly different at the .05 level.

Bud Diameter

The biological explanation for the interaction LD * SOD will not be accepted as a definite result, since the overlapped confidence intervals of the means (Figure 25) clearly show the lines could be parallel if the experiment were rerun.

A Tukey's HSD test (Table 29) shows a significant difference only between the two highest responses and the two lowest ones. It means that the best treatment combination can be either one of the higher responses, excluding only LONG DAY 1 with SOWING DATE 1 and 3.

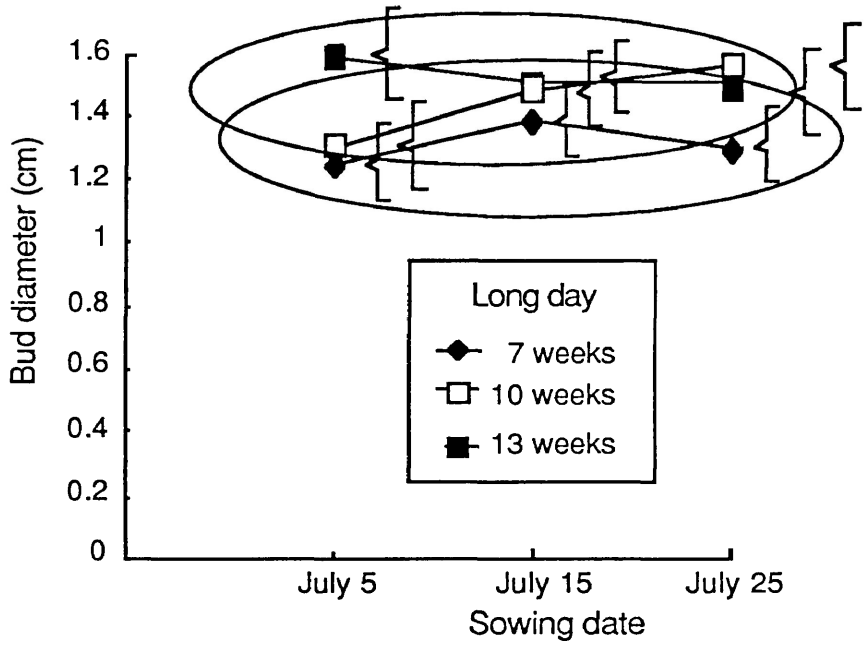

Figure 25. Confidence intervals of the LONG DAY * SOWING DATE means for the variable bud diameter. Means located within the same circle are not significantly different (Tukey's HSD).

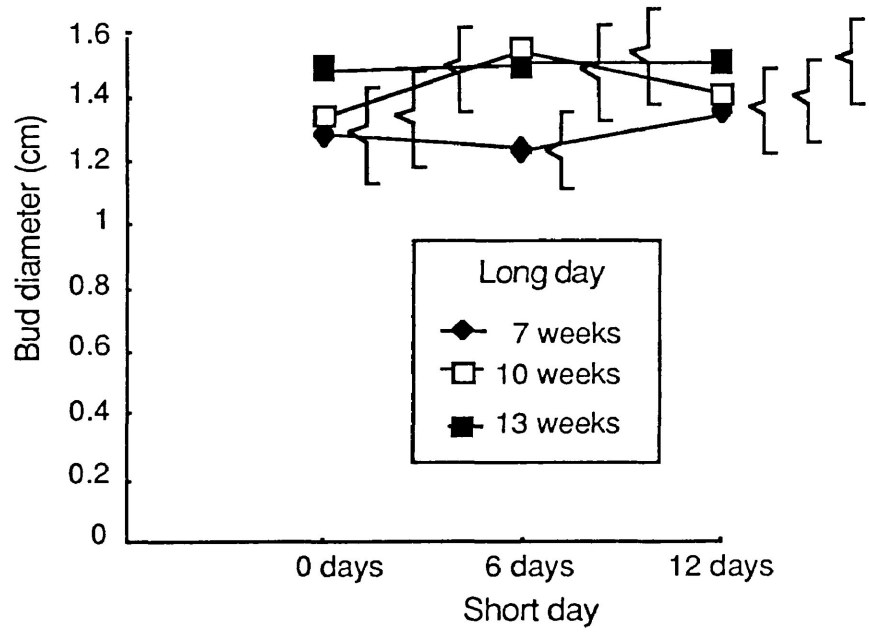

Figure 26. Confidence intervals of the LONG DAY * SHORT DAY means for the variable bud diameter.

Table 29. Tukey's HSD procedure for LD * SOD interaction for the response bud diameter.

Groups	1	3	4	2	5	9	8	6	7
1									
3									
4									
2									
5									
9									
8									
6	*								
7	*	*							

* Denotes pairs of groups significantly different at the .05 level.

In figure 26, the almost significant interaction LD * SHD is determined by the special response given by SHORT DAY 2 with LONG DAY 2, which does not follow the pattern of SHORT DAY 1 and 3, where the longer the LONG DAY treatment the bigger the bud diameter (expected result). Considering the slight difference between means (only 20% between the highest and the lowest), as well as the high overlapping shown by the confidence intervals (Figure 26), the analysis of the LD * SHD interaction does not provide meaningful results.

Root Dry Weight

The crossing lines for seedlings sown on July 5 can be explained as follows (Figure 27). Seedlings held under long days for 7 weeks gave the lowest response because of their poor physiological condition. Thirteen-week LONG DAY

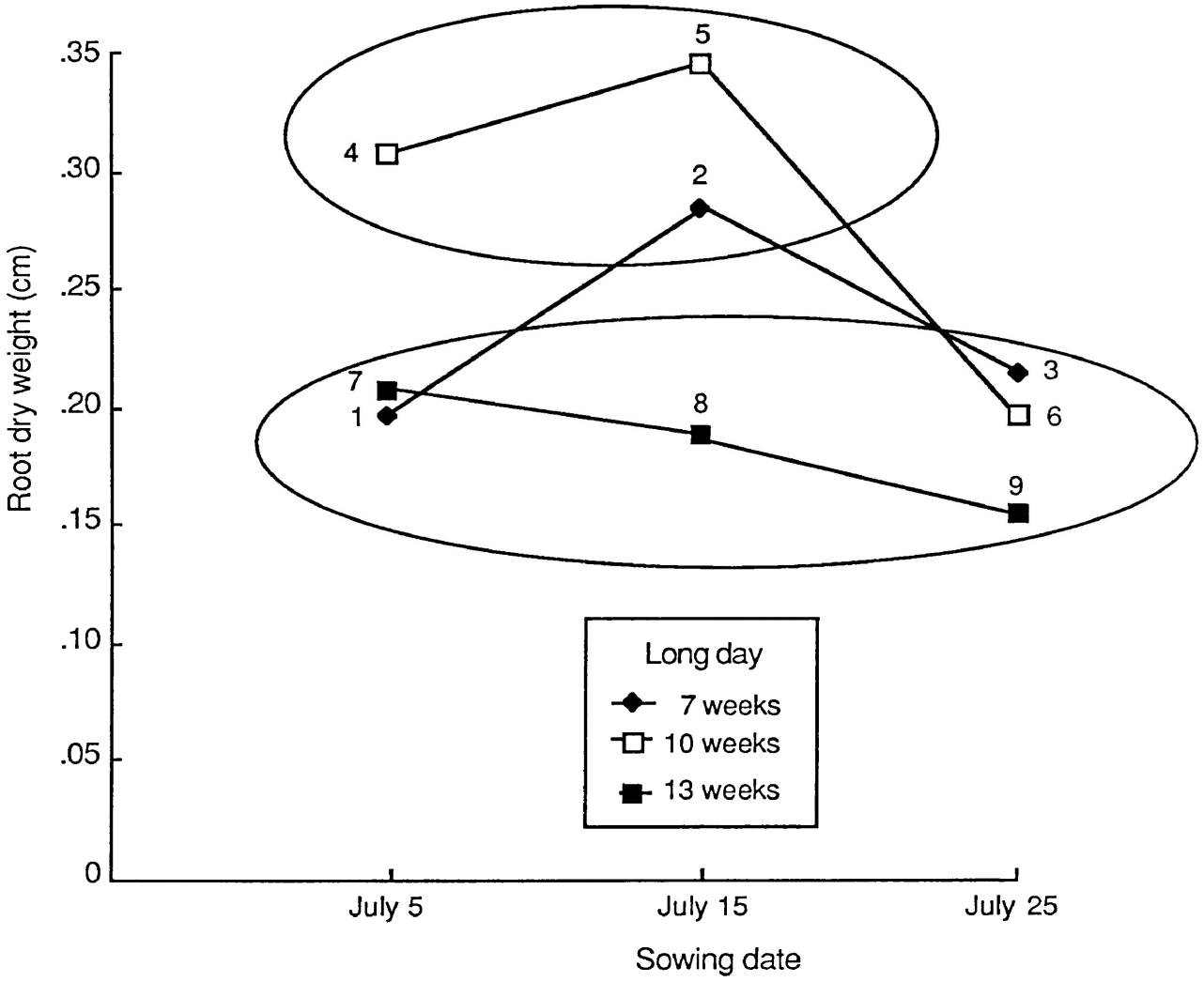

Figure 27. LONG DAY * SOWING DATE means for the variable root dry weight. Means locatedwithin the same circle are not significantly different (Tukey's HSD).

seedlings also gave a low response due to their potbound problem (seedlings too big for the size of the container). Thus 10-week specimens gave the best response for SOWING DATE 1.

For seedlings sown on July 15, although also 10-week LONG DAY seedlings gave the best response, 7-week specimens were almost just as good. Thirteen-week seedlings did not perform very well because the containers were too small for them.

The fact that seedlings of all ages gave almost the same response when sown on July 25 does not seem to have a reasonable explanation.

The contrast for the means are given in Table 30.

Table 30. Tukey's HSD procedure for LD * SOD interaction for the response root dry weight.

Group	9	8	6	1	7	3	2	4	5
9									
8									
6									
1									
7									
3									
2	*	*	*						
4	*	*	*	*	*	*			
5	*	*	*	*	*	*			

* Denotes pairs of groups significantly different at the .05 level.

SUMMARY AND APPLICATIONS OF THE MAIN RESULTS

It would not be wise to draw strong recommendations from the results because of the abundant noise in the experiment. However, after doing a cautious and thorough analysis, the main findings and their practical applications can be presented as follows.

The factor LONG DAY showed the most dramatic results. To obtain the biggest possible seedlings, without reaching a potbound situation due to the small volume of the medium, a LONG DAY treatment of 10 weeks seems to be the best choice. Even though 7-week LONG DAY seedlings produced more primordia than 10-week specimens, the difference was too small to take into consideration (16%).

No important differences were found among seedlings from different SOWING DATES. However, considering the use of a heating system is expensive, and the optimum growth temperature for black spruce seedlings is 25° C (either for dry matter production or primordia initiation), trays should be sown as soon as the winter crop is out of the greenhouse. If a black out system is not available, seedlings should not be full grown before the middle of August when the days are short enough to induce bud setting. In this case, if it is desired to grow seedlings under a LONG DAY treatment for 10 weeks, they should not be sown before the beginning of June.

The provision of a SHORT DAY (8-h photoperiod) for six or 12 days to induce bud-set is not useful when the seedlings are placed under natural photoperiod after the middle of August, since the natural day length is already short enough to induce bud-set. Although the seedlings will stop growing five days earlier when placed under an 8-h photoperiod for 12 days, this difference is insufficient to justify the use of a black-out system.

After an extended greenhouse culture period of 11 weeks all the seedlings in the experiment were dormant and, therefore, frost hardy and ready to be moved outside or into cold storage (Colombo et al. 1982). The dormant state of the seedlings after nine or 10 weeks of extended greenhouse culture could not be studied. However, it would be useful to know exactly when the seedlings become dormant, so that trays could be moved outside as soon as possible, thus reducing the risk of exposure to colder temperatures late in the fall. The Ministry of Natural Resources has laboratories for frost hardiness testing in the Swastika and Dryden nurseries. However, if the nurseryman wants to run his own test, Colombo (1984) published an operational manual which thoroughly describes an effective technique.

ADVANTAGES AND DISADVANTAGES OF THE SUMMER CROP

Seedlings from the better treatments of this summer crop were significantly bigger than the ones sown in outdoor beds at the Thunder Bay nursery last spring (1+0 seedlings) (Gonda 1986). Thus, it is possible to obtain shippable bareroot seedlings after two growing seasons, one year sooner than the traditional 1 1/2 + 1 1/2 transplanted bareroot stock. Furthermore, 1+0 seedlings initiated only a few primordia (10 - 15), usually bud-scale-like, whereas specimens from the best treatments of the summer crop experiment produced about 100 primordia.

Pregerminated seedlings had a germination rate of two seeds sown for every plant obtained at the end of the greenhouse period. Assuming a 50 percent loss on the bed the subsequent years the germination rate is still much higher than the one for seedlings sown outdoors (in the Thunder Bay nursery 12 seeds are sown for every shippable seedling). Due to the characteristics of the containers and the controlled environment in the greenhouse, it is expected to obtain more homogeneous seedlings. This is very important since the differences in size at time of planting remain the same for at least 10 more years (Armson 1974).

The main disadvantages of the summer crop are related to the container. Since the volume of the medium is only 8 cm^3 it tends to dry out fast. Thus, the container has to be

watered quite often during hot sunny days, and when the seedlings are more than 7 weeks old. Since the tray cavities are open at the bottom, some roots die by protruding from the medium. Due to the medium's special rubber-like consistency, if it dries out it shrinks and does not recover its original size when rewetted. The air space left around the medium becomes a deadly trap for protruding roots. Though these problems are mentioned as disadvantages, most may be overcome with time and experience.

A possible improvement for the summer crop is also related to the container size. The use of a bigger container may decrease the risk of frost heaving in the field by promoting the development of a bigger root system. Furthermore, a bigger container also has the potential of producing shippable seedlings after only one growing season in the field. This has been done already in Oregon, with encouraging results, for Douglas-fir, under the name of Plug + 1 seedlings production (Hahn 1984). The Plug + 1 system maximizes the advantages of accelerated transplants since nurseries are able to adjust their production targets annually instead of having to plan crop sizes three years in advance. However, a bigger container would require more greenhouse space thereby reducing greenhouse output. Hence, a thorough study should be conducted in order to find out which is the biggest

container size that can keep this production system economically feasible.

CONCLUSIONS

Even though this accelerated transplant system is not going to be the solution for all the reforestation problems, it is a valuable effort towards shortening the nursery production cycle and improving the quality of the black spruce bare root stock production in Ontario nurseries.

The best treatments of this summer crop hold promise as an effective growing regime to maximize the greenhouse output for this particular size of Castle and Cooke containers.

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APPENDICES

APPENDIX I

TREATMENT COMBINATIONS OF THE FLATS KEPT IN THE NURSERY AND UNIVERSITY COOLERS

	Nursery cooler				University cooler		
	Factor levels				Factor levels		
Flat N	A	SHD	SOD	Flat N	A	SHD	SOD
1	1	1	1	23	2	1	3
2	1	1	2	24	2	1	4
3	1	1	3	25	2	2	3
4	1	1	4	26	2	2	4
5	1	2	1	27	2	3	3
6	1	2	1	28	2	3	4
7	1	2	2	29	3	1	1
8	1	2	2	30	3	1	2
9	1	2	3	31	3	1	2
10	1	2	4	32	3	1	3
11	1	3	1	33	3	1	4*
12	1	3	2	34	3	2	1
13	1	3	3	35	3	2	2
14	1	3	3	36	3	2	3
15	1	3	4	37	3	2	4*
16	2	1	1	38	3	3	1
17	2	1	2	39	3	3	2
18	2	2	1	40	3	3	2
19	2	2	2	41	3	3	3
20	2	3	1	42	3	3	4*
21	2	3	2				
22	2	3	2				

* missing flats

APPENDIX II

RESPONSE VARIABLE MEANS FOR ALL RESPONSES AND EXPERIMENTAL UNITS

Factor levels			Response Variables (99.99=missing data)								
A	SHD	SOD	H (cm)	BAS CAL (mm)	BUD DIA (mm)	RDW (g)	TDW (g)	N.of PRIM	CES GRO (n.	BUD INI of	ADV INI days)
1	1	1	1.91	0.6900	1.2833	.1796	.1326	095.50	30	48	66
1	1	2	3.19	0.8036	1.3333	.2301	.1802	139.60	30	30	54
1	1	3	2.75	0.6627	1.2500	.2364	.1453	126.50	30	36	54
1	1	4	2.81	0.8109	1.2667	.3073	.1877	112.67	24	36	42
1	2	1*	1.71	0.7164	1.0500	.2139	.0977	050.44	30	54	66
1	2	1	1.79	0.7400	1.1833	.2205	.1035	110.27	30	60	66
1	2	2*	3.01	0.7854	1.4000	.2499	.1448	143.00	24	36	54
1	2	2	2.89	0.9373	1.3750	.3243	.2124	122.70	24	36	54
1	2	3	2.77	0.7609	1.1833	.2098	.1445	116.90	24	36	54
1	2	4	3.94	0.8373	1.3167	.2687	.2408	119.89	30	24	42
1	3	1	1.67	0.6773	1.4000	.1776	.1073	073.20	24	48	54
1	3	2	3.07	0.8300	1.3917	.3330	.2036	147.87	24	36	54
1	3	3*	2.83	0.8290	1.4500	.2218	.1432	127.40	24	36	54
1	3	3	2.24	0.6975	1.2083	.1887	.1096	120.00	18	36	54
1	3	4	3.50	0.7682	1.3417	.2460	.1574	105.17	24	24	42
2	1	1	3.20	1.0291	1.2500	.2735	.1572	108.00	30	36	54
2	1	2	5.75	1.0054	1.2833	.3265	.3749	096.81	18	30	42
2	1	3	5.75	0.7600	1.4750	.1777	.3488	089.37	24	24	42
2	1	4	4.70	0.7545	1.2083	.1846	.2939	083.70	18	24	42
2	2	1	3.25	0.8800	1.3417	.3040	.2780	103.70	24	36	54
2	2	2	5.27	0.9454	1.7000	.4114	.4127	111.80	18	24	42
2	2	3	5.23	0.7600	1.6083	.1896	.2863	125.40	24	24	42
2	2	4	4.27	0.6973	1.4167	.1665	.2568	070.62	30	24	42
2	3	1	3.11	0.8636	1.2333	.3487	.1650	115.40	18	36	54
2	3	2*	5.11	0.8473	1.3667	.2942	.3631	090.43	18	24	42
2	3	2	5.02	0.9109	1.5083	.3535	.3378	094.90	18	30	42
2	3	3	4.51	0.8018	1.5833	.2212	.3026	097.33	18	24	42
2	3	4	4.35	0.8800	1.2917	.2035	.2069	079.89	12	24	42
3	1	1	4.40	0.7464	1.5583	.1758	.2751	085.00	24	24	42
3	1	2*	7.15	0.8473	1.3667	.1748	.4767	088.75	24	12	30
3	1	2	7.31	0.9273	1.4333	.2203	.4844	094.25	30	12	30
3	1	3	6.89	0.7173	1.6083	.1559	.4191	105.45	18	6	24
3	1	4	6.05	99.99	99.99	99.99	99.99	071.30	18	24	42
3	2	1	3.36	0.7900	1.5917	.1954	.2300	104.00	18	24	42
3	2	2	6.63	0.8527	1.4417	.1652	.4373	073.11	24	12	42
3	2	3	6.74	0.7336	1.4917	.1296	.3918	097.80	18	12	20
3	2	4	6.42	99.99	99.99	99.99	99.99	061.87	18	24	42
3	3	1	5.39	0.7764	1.5667	.2516	.3802	100.33	18	24	42
3	3	2*	6.05	0.7909	1.6083	.1718	.4651	075.80	18	12	30
3	3	2	6.09	0.9054	1.5667	.2039	.4635	068.80	18	12	30
3	3	3	7.25	0.7827	1.3250	.1777	.3925	064.50	18	12	30
3	3	4	5.43	99.99	99.99	99.99	99.99	045.25	18	18	42

* duplicated treatment combinations.