EFFECTS OF POST-BUDSET FERTILIZATION ON SECOND-CROP BLACK SPRUCE CONTAINER STOCK

by

Stephen R. Warrington©

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

Supervisor: Kenneth M. Brown

School of Forestry
Lakehead University

August, 1992

ISBN 0-315-78949-2

ABSTRACT

Warrington, S.R. 1992. Effects of post-budset fertilization on second-crop black spruce container stock. 50 pp. M.Sc.F. Thesis, Lakehead University, Major Advisor: Dr. K.M. Brown

Keywords: "Finisher" fertilizer, black spruce container stock, cold hardiness, Extended Greenhouse Culture, bud development, root development.

Second crop black spruce (Picea mariana (Mill.) B.S.P.) container stock is often treated with finisher fertilizer after the induction of budset. The objective of this experiment was to study the effect of three components of a finisher fertilizer (monoammonium phosphate, potassium nitrate and potassium sulphate) on bud development, root development and the induction of cold hardiness in second-crop black spruce container stock. Fifteen fertilizer treatments were applied to black spruce seedlings at three private seedling growers in the fall of 1988.

The analysis showed no significant difference in bud and root development among the fertilizer treatments. However, the fertilizer treatments may have had a small effect on the induction of cold hardiness. The hardiest seedlings in the experiment were treated with a fertilizer high in phosphorus and ammonium. A response surface analysis indicated that seedling hardiness may be further improved with fertilizer combinations outside of the experimental region in the direction of the strongest response.

TABLE OF CONTENTS

Page
ABSTRACT....ii

LIST OF TABLES....v

LIST OF FIGURES....vi

ACKNOWLEDGMENTS....vii

INTRODUCTION....1

LITERATURE REVIEW....3
DETAILS OF SECOND CROP CULTURE....3
The Germination Phase....3
The Juvenile Phase....4
The Height Growth Phase....4
The Induction Of Bud Set Phase....5
The Extended Greenhouse Phase....6
Promotion Of Bud Development....6
Promotion Of Root Development....7
Promotion Of Cold Hardiness....8
Freezing process....8
Stages of hardening....9
Root cold hardiness....10
Effects of fertilizer on cold hardiness....11
Measures of cold hardiness....12
Finisher Fertilizers....13

METHODS....14
EXPERIMENTAL DESIGN....14
EXECUTION OF EXPERIMENT....16
Stage 1....16
Stage 2....17
Freezing Seedlings....17
Assessment of Freezing Damage....19
Dry Weight Percent....21

RESULTS....22
PRELIMINARY ANALYSIS....22
ANALYSIS OF VARIANCE....23
RESPONSE SURFACE ANALYSIS....24
COMPARISON OF GROWERS....26

DISCUSSION....31
BUD AND ROOT DEVELOPMENT....31
COLD HARDINESS....33
COMPARISON OF GROWERS....36

CONCLUSIONS....38

LITERATURE CITED....39

APPENDIX 1: CENTRAL COMPOSITE EXPERIMENTAL DESIGN....44

APPENDIX 2: ONE-WAY UNIVARIATE ANALYSIS OF VARIANCE....46

APPENDIX 3: FACTORIAL ANALYSIS OF VARIANCE OF CT3....47

APPENDIX 4: RESPONSE SURFACE ANALYSIS OF VARIABLE CT3.....48

APPENDIX 5: LIST OF DATA USED IN THE ANALYSIS.....51

LIST OF TABLES

Page

Table 1. Important dates in growers' schedule 15

Table 2. Fertilizer treatment combinations.....16

Table 3. The dates on which fertilizer treatments were applied.....17

Table 4. Dates of freezer tests on finisher fertilizer treated seedlings.....19

Table 5. Freezing damage assessment codes.....20

Table 6. List of morphological and physiological variables considered for analysis.....22

Table 7. The correlation matrix of the nine response variables.....23

Table 8. The two significantly different CT3 treatment means.....23

Table 9. Number of needle primordia before and after the fertilizer applications.....27

Table 10. A comparison of the morphological and physiological attributes of the seedlings in the experiment by grower.....27

Table 11. A comparison of the 1988 black spruce "extended" crop from the three private seedling growers that cooperated in the experiment.....29

Table 12. The cultural techniques used by the growers to grow their 1988 black spruce "extended" crop.....30

Table A2.1. Results of the univariate analysis of variance for 8 response variables.....46

Table A3.1. Results of the analysis of variance for the critical temperature on the third test date.....47

Table A4.1. Codes used in the analysis for the 15 treatment combinations.....48

Table A4.2. Regression coefficients and associated statistics for the second-order analysis of variable CT3'.....49

Table A5.1. List of data.....51

LIST OF FIGURES

Page

Figure 1. Determining critical temperature using the whole tree assessment method.....21

Figure 2. Interaction effect of $NH_4H_2PO_4$ and KNO_3 to the critical temperature response on the third test date.....24

Figure 3. Estimated critical temperature response (°C), when K_2SO_4 = 0.....26

Figure 4. A system diagram showing how the uptake of N effects cold hardiness.....34

Figure A1.1 Central composite design with two factors (x1 and x2).....44

ACKNOWLEDGEMENTS

I would like to thank the three seedling growers, Hills Greenhouses Ltd., Hodwitz Enterprises Ltd. and Creekside Nursery Ltd., for cooperating in this experiment. I am also indebted to Dr. K.M. Brown, Dr. K.M. McClain and Dr. Al Macdonald for their advice in the planning stages and for their helpful comments on earlier drafts of this thesis. My thanks also go to the Northern Forest Development Group of the Ontario Ministry of Natural Resources (OMNR) in Timmins for their financial support, and to the OMNR, Thunder Bay District for supplying the tree seedlings for the experiment.

SRW

INTRODUCTION

Double cropping is one of several options to produce black spruce (Picea mariana (Mill) B.S.P.) container stock seedlings in greenhouses (McClain, pers. comm., 5 June 1991). The double cropping system involves growing two crops of seedlings in the same greenhouse in one year. The first crop is sown in early February, and is grown for field outplanting about four months later. The second crop is sown in late May after the first crop has been moved out of the greenhouse. The second crop is grown through the summer, stored overwinter and outplanted in the spring.

The second crop is often held in the greenhouse for an extended period in the fall (early September to late October) in order to develop new buds and condition the crop for overwinter storage. Growers refer to this period as the Extended Greenhouse Culture phase and they refer to the crop as an extended crop. The application of fertilizers is one of the treatments used to condition the crop in the Extended Greenhouse phase. The fertilizers applied during the Extended Greenhouse phase are commonly called finisher fertilizers because they are the last fertilizers to be applied to the greenhouse crop.

In the black spruce double cropping system, the risk of mortality for the second crop is higher than it is for the first crop (Anon. 1987). In the Thunder Bay area in 1987, overwintered container seedling mortality numbered 6.9 million seedlings (Anon. 1987). This unacceptable level of seedling loss had a direct cost of $600,000. Seedling mortality resulted from desiccation (drying out) because of inadequate root systems and inadequate seedling hardiness in second crop seedlings (Anon. 1987). The report by the Ontario Ministry of Natural Resources (Anon. 1987) recommended refinements to the Extended Greenhouse Cultural technique.

This thesis reports my attempt to study the fertilizer component of the Extended Greenhouse Culture phase. The purpose of my study was to determine the effects of three nutrients [monoammonium phosphate ($NH_4H_2PO_4$), potassium nitrate (KNO_3), and potassium sulphate (K_2SO_4)] on these response variables of second crop black spruce container stock:

1) bud development
2) root development
3) the induction of cold hardiness

The experiment consisted of a three-factor, central composite design executed at each of three cooperating private growers.

My major conclusion is that the finisher fertilizer treatments studied had no statistically significant effect on either bud or root development, and little effect on the induction of cold hardiness, over a wide range of levels of the component nutrients.

LITERATURE REVIEW

DETAILS OF SECOND CROP CULTURE

To produce a successful second crop of black spruce container stock, growers must achieve three production goals. First, the crop must meet the minimum criteria for size specified in the contract between the grower and the client. For example, Hills greenhouses had to meet the following seedling size standards for their 1988 extended black spruce crop: minimum height of 10 cm, oven dry weight of 400 mg and shoot/root ratio of 4.5:1. Second, the crop must be conditioned to survive overwinter storage. Crops are moved outside for overwinter storage only after they have been conditioned to survive temperatures below -15°C (Colombo et al. 1984). Third, the crop must be conditioned to survive outplanting and to initiate vigorous growth in the field. Colombo and Odlum (1984) suggest that growers aim for a minimum of 150 needle primordia in terminal buds to enhance shoot growth after outplanting.

The second crop cultural regime contains 5 distinct phases: the germination phase, the juvenile phase, the exponential height growth phase, the induction of budset phase and the extended greenhouse phase. Each phase has its own cultural objectives.

The Germination Phase

Germination of the second crop of black spruce container stock takes place in the greenhouse in late May or early June. During this phase the crop germinates and the germinants put down a radicle (Tinus and McDonald 1979). The most important variables to germination are temperature and the availability of moisture. The seed must be kept moist with frequent light watering, the temperature reasonably warm (18-21°C) and the humidity of the greenhouse atmosphere should be 60 to

80% (Tinus and McDonald 1979; Tinus 1981). Covering the seed with a coarse-textured material (e.g. perlite or granite grit) protects the seed from drying, inhibits weed growth, prevents the seed from being dislodged and does not interfere with germination (Tinus 1981). Although moisture is important, too much moisture may encourage fungal infections. Fertilizer is not needed at this stage as the germinant is supplied with food and mineral nutrients stored in the seed (Tinus 1981).

The Juvenile Phase

During the juvenile phase the seed coat is shed, therefore light becomes important because the seedling must now produce its own photosynthate (Tinus 1981). The high light intensity and long daylengths in the month of June are sufficient for the photosynthesis requirements of the juvenile seedling. However, high light intensity may increase moisture stress which can be fatal at this stage (frequent light watering can eliminate this problem) (Tinus and McDonald 1979). Light fertilization (50-75 ppm nitrogen) starts in the juvenile stage after the stored reserves in the seed have been used. A fertilizer called a starter, which is low in nitrogen (N), high in phosphorus (P) and moderate in potassium (K) (e.g. 10-52-10), is applied at the juvenile stage (Scarratt 1986). High P is used to promote root development, which is important at this stage.

The Exponential Height Growth Phase

Once seedlings are firmly established, they are capable of growing exponentially (the bigger they get the faster they grow) when growing conditions are near optimum (Tinus 1981). During this growth phase, the crop is fertilized, watered, provided with heat and sometimes given artificially-long days. The second crop is grown through the summer, and so there is usually enough heat and daylight for good growth. The optimum temperatures for height growth are 20 to 25°C in the daytime and 17-19°C in the night. Fans are used to cool

the greenhouses when temperatures become too hot (30°C). A grower fertilizer is used to meet seedling demands during the exponential growth phase. A grower fertilizer is usually a balanced fertilizer (e.g. 20-20-20) or one high in nitrogen (e.g. 34-0-0). Nitrogen is important for growth in seedlings; it forms the structure of the protoplasm for new cells and it is required to synthesize chlorophyll, needed for photosynthesis (Lavender 1984; Kramer and Kozlowski 1960). Phosphorus is important for growth as it is part of the chief medium used in energy transfer (Adenosine triphosphate) (Morrison 1974). Potassium is involved in enzyme activity and a deficiency in K will hinder translocation of carbohydrates and nitrogen metabolism (growth will be reduced) (Kramer and Kozlowski 1960; Morrison 1974). The grower fertilizer is applied at a relatively high concentration (150-300 ppm based on N) to meet the demands of the rapidly growing seedlings (Scarratt 1986).

Under natural conditions, height growth of black spruce ceases and buds are set in mid to late July (Day 1987a). In second crop greenhouse culture seedlings rarely reach a minimum height requirement by the natural budset time of mid-July and seedlings must be encouraged to grow until mid-August (Lavender 1984). Seedlings are kept growing by the maintenance of optimum growing temperatures, regular irrigation and a high concentration of grower fertilizer. Artificial lighting may be used to lengthen photoperiod (to 16 or 18 hours) when the natural photoperiod becomes shorter in August (if grower is equipped with lights). The longer photoperiod will inhibit budset and thus maintain height growth, but it is not necessary if other variables are optimum for height growth.

The Induction of Budset Phase

Budset can be induced through nutrient stress, moisture stress, by shortening the photoperiod and by reducing greenhouse temperature (Day 1987a; Glerum 1985; Tinus and McDonald 1979; Macey and Arnott 1986). Treatments are applied to set buds when the seedlings are

approximately one centimetre short of their required height. Seedlings will grow to their required height by the time these treatments take affect.

The natural shortening of the photoperiod through the summer is what triggers the induction of budset in nature. The quickest way to induce budset in the greenhouse is through the use of short photoperiod; blackout curtains are used to block light after eight hours of daylight (Colombo 1989; Macey and Arnott 1986).

Nutrient stress involves leaching the soil of nutrients and withholding fertilizer. The principle behind nutrient stress is that nutrients (especially N) are needed for growth, therefore if nutrients are leached from the soil height growth will stop. Moisture stress (withholding water) causes bud scales to initiate faster than nutrient stress (Macey and Arnott 1986). The formation of bud scales indicates the start of the bud set phase.

The Extended Greenhouse Phase

The extended greenhouse phase has three cultural objectives: bud development, root development and the induction of cold hardiness. The cultural regime is tailored to provide for each of these objectives as follows:

Promotion Of Bud Development.

Studies indicate that bud development is promoted by maintaining soil moisture at field capacity, applying fertilizers, maintaining temperature at 20°C and maintaining an eight hour photoperiod (Tinus and McDonald 1979; Colombo and Odlum 1984; Colombo et al. 1982; Colombo et al 1989). Shutting out light with blackout curtains may be necessary to shorten the photoperiod. Short photoperiod indirectly increases needle initiation by allowing more time for initiation to occur. Short photoperiod (eight hours) induces budset quickly compared to natural photoperiods (12-14 hours) yielding a longer period for bud development in the fall (Colombo and Odlum 1984; Colombo et al. 1982).

Pollard and Logan (1977) observed that photoperiods between six and fifteen hours did not effect the initiation of leaf primordia in Picea species after budset. However, there was a decrease in the rate of needle initiation as photoperiod decreased under six hours (Pollard and Logan 1977).

Like height growth, bud development also needs mineral nutrients and growing temperatures to create new cells. Experiments with black and white spruce (Pollard and Logan 1977 and 1979) concluded that needle initiation was increased 70 to 80% over the temperature range of 15° C to 25° C. Colombo et al. (1981) found that seedlings stored in low outdoor temperatures in the fall produced less than half as many needle primordia as seedlings stored in temperature controlled greenhouses (temperature maintained at 20°C). The rate of needle initiation in black spruce fed distilled water only (after bud set) was 60% of the rate in black spruce fed with a standard nutrient regime (Pollard and Logan 1979).

The number and rate of leaf primordia formation is also sensitive to the size and age of seedlings at the time of bud set (Macey and Arnott 1986; Pollard 1974a). For example a 10-week old 10-cm seedling will produce more primordia than a 16-week old 10-cm seedling (Pollard 1974a).

Promotion Of Root Development.

Root meristems exhibit two peak periods of growth each year. The first begins in late winter and continues until shortly after bud break. The second extends from late summer until mid-fall (Lavender 1984). Roots and shoots compete for the same limited supply of food. When shoots are actively growing roots are inactive. So to promote root growth, growers must first shut down shoot growth (Timmer and Martin 1982). One way to do this is by depriving seedlings of N (nutrient stress) (Timmis 1974). Others have found that root growth is enhanced in seedlings fertilized with phosphorus (P) in the fall (Waggaman 1969; Brix and van den Driessche 1974).

Seedlings with higher root growth capacity (RGC) have a better chance of survival and improved growth after spring outplanting. A fall fertilizer application improves the post-winter RGC of conifer seedlings (Brix and van den Driessche 1974; Donald and Simpson 1985).

Promotion of cold hardiness.

Freezing process. Plant tissue can be damaged by freezing if the plant is not hardy enough to withstand one or more of the following: low temperature, a fast rate of temperature decline, or a long freezing time (Asahina 1978). There are two types of freezing in plant tissue; intracellular and extracellular freezing.

Intracellular freezing occurs when ice crystals form rapidly in the protoplasm of the cell (within the cell membrane). Growing ice crystals destroy the structure of the protoplasm and probably pierce the cell membrane (Levitt 1978; Asahina 1978). In laboratory experiments where intracellular freezing was induced, plant tissue was always killed. This type of freezing occurs rarely if at all in nature as natural cooling is usually too slow (Levitt 1978), therefore it will not be discussed in detail (Refer to Franks 1981; Asahina 1978; Levitt 1978; Larcher 1980; Burke et al. 1976 for further details on intracellular freezing).

Extracellular freezing is the formation of ice crystals in the spaces between cells and spaces between cell walls and protoplasm (outside of the protoplasm). This type of freezing occurs naturally in all boreal forest tree and shrub species during winter freezes (Levitt 1978; Burke et al. 1976). Freezing starts in extracellular spaces and as cooling continues water is drawn out from the protoplasm and freezes extracellularly (Larcher 1980). Water is withdrawn from the protoplasm because the vapour pressure of the extracellular ice is lower than the supercooled solution (cooled below the freezing point without freezing) in the cell. During the process of extracellular freezing, the protoplasm is dehydrated in the same manner as evaporative dehydration

or desiccation, and the concentration of dissolved substances in the cell increases (Larcher 1980; Levitt 1978; Asahina 1978).

The nature of the injury caused by extracellular freezing is not fully understood, but several theories exist (Levitt 1978). Desiccation of the protoplasm may cause irreversible injury if enough water is drawn from the cell by freezing (Asahina 1978). However, Burke et al. (1976) believe that desiccation alone is not the cause of freezing injury. They observed that damage occurs in the membrane of most frost-damaged cells and that membrane-bound proteins may be denaturated by freezing. Further, tearing and disruption likely occur as the ice crystals grow and the desiccating protoplasm shrinks (Burke et al. 1976). Franks (1981) suggests that the increase in the intracellular solute concentration, which accompanies extracellular freezing, may have toxic effects on the cell.

The ability of cells to withstand extracellular freezing depends on the amount of cell water that is bound water and on the ability of free water to pass easily through the membrane (Larcher 1980). The cells of hardy boreal species are able to withstand a remarkable amount of shrinking and extracellular freezing (Asahina 1978). Bound water, making up about 30% of the total water in hardy tissue, is the only water that doesn't freeze at low temperatures (-30 to -40°C) (Burke et al. 1976). Bound water is comprised of water molecules bound to protoplasmic components to the extent that they are prevented from migrating to the ice surface and participating in the crystallization process (Larcher 1980; Franks 1981). After the free water has frozen extracellularly, the remaining supersaturated protoplasmic solution (containing bound water) is able to supercool to an apparently unlimited extent (Franks 1981). When hardy cells are warmed after freezing, the contracted cells absorb the water from the melting extracellular ice to recover their normal appearance and activity (Asahina 1978).

Stages of cold hardening. Cold hardiness in boreal forest

species is the ability to withstand extracellular freezing. Cold hardiness is developed in the fall in preparation for freezing winter temperatures, then lost in the spring when freezing temperatures are no longer a threat. Decreasing daylength and low night temperatures (< 10°C) induce the conclusion of shoot height growth and the transition to the dormant (hardy) state (Larcher 1980). The leaves (or needles) are the site of detection of the short-daylength stimulus which initiates the prehardening stage of acclimation (Weiser 1970). During the prehardening stage, sugars and other protective substances accumulate in the protoplasm while the amount of water in the cell decreases. Prehardening allows the cell to withstand temperatures just below zero (-3 to -5°C) without freezing (Larcher 1980; Levitt 1978). In the next stage of hardening, which is induced by the temperature regularly falling just below zero, biomembrane structure and enzymes are reorganized so that the cell can withstand the removal of water by ice formation (Levitt 1978; Larcher 1980). However, Colombo (1989) found that cold hardiness increases without cold temperature exposure in black spruce container stock. The last stage of hardening, when protoplasm achieves maximal frost hardiness, is induced by prolonged freezing temperatures below -5°C (Larcher 1980). Inhibition of bud activity increases steadily as the hardening process progresses until in November complete shoot dormancy occurs. Once dormant, a shoot can not be induced to sprout with warm temperatures until a chilling requirement is met (Larcher 1980). A chilling requirement involves exposure to low temperature for a certain period of time (usually < 0°C for three to eight weeks).

Root cold hardiness. Root cold hardiness is different from shoot cold hardiness for the following reasons: 1) roots are generally less cold hardy than shoots, 2) photoperiod has no effect on root growth, 3) root hardiness is controlled by temperature alone, 4) entire root hardiness can be lost within 24 hours of exposure to warm temperature (deacclimation takes four to six days in shoots), and 5) roots do not

have a chilling requirement when they become dormant (Green and Fuchigami 1985). Roots are more susceptible to freezing temperatures because they develop frost hardiness later than shoots and they lose hardiness quickly when exposed to warm temperatures.

Effects of fertilizer on cold hardiness. The effect that nutrients have upon cold hardiness is ambiguous. N can reduce frost hardiness when applied before bud set (Glerum 1985; Duryea 1984; Koskela 1970) by prolonging height growth and delaying bud set. However, N applied in the fall after bud set can reduce frost damage in some cases (Duryea 1984; Anderson and Gessel 1966; Benzian et al. 1974; Benzian 1966; Aldhous 1972; Roberts and Miska 1980; Brix and van den Driessche 1974). Phosphorus fertilizer applied to Sitka spruce seedlings before bud set delayed hardening in the fall (Malcolm and Freezaillah 1975). Some studies found that the application of phosphorus and potassium (K) fertilizer after bud set improved frost hardiness in conifer seedlings (Duryea 1984; Levitt 1956; Aldhous 1972; Benzian 1966; Koskela 1970; Roberts and Miska 1980). Timmis (1974) believed that the balance of K and N was important to the level of cold hardiness attained. Other reports, however, showed that N, P and K fertilizers have no effect on frost hardiness when applied after bud set (Blake et al. 1979; D'Aoust and Cameron 1981; Christersson 1973; Donald and Simpson 1985) and before bud set (van den Driessche 1980).

Various authors have discussed the possible roles of K and N in the development of cold hardiness even though experiments have not been conclusive in supporting their theories. Nitrogen is necessary for protein synthesis leading to the augmentation of the protoplasm and membranes during hardening (Timmis 1974). Potassium increases cold hardiness indirectly by increasing drought resistance, thus avoiding winter drying when the soil is frozen (Lavender 1984; Brix and van den Driessche 1974). Potassium is believed to increase cell permeability to water and increase soluble carbohydrate content in the cell (Brix and van den Driessche 1974).

The rate of fertilizer application affects cold hardiness in containerized black spruce seedlings (Colombo and Smith 1987). Colombo and Smith (1987) found freezing damage increased with fertilizer applied at a rate of up to 3 times the normal rate. Bud development and frost hardiness development were best at 9 times the normal fertilizer rate because high salt levels stressed seedlings into setting bud early (Colombo and Smith 1987).

Measures of cold hardiness. Shoot moisture content and dry matter content have been used to indicate the state of hardiness in seedlings. Colombo (1989) reported that shoot moisture content declined as frost hardiness increased in black spruce containerized seedlings. Under a short-day treatment, the dry matter content increased (moisture content decreased) in conifer seedlings as did the frost hardiness (Rosvall-Ahnebrink, 1977). Frost resistant plants have less water content than do tender plants, retain water better than do tender plants, and they contain a high proportion of bound water relative to free water (Roberts and Miska 1980). Seasonal decline in shoot moisture content can be attributed to an increase in shoot dry matter due to cell wall thickening, xylem cell wall lignification, and the augmentation of proteins and sugars in the protoplasm (Colombo 1989).

The cold hardiness of seedlings can also be measured by rating seedling tissues damage after exposure to freezing temperatures (Glerum 1985). This method is called "the growth and browning test" or "the whole tree seedling assessment method" and has been used in various studies (Timmis 1977; Blake et al. 1979). The method involves rating tissue damage on a number scale (e.g. 1 to 10). Damage is observed as tissue colour: healthy tissue is fresh green and injured tissue changes colour over time (Glerum 1985).

There are other methods of measuring cold hardiness, but they are not discussed because they were not used in my experiment. Glerum (1985) discusses several different methods of evaluating cold

hardiness.

Finisher Fertilizers

When fertilizing resumes after bud set, it may be with one of the so-called "finisher" fertilizers. Plant Products 8-20-30 is one example of a finisher fertilizer. This particular fertilizer is intended to harden off conifer seedlings in the fall. It is low in nitrogen to reduce the potential for top growth; it is high in phosphorus to encourage root growth; and it is high in potassium to increase resistance to frost (Day 1987b).

METHODS AND MATERIALS

The objective of the experiment was to determine what levels of three combined nutrients ($NH_4H_2PO_4$, KNO_3 and K_2SO_4), applied after budset, produced seedlings with the most needle primordia, the largest roots (by dry weight) and the greatest cold hardiness. Three hypotheses were derived from the literature review:

1) Bud development in black spruce seedlings should be enhanced by a fertilizer with a higher concentration of N.

2) Root development should be increased by a fertilizer with a high proportion of P applied at a low concentration.

3) Cold hardiness should be increased by a fertilizer balanced in N and K applied at a high concentration.

EXPERIMENTAL DESIGN

Finisher fertilizer treatments were applied to second crop black spruce container stock in a central composite experimental design (Khuri and Cornell 1987). The experiment was replicated in 3 complete blocks with 1 block at each of 3 cooperating private growers in the vicinity of Thunder Bay. The cooperators were: Hills Greenhouses Ltd., Hodwitz Enterprises Ltd. and Creekside Nursery Ltd.

All 3 cooperating nurseries seeded their 1988 summer crops in Japanese paperpots in late May to early June of 1988 (Table 1). Treatments were applied to stop height growth and induce budset when the stock was approximately one centimetre short of the target height (Table 1). Contracts typically specify black spruce seedlings be a minimum of 10 cm tall. The growers' cultural regimes are compared in the results section.

A complete set of fertilizer treatments was applied at each of

the 3 cooperating growers as soon as 100 percent budset was achieved (Table 1). The time of 100 percent budset was determined by Ontario Ministry of Natural Resources personnel who monitored the crops. The experimental units were entire trays of 350 Japanese paperpot seedlings.

Table 1. Important dates in growers' schedules (1988).

Grower	Crop seeded	Induction of bud set	100% bud set
Hills	May 25	August 25-31	September 15-20
Hodwitz	May 29-31	August 25-31	September 15-20
Creekside	June 1-6	August 25-31	September 15-20

The central composite experimental design consisted of a 2^3 factorial array of treatments, plus 6 "star points" and a centre point (8 + 6 + 1 = 15 treatment combinations) (See Appendix 1 for additional details). Each star point was replicated twice and the centre point was replicated 3 times (Draper 1982). Replication of some of the treatments allowed experimental error to be estimated. Altogether, 23 experimental units were needed at each grower. One tray of paperpot seedlings represented one experimental unit (300 to 350 seedlings per tray), therefore 23 trays were set aside in a greenhouse at each of the cooperating nurseries.

The 15 treatment combinations are listed in Table 2. In addition to the 3 primary nutrients [monoammonium phosphate ($NH_4H_2PO_4$), potassium nitrate (KNO_3), and potassium sulphate (K_2SO_4)] an equal amount of chelated trace elements (5.175 g/100 l water) was added to each treatment.

For each treatment, a concentrated nutrient solution was created by mixing the nutrient amounts from Table 2, plus 5.175 g of chelated trace elements, with 10 l of water. Each concentrated solution was then diluted to 1 part concentrate and 9 parts water, before it was applied to the experimental unit. The final dilution allowed the treatments to be applied at the rates shown in Table 2.

Table 2. Fertilizer treatment combinations.

Treatment number[a]	Nutrient concentration			Nominal formulation	Applic. rate
	$NH_4H_2PO_4$	KNO_3	K_2SO_4		
	..... g/100 l water			N-P-K	ppm N
1	24.30	23.25	22.28	8-20-30	62
2	7.11	6.81	6.52	7-17-26	18
3	7.11	6.81	38.03	3-8-42	18
4	7.11	39.69	6.52	11-8-13	64
5	7.11	39.69	38.03	7-5-43	64
6	41.49	6.81	6.52	10-43-11	60
7	41.49	6.81	38.03	7-28-26	60
8	41.49	39.69	6.52	11-28-24	106
9	41.49	39.69	38.03	8-21-31	106
10	0.00	23.25	22.28	6-0-45	32
11	48.60	23.25	22.28	9-30-23	91
12	24.30	0.00	22.28	6-29-23	30
13	24.30	46.50	22.28	10-15-34	94
14	24.30	23.25	0.00	12-28-21	62
15	24.30	23.25	44.55	6-15-36	62

[a]Treatment number 1 is the centre point of the design; treatments 2 to 9 are the 2^3 factorial experiment; treatments 10 to 15 are the star points of the design.

EXECUTION OF EXPERIMENT

The experiment was executed in 2 stages:

Stage One

The objective of the first stage was to determine the effect of the finisher fertilizer treatments on the growth and development of seedling terminal buds and seedling root systems. The finisher fertilizer treatments were applied 4 times at each grower between the date when 100 percent bud set was achieved and the cessation of bud growth (Table 3). Fertilizer treatments were applied by hand watering whenever the crops required water.

Sample seedlings were taken from each experimental unit to determine the state of bud and root development after the last fertilizer application. Terminal buds (from 15 seedlings per experimental unit) were fixed immediately in a mixture of formalin, acetic acid and alcohol (FAA). These buds were dissected and measured for bud diameter and number of needle primordia in February and March, 1989.

The number of needle primordia were estimated using a technique

described by Pollard (1974a). The technique involved counting the number of primordia in one spiral from the apex to the base of the bud and then multiplying by the number of spirals. Root dry weight and shoot/root ratio were used as measures of root development. Root and shoot fresh and oven dry weights were measured for 20 seedlings per experimental unit from November 7-12, 1988. The fresh and oven dry weight of the shoot were needed to determine the dry matter content of the shoot, which will be discussed in stage two.

Table 3. The dates on which fertilizer treatments were applied (1988).

	Date of treatment application			
Grower	1st	2nd	3rd	4th
Hills	Sept. 27	Oct. 3	Oct. 12	Oct. 19
Hodwitz	Sept. 27	Oct. 4	Oct. 12	Oct. 19
Creekside	Sept. 27	Oct. 3	Oct. 12	Oct. 21

Stage Two

Following the final fertilizer application, the growers continued to reduce greenhouse temperatures to further induce cold hardiness. The second stage of the experiment covered this period of temperature reduction (October 24 to November 14, 1988). The objective of this phase of the study was to determine whether finisher fertilizer treatments affected the induction of cold hardiness in black spruce seedlings.

Freezing Seedlings. Seedling cold hardiness was determined by a whole seedling assessment method. A temperature-controlled freezer (Constant Temperature Control, Ltd.) located at the Lakehead University greenhouse, which can be set to any freezing temperature down to -80°C, was used to freeze sample seedlings. In addition, the freezer temperature can be raised or lowered at specified rates. The temperature inside the freezer was monitored by three temperature probes: two air temperature probes and one soil temperature probe. Seedlings were tested by grower as the freezer was only large enough to

accommodate the sample seedlings from a single grower at one time.

Seedlings from each experimental unit were subjected to four different freezing temperatures: -10°C, -20°C, -30°C and -40°C. At each grower, each of the 23 experimental units was sampled to obtain four sets of six seedlings. Each set of six seedlings was placed upright in a small paper bag, and the bag was labelled with the treatment combination and replicate. There were 92 (4 x 23) such bags in all. The bags were placed in the test freezer at an initial temperature of +5°C.

The temperature inside the freezer was lowered at a rate of 5°C per hour until -10°C was reached. Air temperature was held at -10°C until the soil temperature inside the paper pots was -10°C (+ or - one degree). When both the air and soil temperature were -10°C, 1 paper bag of six seedlings from each experimental unit was taken out of the freezer. The temperature inside of the freezer was then lowered at a rate of 5°C per hour to -20°C. The temperature was held at -20°C for two hours at which time another bag of six seedlings from each experimental unit was removed from the freezer. This procedure was repeated for -30°C and -40°C.

As each group of frozen seedlings was removed from the test freezer, it was placed in a separate plastic bag and labelled so the seedlings in the bag could be identified as to grower, experimental unit, and test temperature. The frozen seedlings in plastic bags were refrigerated at +5°C so they would thaw slowly. At the same time, a fifth set of six seedlings, was taken from each experimental unit, placed in a labelled plastic bag and refrigerated at +5°C. This control sample was not exposed to freezing temperatures.

The freezing procedure above was performed three times for each of the three growers over a period of three weeks. This allowed the seedlings to be tested at different periods of their acclimation. Test dates are reported in Table 4.

Table 4. Dates of freezing tests on finisher fertilizer treated seedlings.

Test number	Grower	Date
1	Hills	October 24-25, 1988
1	Hodwitz	October 25-28, 1988
1	Creekside	October 29-30, 1988
2	Hills	November 1-3, 1988
2	Hodwitz	November 4-6, 1988
2	Creekside	November 7-8, 1988
3	Hills	November 9-11, 1988
3	Hodwitz	November 11-13, 1988
3	Creekside	November 13-15, 1988

Assessment of Freezing Damage. Upon completion of the freezer test at Lakehead University, the test seedlings were taken to the Thunder Bay Forest Nursery where they were held in cold storage at -4°C for two months. This was sufficient to satisfy their chilling requirement (Nienstaedt 1967). After cold storage, the seedlings were thawed slowly in the refrigerator at the Lakehead University greenhouse. During the period of January 17 to February 10, 1988, each group of 6 test seedlings was planted in a labelled pot containing a 2:1, peat:vermiculite mixture. The pots were placed in a greenhouse at Lakehead University, where the temperature was maintained at about 20°C. The seedlings were grown for three to four weeks and then assessed for freezing damage.

Freezing damage assessment was conducted between February 19 and March 24, 1988. Four seedling tissues were inspected for damage: needles, buds, roots and stem. Each tissue was rated on a scale of 1 to 5 as indicated in Table 5.

An average damage code was calculated for each tissue, within each group of six seedlings. Then, a preliminary analysis was done to detect any correlation between the damage codes of different tissues. I discovered from the analysis (Pearson's correlation) that the damage codes of the four seedling tissues were highly correlated ($r > 0.93$).

Therefore, the damage codes for the four tissues were averaged to give a damage code for the whole seedling. Thus, each experimental unit had a whole-seedling damage code for each freezing-test temperature.

Table 5. Freezing damage assessment codes.

Tissue	Damage code	Diagnostic characteristic
Needles	1	- healthy and green
	2	- 1/4 of needles dead or brown
	3	- 1/2 of needles dead
	4	- 3/4 of needles dead
	5	- all of needles dead
Buds	1	- all or most buds have flushed
	2	- 3/4 of the buds have flushed
	3	- 1/2 of the buds have flushed
	4	- 1/4 of the buds have flushed
	5	- no buds have flushed
Roots	1	- roots healthy (many long white roots)
	2	- white roots shorter and less vigorous
	3	- some dead roots and few new white roots
	4	- few roots alive (some white root tips)
	5	- all roots dead (dark brown)
Stem	1	- whole stem alive (green under bark)
	2	- top 1/4 of stem dead
	3	- top 1/2 of stem dead
	4	- top 3/4 or bottom 1/4 of stem dead
	5	- whole stem dead (brown under bark)

I decided to use the temperature at which a damage code of 4 occurred as the cold hardiness response variable. This temperature was determined by graphing the whole seedling damage codes, for an experimental unit, over freezing temperature and interpolating between the 2 observed values on either side of code 4. The temperature at which code 4 seedling damage occurred was called the critical temperature. Figure 1 illustrates how the critical temperature was calculated.

In a few cases, the test seedlings were so tender that code 4 damage occurred above -10°C. This was the case in the earliest of the three frost hardiness tests, therefore the data from this test was not analyzed. In a few other cases, the test seedlings were so hardy that code 4 damage was not observed even at the lowest test temperature of

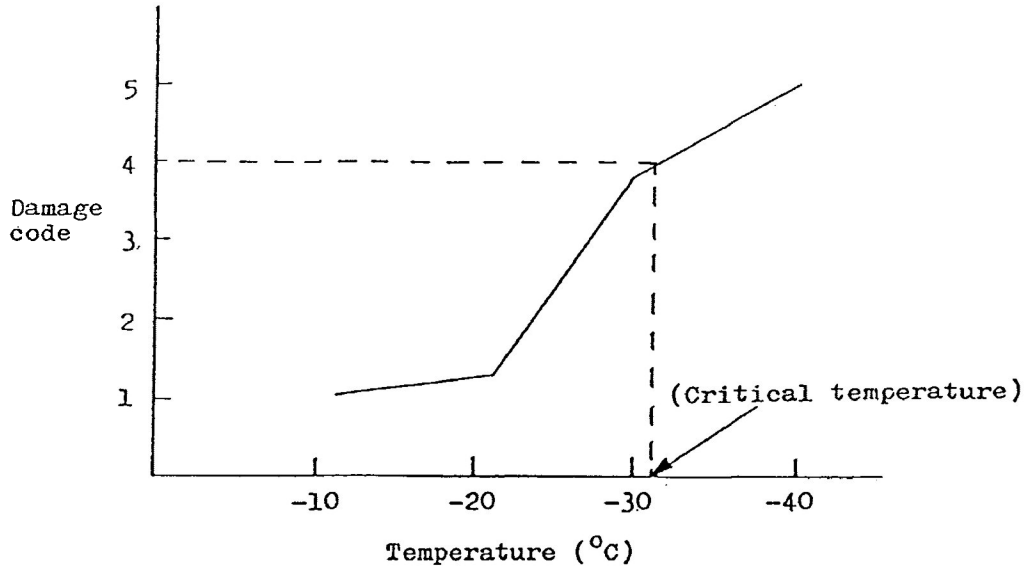

Figure 1. Determining critical temperature using the whole tree assessment method.

-40°C, so critical temperature was estimated by extrapolation.

Dry Weight Percent. Each time the experimental units were sampled to test for cold hardiness, 10 additional seedlings were taken to measure seedling fresh and dry weights. The fresh and dry weights of roots and shoots were measured for seedlings on the following dates:

test 1 - November 7-12, 1988
test 2 - November 13-18, 1988
test 3 - November 18-23, 1988

The fresh and dry weights of the shoots were used to calculate the dry weight percent of the shoots as follows:

$$DWP = (SDW/SFW)*100$$

where,

DWP = shoot dry weight percent
SDW = shoot dry weight
SFW = shoot fresh weight

RESULTS

PRELIMINARY ANALYSIS

Initially, 10 morphological and physiological variables were considered for analysis (Table 6).

Table 6. List of morphological and physiological variables considered for analysis.

Variable type/Symbol	Variable name
Morphological	
NP	number of needle primordia
BD	bud diameter
S/R	shoot/root ratio
RDW	root dry weight
DWP1	dry weight percent on the 1st test date
DWP2	dry weight percent on the 2nd test date
DWP3	dry weight percent on the 3rd test date
Physiological	
CT1	critical temperature for 1st test date
CT2	critical temperature for 2nd test date
CT3	critical temperature for 3rd test date

The CT1 could not be calculated for most treatments because most of the seedlings in that test group were killed by the first test temperature, -10°C. Consequently, CT1 was dropped from the analysis.

A correlation analysis was carried out on the remaining nine response variables. The NP and BD were found to be so highly correlated (r=0.95), that NP was dropped from further analysis. On the other hand, the correlation analysis revealed that each of the last seven variables was essentially independent of all other variables (Table 7).

Table 7. The correlation matrix of the nine response variables.

	NOPRIM	BD	S/R	RDW	DW1	DW2	DW3	CT2	CT3
NOPRIM	1.00								
BD	0.95	1.00							
S/R	-0.26	-0.20	1.00						
RDW	-0.13	-0.06	-0.29	1.00					
DW1	-0.31	-0.21	0.22	0.22	1.00				
DW2	-0.21	-0.14	0.38	0.22	0.13	1.00			
DW3	-0.37	-0.30	0.27	0.20	0.35	0.29	1.00		
CT2	0.28	0.25	-0.10	0.01	-0.08	-0.24	-0.23	1.00	
CT3	0.15	0.07	-0.05	-0.26	-0.15	-0.19	-0.31	-0.33	1.00

ANALYSIS OF VARIANCE

The eight independent response variables (BD, S/R, RDW, DWP1, DWP2, DWP3, CT2, and CT3) were analyzed separately under a one-way, univariate analysis of variance (Appendix 2). The analyses revealed no statistically significant differences between any of the 15 fertilizer treatments for seven of the eight response variables. The analysis of CT3 showed a significant effect due to fertilizer treatments.

A multiple range test (Tukey's procedure) of the CT3 treatment means revealed a significant difference between only two of the treatments. The treatment with the most monoammonium phosphate ($NH_4H_2PO_4$), and the mean level of potassium nitrate (KNO_3) and potassium sulphate (K_2SO_4) produced seedlings significantly more frost hardy than the seedlings treated with the most KNO_3, and the mean level of $NH_4H_2PO_4$ and K_2SO_4. The N-P-K formulation for these two treatments is presented in Table 8.

Table 8. The two significantly different CT3 treatment means.

Treatment number	Nutrient concentration			Nominal formulation	Applic. rate	CT3
	$NH_4H_2PO_4$	KNO_3	K_2SO_4			
	..... g/100 l water			N-P-K	ppm N	°C
11	48.60	23.25	22.28	9-30-23	91	-37.0
13	24.30	46.50	22.2	10-15-34	94	-25.6

A second ANOVA was done on the CT3 measurements using only the 2^3 factorial part of the design (Appendix 3). The analysis revealed that the interaction between nutrients $NH_4H_2PO_4$ and KNO_3 produced the most significant affect. This interaction is illustrated in Figure 2.

RESPONSE SURFACE ANALYSIS

A response surface analysis was conducted to further explore the effects of the three study nutrients on CT3. The data were adjusted prior to the analysis to remove the effect of grower. To make this adjustment, the overall mean CT3 was computed for each grower and subtracted from the CT3 values for that same grower. The adjustment created a new variable referred to as CT3′.

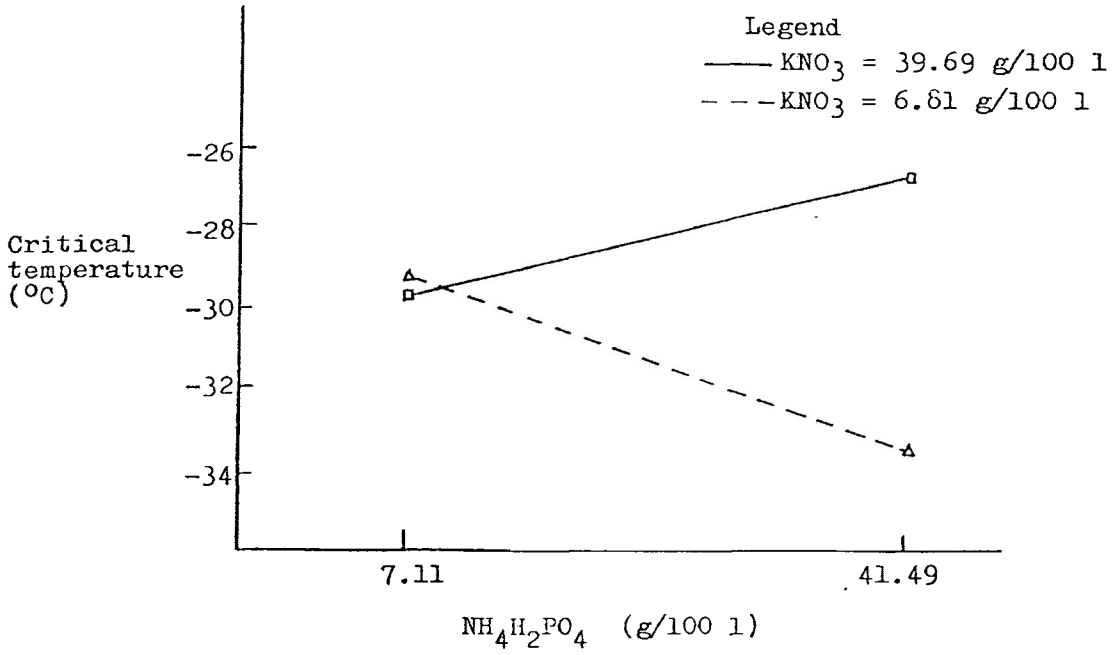

Figure 2. Interaction effect of $NH_4H_2PO_4$ and KNO_3 to the critical temperature response on the third test date.

The regression equation below resulted from fitting a second-order regression model to the adjusted response data (Appendix 4). The fitted model is;

$$CT3' = 0.1798 - 0.9966\ MP + 1.6068\ PN + 0.5354\ PS - 1.2266\ (MP)^2 + 2.1505\ (PN)^2 - 1.1850\ (PS)^2 + 1.7415\ MP*PN + 1.5424\ MP*PS + 0.5144\ PN*PS$$

where

CT3′ = adjusted critical temperature response on third test date
MP = coded variable for monoammonium phosphate
PN = coded variable for potassium nitrate
PS = coded variable for Potassium sulphate

The fitted model provided a compact summary of the CT3′ response surface, and facilitated the exploration of that surface. Only 3 of the independent variables in the model were statistically significant: PN, $(PN)^2$ and MP*PN (Appendix 4). And these 3 model variables involve only 2 of the controlled factors: PN and MP. Since PS appears to have no statistically significant effect on CT3′, the response surface over the PN-MP factor space was analyzed while holding the level of PS at 0 g/100 l (Figure 3). Figure three indicates that the critical temperature becomes lower as the $NH_4H_2PO_4$ increases and the KNO_3 decreases.

A canonical analysis (Appendix 4) was performed on the CT3′ response surface. The goal of this analysis was to find the levels of PN and MP (within the experimental region) that produce the minimum critical temperature. The coordinates of the point at which minimum critical temperature is predicted are:

$NH_4H_2PO_4$ = 40.97 g/100 l
KNO_3 = 19.30 g/100 l
K_2SO_4 = 0 g/100 l

In conventional N-P-K formulation, this fertilizer is approximately 12-38-14 at 77.5 ppm based on N.

The minimum CT3 on the response surface is -38.81°C, 7.67 degrees less than the mean CT3 of -31.14°C and 13.21 degrees less than the highest CT3 of -25.6°C. The highest CT3 resulted from the fertilizer treatment with the formulation of 10-15-34 at 94 ppm based on N.

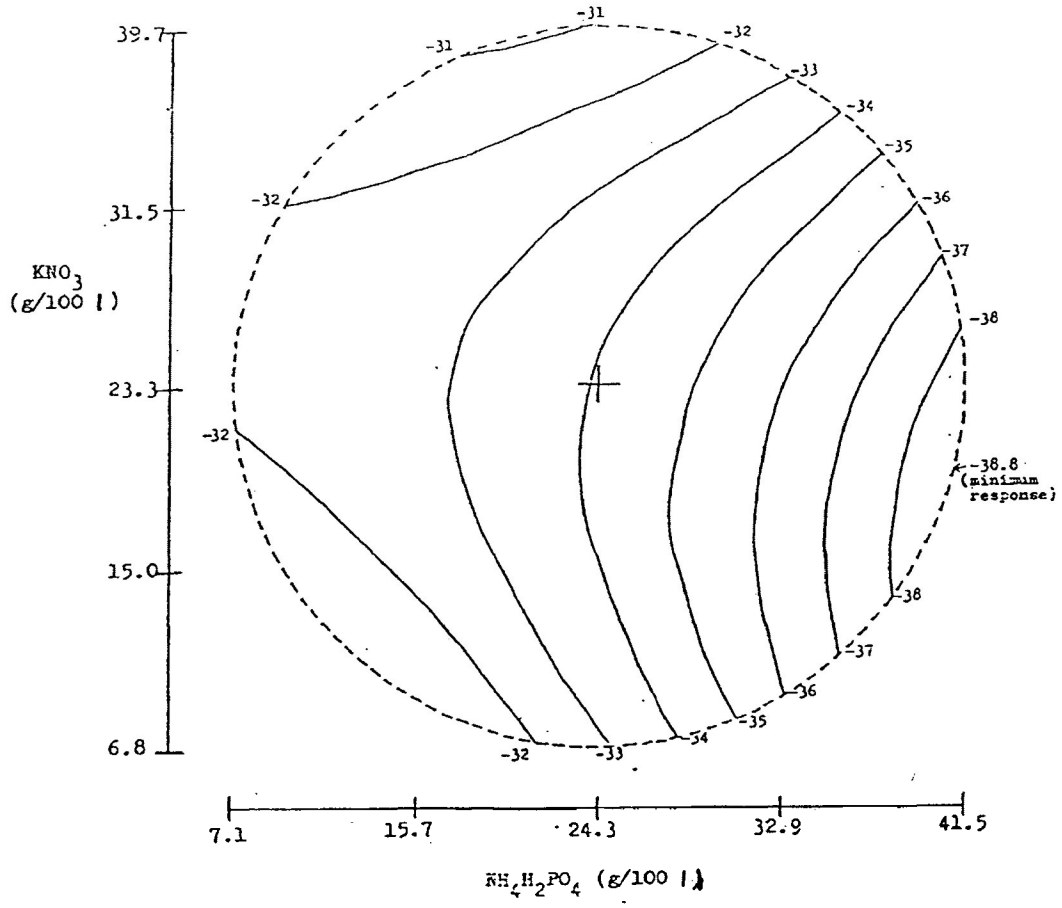

Figure 3. Estimated critical temperature response (°C), when K_2SO_4 = 0 g/100 l.

COMPARISON OF GROWERS

Although there were not many differences between fertilizer treatments, there were big differences between growers. My experiment was not designed to test statistically for differences between growers, but rough comparisons can be made by simply looking at the numbers.

Table 9 shows the average number of needle primordia from experimental seedlings for each grower prior to the first fertilizer application (September 27, 1988) and after the last fertilizer application (October 21, 1988). At Hodwitz and Creekside over half the needle primordia had formed in the terminal buds before the fertilizer treatments were applied.

Table 9. Number of needle primordia before and after the fertilizer applications.

Grower	Pre-fertilizer	Post-fertilizer
	.....average number of needle primordia.....	
Hills	44.0	102.2
Hodwitz	62.6	87.3
Creekside	68.3	134.0

Table 10 compares the morphological and physiological characteristics of the seedlings used in the experiment from the three growers.

Table 10. A comparison of the morphological and physiological attributes of the seedlings in the experiment by grower (October 23-28, 1988).

Attribute	Grower					
	Hills		Hodwitz		Creekside	
	mean	sd	mean	sd	mean	sd
needle prim. (No.)	102.2	6.5	87.3	5.9	134.0	9.6
shoot/root(dry wt)	5.6	0.7	4.9	0.7	4.7	0.5
root dry wt. (mg)	93.3	12.0	86.8	13.1	82.6	8.2
DWP3 (%)	36.7	1.9	36.7	1.9	33.9	1.5
CT3 (°C)	-33.8	6.8	-30.0	5.6	-29.6	4.7

The morphological and physiological characteristics of the growers' black spruce extended crops for 1988 are reported in Table 11. The information in this table was collected by the Ontario Ministry of Natural Resources (OMNR) Thunder Bay regional office. The OMNR data for RCD, height, shoot/root and total dry weight were not collected on the same date for all growers. The date on which each measurement was taken is listed beside the measurement in Table 11. Note that some of the measurements for the seedlings at Hodwitz were taken over a month earlier (Sept. 6) than those for the other two nurseries (Oct. 11 and 18). I assume that the height, RCD and dry weight of the Hodwitz seedlings would have increased and the shoot/root ratio decreased over the months of September and October. The difference in dates makes

Table 11. A comparison of the 1988 black spruce extended crop from the three private seedling growers that cooperated in the experiment.

Morphological and Physiological Attributes	Grower					
	Hills		Hodwitz		Creekside	
	Date	Value	Date	Value	Date	Value
Height (cm)	Oct.18	15.3	Sept.6	16.9	Oct.11	14.6
RCD[a] (mm)	Oct.18	1.6	Sept.6	1.2	Oct.11	1.6
Total dry wt.(mg)	Oct.18	504.7	Sept.6	393.7	Oct.11	542.0
shoot/root(dry wt)	Oct.18	5.6	Sept.6	7.9	Oct.11	4.0
needle primordia	Oct.17	92.0	Oct.17	105.0	Oct.17	122.0
Index of Injury(%)	Oct.17	0.0	Oct.17	0.0	Oct.17	2.6
Survival[b] (%)	Jan.6/89	70.0	Jan.6/89	83.0	Jan.6/89	96.0
"	Feb.7/89	56.0	Feb.7/89	52.0	Feb.7/89	93.0
Root growth[c](days)	Jan.6/89	11.0	Jan.6/89	9.0	Jan.6/89	7.0
"	Feb.7/89	6.0	Feb.7/89	6.0	Feb.6/89	6.0

Source: Ontario Ministry of Natural Resources, Thunder Bay Region.
[a] Root Collar Diameter
[b] Seedlings that were healthy after they were taken from overwinter storage and grown in a greenhouse for 28 days. (source: McClain and Elliot 1989)
[c] The number of days for seedlings to attain a considerable number of white root tips after being taken from overwinter storage and planted in a greenhouse.(source: McClain and Elliot 1989)

comparison between growers more difficult.

The information for the Hills crop was for the crop of seedlings that was in the same greenhouse as my experimental seedlings. The shoot/root ratio and number of primordia for the Hills crop were similar to the experimental seedlings (comparing Tables 10 and 11). The OMNR did not have data for the Hodwitz crop grown in the same greenhouse as my experimental seedlings, so the data in Table 11 under Hodwitz is for an extended crop grown in a different greenhouse at the Hodwitz nursery. The experimental seedlings at Creekside Nursery were moved from the greenhouse they were grown in to an empty greenhouse before the experiment was started. Therefore, the data in Table 11 under Creekside represents all three greenhouses that contained extended black spruce crops at the Creekside nursery.

Table 12 compares the cultural regimes used by the growers to grow their 1988 extended black spruce crops (as reconstructed in November, 1991). Ron Vilim, of Hills Greenhouses, had the most accurate records

of the three growers for the 1988 regime. Dan Hodwitz (of Hodwitz Enterprises) and Dennis Travesinutto Jr. (of Creekside Nursery) provided information from memory as they had no written records of the 1988 crop. Other information was obtained from the OMNR, Thunder Bay regional office. The information for Table 12 was collected in November of 1991.

Table 12. The cultural techniques used by the growers to grow their 1988 black spruce "extended" crop.

Cultural Techniques	Growers		
	Hills	Hodwitz	Creekside
Germination date	June 1	June 6	June 11-16 (estimate)
Growing temperature (°C)			
- daytime	27	26	22
- nighttime	20	21	19
Artificial Light	Aug. 12 -26 (18 hour day)	none	none
Start of fertilization	June 12	June 18 (estimate)	June 23 (estimate)
Fertilizer and Rate (N-P-K)			
- Starter	20-8-20 @ 125-150 ppm	10-52-10 @ 50-70 ppm	10-52-10 @ 75 ppm
- Grower	20-20-20 @ 150-200 ppm 34-0-0 @ 150-200 ppm 20-8-20 @ 200 ppm	20-20-20 @ 100-150 ppm	20-8-20 @ 150 ppm
-Finisher	20-8-20 @ 100 ppm 12-0-44 @ 100 ppm	10-52-10 @ 100 ppm	8-20-30 @ 100 ppm
Techniques used to stop height growth	- shut off artificial lighting - leach nutrients from soil - stop heating - stop fertilizing	- leach nutrients from soil - stop heating - stop fertilizing	- stop heating - soil was <u>not</u> leached - fertilizer application was continued
When above techniques where applied	- last week of August	- last week of August	- last week of August
100% bud initiation	- Sept. 15-20	- Sept. 15-20	- Sept. 15-20
Seedlings moved outside	Oct.12	Nov.15	Oct.15

DISCUSSION

BUD AND ROOT DEVELOPMENT

The fertilizer treatments studied in this experiment seemed to have had little, if any effect on bud development, root dry weight or dry weight percent, and only a slight effect on frost hardiness. Scarratt (1986) found that the growth (height, diameter, dry weight and root area index) of jack pine container stock was not affected by the type of commercial fertilizer used. He found no evidence that special starter, grower or finisher fertilizers were needed under optimum growing conditions. In Scarratt's (1986) experiment, all the fertilizers had N, P, and K in different proportions, starters were applied at rates of at least 50 ppm N, growers were applied at rates of at least 100 ppm N and finishers were applied at rates of at least 25 ppm N. Seedlings need only a certain amount of N, P and K for growth and development under optimum conditions and all the fertilizer treatments in my experiment and in Scarratt's experiment seemed to satisfy the seedlings needs.

Colombo and Odlum (1984) suggested that bud development in black spruce containerized seedlings could be maximized by maintaining temperature at 20 to 30°C, maintaining soil moisture at field capacity and applying a complete fertilizer in the fall. In my experiment, soil was kept moist and fertilizers were applied, however the greenhouse temperature was not maintained at 20 to 30°C. The greenhouse heaters at all three growers were used to maintain the growing temperatures during the height growth phase. The heaters were then shut off during the last week of August so that natural night temperatures (5 to 10°C) would help induce budset (see Table 12). After budset, the heaters were not turned back on; buds developed under natural fall

temperatures. According to records from Hills Greenhouses, the temperatures ranged from 10 to 30°C during the day and -3 to +10°C at night from September 15 to October 12, 1988 (temperatures below zero did not occur until October). Under these low temperatures the metabolism of the seedling slows and so does bud development (Colombo et al. 1981). Slower bud development requires less nutrients and all the fertilizer treatments seemed to have been sufficient in providing these nutrients.

The lack of bud-development response to my fertilizer treatments may also be due to the fact that my treatments were applied too late in the bud-development cycle. Unless blackout curtains are used, 100 percent bud initiation takes 2 to 3 weeks (Colombo 1989). During bud initiation, fertilizer should be withheld to minimize the risk of additional shoot growth. As a result, by the time 100 percent budset is achieved some terminal buds have already developed many needle primordia, and the opportunity for finisher fertilizers to effect bud development has passed. Needle initiation is highest at the beginning of bud development and gradually diminishes until initiation stops several weeks later (Pollard 1974). Table 9 showed that bud development was well along by the time fertilizer treatments were applied. The fertilizer treatments were not applied until a week after 100 percent budset occurred because the crops did not require watering until then. Water evaporates slower from soil and seedlings as temperatures decrease, thus watering is needed less frequently in the fall months.

Fertilizers high in P (e.g. 10-52-10) are used in the fall to increase root growth of container grown seedlings (Brix and van den Driessche 1974). My study failed to show a root growth response to P. Instead, I found that the fertilizer treatments without P had the same effect on fall root growth as did the treatments high in P. The role of P in root growth is not well documented. The main role of P in plants seems to be as a component of phosphate groups which are found in Adenosine Triphosphate (ATP) the chief medium for energy transfer

(Kramer and Koslowski 1960).

Timmis (1974) found that depriving Douglas-fir seedlings of N fertilizer stimulated root growth. Gagnon et al. (1988) observed no difference in root dry weight of black spruce seedlings fertilized with 3 levels of N fertilizer. The various levels of N in my experiment also had no effect on root dry weight.

My study did not contain an examination of field growth and survival. Other studies have shown, however, that fall fertilization of container grown seedlings may improve stock performance after spring outplanting. For example, stock that was fertilized in the fall has shown better spring shoot and root growth than unfertilized controls (Timmer and Munson 1989, Brix and van den Driessche 1974, Benzain 1966, Donald and Simpson 1985). Further, rapid root extension after outplanting has been shown to improve the survival of outplanted container stock (Brix and van den Driessche 1974, McCreary and Duryea 1987). During bud break and shoot expansion in the spring respiration may exceed photosynthesis, demonstrating that nutrients and organic reserves must be mobilized from last-year's shoot to provide energy material (Krueger 1967). Fertilizing in the fall provides stored nutrients that may be used by the seedling when buds break in the spring.

COLD HARDINESS

My analysis of the critical temperature data suggested a relatively weak cold hardiness response to nutrients $NH_4H_2PO_4$ and KNO_3 and no response to nutrient K_2SO_4. The most favourable (ie., minimum) critical temperature response (-38.81°C) was predicted to occur at a formulation of

$$\begin{aligned} NH_4H_2PO_4 &= 40.97 \text{ g/100 l} \\ KNO_3 &= 19.30 \text{ g/100 l} \\ K_2SO_4 &= 0 \text{ g/100 l} \end{aligned}$$

which is approximately a 12-38-14 fertilizer applied at 77.5 ppm based on N. The fertilizer formulation that produced the least favourable

temperature response (-25.6°C) was

$NH_4H_2PO_4$ = 24.30 g/100 l
KNO_3 = 46.50 g/100 l
K_2SO_4 = 22.20 g/100 l

which is approximately 10-15-34 applied at 94 ppm N.

Howell and Dennis (1980) stated that an optimum range of each nutrient exists for each species and if the concentration of the essential nutrients (N,P and K) lies outside this range, hardiness will be adversely affected. The contour map of the critical temperature response surface for the nutrients $NH_4H_2PO_4$ and KNO_3 (Figure 3) indicates that the optimum range of these two nutrients lies to the "south-east" of this map. In the direction of the optimum range the amount of KNO_3 is slowly decreasing and the amount of $NH_4H_2PO_4$ is increasing rapidly.

The source of the N fertilizer may be a factor in the results of the frost hardiness test. In my study, nitrogen came from two sources: ammonium (NH_4) and nitrate (NO_3). The seedlings with the lowest critical temperature were fertilized with a large amount of NH_4 from monoammonium phosphate and a smaller amount of NO_3 from potassium nitrate. Various studies have indicated that N in the form of NH_4 is taken-up more readily by conifer seedlings than N in the form of NO_3 (McFee and Stone 1968, Swan 1960, Christersson 1972, van den Driessche 1971). A system diagram (Figure 4) helps to explain why seedlings treated with more NH_4 than NO_3 were more frost hardy than seedlings treated with more NO_3 than NH_4 in my experiment.

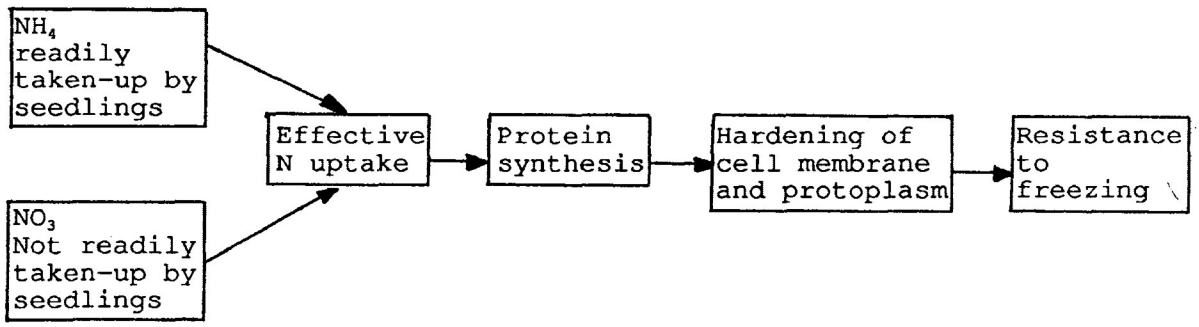

Figure 4. A system diagram showing how the uptake of N effects cold hardiness.

Seedlings fertilized with more NH_4 may have taken-up more N, although I did not verify this. Nitrogen is necessary for protein synthesis and protein is needed for the changes in the cell membrane and protoplasm (Timmis 1974). The cell membrane and protoplasm change during hardening so the cell can withstand extracellular freezing.

Contrary to most studies, Radwan et al. (1971) found that urea and a NO_3 fertilizer produced better growth and survival in Douglas-fir seedlings than an NH_4 fertilizer. The most favourable N source often changes with plant age, pH, the concentration of other nutrients and the carbohydrate contents of the plant (Christersson 1972).

Timmis (1974) believed that frost hardiness was related to a K/N balance. He found that one half the regular dose of K (75 ppm K) and a regular dose of N (50 ppm N) applied through the growing season produced hardier Douglas-fir seedlings than did the regular levels of N and K (50 ppm N and 150 ppm K). My results suggested the same effect. The increasing cold hardiness on the response surface corresponded to a decreasing K/N ratio. Along the steepest critical temperature gradient on the response surface, the amount of $NH_4H_2PO_4$ increases rapidly, the amount of KNO_3 decreases slowly and the amount of K_2SO_4 decreases rapidly. Timmis (1974) recommends a K/N ratio of approximately 0.6 for Douglas-fir seedlings. The K/N ratio that produced the hardiest black spruce seedlings in my experiment was 1.2. The fertilizer commonly used as a finisher fertilizer (8-20-30 at 60 ppm N) has a K/N ratio of 3.75.

Some studies indicate that P fertilizer should be applied after bud set to increase root growth or potential root growth (Brix and van den Driessche 1974, Waggaman 1969). My study indicated that root dry weight was unaffected by the amount of post-budset P. However, the results of the frost hardiness test indicate that a finisher fertilizer with a large amount of P (102 ppm P) may improve frost hardiness. I cannot say whether the improved frost hardiness was due to 1) the increase in P, 2) the easier uptake of NH_4 compared to NO_3, or 3) the decreasing K/N ratio.

Colombo and his coworkers (Colombo 1989, Colombo et al. 1981) have found a strong correlation between bud development and frost hardiness in black spruce. Those seedlings that were slowest to initiate buds were also the last to develop high levels of frost hardiness (Colombo and Smith 1984). Colombo (1989) also found that the dry matter content of black spruce seedling shoots was correlated with frost hardiness. Cold hardiness in seedlings starts to develop only after height growth stops and buds are initiated. When height growth stops mineral nutrients and photosynthate become available to develop roots, buds, cold hardiness and increase dry matter content.

COMPARISON OF GROWERS

My study suggested that finisher fertilizer treatments were not as important to bud development as other uncontrolled factors. Differences in the number of needle primordia were found, but the biggest differences were found between growers and not between fertilizer treatments within growers (Tables 9, 10 and 11). Presumably, differences between growers were due to differences in growing regimes used by the growers. Colombo and Smith (1987) noted that black spruce seedlings with the greatest stem diameters also had the greatest numbers of needle primordia. Therefore, cultural regimes aimed at increasing seedling stem diameter may have the carryover effect of improving bud development.

The seedlings at Creekside nursery had 15 to 20% more needle primordia in their terminal buds than the seedlings from the other two nurseries. Table 12 showed that the biggest difference in Creekside's growing regime (compared to the other two nurseries) was that fertilizers were still applied through the induction of budset. Budset at Creekside was induced by natural fall photoperiod and temperature. A study of 3 methods of inducing budset showed that each method affected bud development differently (Macey and Arnott 1986). Induction of bud set using moisture stress or nutrient stress resulted in

seedlings with fewer needle primordia than seedlings induced to set bud using a shortened photoperiod (Macey and Arnott 1986). Nutrient stress was used to induce budset at Hills and Hodwitz, and seedlings at these nurseries had fewer needle primordia than seedlings at Creekside where nutrient stress was not used.

In January and February of 1989, Creekside nursery had a higher percent of healthy seedlings in overwinter storage than the other nurseries (Table 11). Ninety-three percent of the seedlings were healthy at Creekside in February, while only 56% and 52% were healthy at Hills and Hodwitz, respectively. The cold hardiness of the seedlings at all three nurseries was similar in late October (Table 10 and 11), therefore changes to the health of the crops must have occurred between November and January.

Table 12 showed that the seedlings at Creekside did not germinate until a week after seedlings at the other nurseries. Therefore, Creekside did not start fertilizing until one week after the others. Even with this late start the seedlings at Creekside went into overwinter storage relatively the same size (height, RCD and dry weight) as seedlings from the other growers.

CONCLUSIONS

The finisher fertilizer treatments had only a slight effect on frost hardiness. There was a trend toward better frost hardiness as more nitrogen was supplied in the form of ammonium (NH_4). The increasing frost hardiness also coincided with a decreasing potassium/nitrogen (K/N) ratio. A map of the response surface for the nutrients $NH_4H_2PO_4$ and KNO_3 showed that the critical temperature was decreasing toward the south-east of the experimental region (Figure 3). Toward the south-east of the experimental region the level of $NH_4H_2PO_4$ is rapidly increasing and the level of KNO_3 is slowly decreasing. Further experiments of the nutrient levels in the area south-east of my experimental region (in Figure 3) will show whether frost hardiness can be improved further.

The finisher fertilizer treatments caused no significant differences in the bud and root development of second-crop black spruce container stock. However, there were differences in bud development between the growers. Bud development was greater in seedlings from Creekside nursery than seedlings from the other two nurseries. This difference was probably due to the different techniques used to induce budset. Hills and Hodwitz used nutrient stress to induce budset while Creekside continued fertilizing seedlings during budset.

LITERATURE CITED

Aldhous, J.R. 1972. Nursery practice. Bull. For. Comm. No. 43, 184 pp. (cited in Malcolm and Freezaillah, 1975).

Anderson, H.W. and S.P. Gessel. 1966. Effects of nursery fertilization on out-planted Douglas-fir. J. For. 64: 109-112.

Anderson, V.L. and R.A. McLean 1974. Design of experiments: a realistic approach. Marcel Dekker, Inc., New York 418 pp.

Anonymous. 1987. Report on the 1987 losses of container stock in the Thunder Bay area. Ont. Min. Nat. Resour. 12 pp.

Asahina, E. 1978. Freezing processes and injury in plant cells. Pp. 17-36 in Li, P.H. and A. Sakai (Eds.) Plant cold hardiness and freezing stress: mechanisms and crop implications. Academic Press, New York, San Francisco, London.

Benzian, B. 1966. Effect of nitrogen and potassium concentrations in conifer seedlings on frost damage. Extr. from Rep. Rothamst. Exp. Sta. (58-9). (Abstract in Roberts and Miska 1980).

Benzian, B., R.M. Brown and S.C.R. Freeman. 1974. Effect of late-season top-dressings of N (and K) applied to conifer transplants in the nursery on their survival and growth on British forest sites. Forestry 47: 153-184.

Blake, J., J. Zaerr and S. Hee. 1979. Controlled moisture stress to improve cold hardiness and morphology of Douglas-fir seedlings. Forest Sci. 25: 576-582.

Brix, H. and R. van den Driessche. 1974. Mineral nutrition of container grown seedlings. Pp. 77-83 in Tinus, R.W., W.I. Stain and W.E. Balmer (Eds). Proceedings of the North American containerized forest tree seedling symposium. Grt. Plains Agric. Council Publ. No. 68, 458 pp.

Burke, M.J., L.V. Gusta, H.A. Quamme, C.J. Weiser and P.H. Li. 1976. Freezing injury in plants. Ann. Rev. Plant Physiol. 27: 507-528.

Christersson, L. 1972. The influence of urea and other nitrogen sources on growth rate of Scots pine seedlings. Physiol. Plant. 27: 83-88.

Christersson, L. 1973. The effect of inorganic nutrients on water economy and hardiness of conifers. Studia Forest. Suecica, 103: 26 (cited in Malcolm and Freezaillah, 1975).

Colombo, S.J. 1989. Cellular and plant physiology: bud dormancy status, frost hardiness, shoot moisture content and readiness of black spruce container seedlings for frozen storage. Ont. Min. Nat. Resour., unpublished. 27 pp.

Colombo, S.J., C. Glerum and D.P. Webb. 1989. Winter hardening in first-year black spruce (Picea mariana) seedlings. Physiol. Plant. 76: 1-9.

Colombo, S.J., C. Glerum and D.P. Webb. 1982. Bud development in black spruce container stock. Ont. Min. Nat. Resour., For. Res. Note No. 32. 5 pp.

Colombo, S.J., D.P. Webb and C. Glerum. 1981. Cold hardiness and bud development under short days in black spruce and white spruce seedlings. Pp. 171-176 in Scarratt, J.B., C. Glerum and C.A. Plexman (Eds). Proceedings of the Canadian containerized tree seedling symposium. COJFRC Sym. Proc., Toronto, Ont. O-P-10. 460 pp.

Colombo, S.J. and K.D. Odlum. 1984. Bud development in the 1982-83 overwintered black spruce container seedling crop. Ont. Min. Nat. Resour., For. Res. Note No. 38. 4 pp.

Colombo, S.J. and W.A. Smith. 1987. Response of containerized black spruce and jack pine seedlings to fertilization rate and growing medium. Ont. Min. Nat. Resour., For. Res. Rep. No. 116. 15 pp.

Colombo, S.J. and W.A. Smith. 1984. Delayed bud initiation in black spruce container seedlings due to accidental day length extention. Ont. Min. Nat. Resour., For. Res. Note No. 37. 4 pp.

D'Aoust, A.L. and C. Hubac. 1986. Phytochrome action and frost hardening in black spruce seedlings. Physiol. Plant. 67: 141-144.

D'Aoust, A.L. and S.I. Cameron. 1982. The effect of dormancy induction, low temperatures and moisture stress on cold hardening of containerized black spruce seedlings. Pp. 153-162 in Scarratt, J.B., C. Glerum and C.A. Plexman (Eds). Proceedings of the Canadian containerized tree seedling symposium. COJFRC Sym. Proc., Toronto, Ont. O-P-10. 460 pp.

Day, R.J. 1987a. How seedling physiology benefits regeneration practices: Nursery management. C6-Tech. Session Paper, Soc. of Amer. Foresters, A. Convention, Minneapolis, Oct.18-21. 16 pp.

Day, R.J. 1987b. A manual of silviculture. 10th edition. School of For. Lakehead Univ., Thunder Bay, Ont. 362 pp.

Donald, D.G.M. and D.G. Simpson. 1985. Shallow conditioning and late fertilizer application effects on the quality of conifer nursery stock in British Columbia. B.C. Min. of Forests, Res. Note, No. 99. 36 pp.

Draper, N.R. 1982. Centre points in second-order response surface designs. Technometrics. 24: 127-133

Duryea, M.L. 1984. Nursery cultural practices: impacts on seedling quality. Chapter 15 in Duryea, M.L. and T.D. Landis (Eds.). Forest nursery manual: production of bareroot seedlings. Martinus Nijhoff/Dr. W. Junk Publishers, Boston, Mass. 385 pp.

Franks, F. 1981. Biophysics and biochemistry of low temperatures and freezing. Pp. 3-19 in Morris, G.J. and A. Clarke (Eds.) Effects of low temperatures on biological membranes. Academic Press, Toronto.

Gagnon, J., C.G. Langlois and J.A. Fortin. 1988. Growth and ectomycorrhiza formation of containerized black spruce seedlings as effected by nitrogen fertilization, inoculum type and symbiont. Can. J. For. Res. 18: 922-929.

Glerum, C. 1985. Frost hardiness of coniferous seedlings: principles and applications. Pp. 107-123 in Duryea, M.L. (Ed.). Proceedings: evaluating seedling quality: principles procedures and predictive abilities of major tests. For. Res. Lab., Oregon State Univ., Corvallis.

Green, J.L. and L.H. Fuchigami 1985. Overwintering container-grown plants. Dep. of Hort., Oregon St. Univ. 12 pp.

Howell, G.S.Jr. and F.G. Dennis, Jr. 1980. Cultural management of perennial plants to maximize resistance to cold stress. Chapter 9 in Olien, C.R. and M.N. Smith (Eds.) Analysis and improvement of plant cold hardiness. CRC Press, Inc., Boca Raton, Florida.

Khuri, A.I. and J.A. Cornell. 1987. Response surfaces: design and analysis. Marcel Dekker, Inc., ASQC Quality Press, New York 405 pp.

Koskela, V. 1970. Frost damage on Norway spruce, Scots pine, silver birch and Siberian larch in the forest fertilizer experiment area at Kivisuo. Folia For. Inst., For. Fenn. No. 78. 25 pp.

Kramer, P.J. and T.T. Kozlowski. 1960. Physiology of trees. McGraw-Hill Book Company, Inc., New York, 642 pp.

Krueger, K.W. 1967. Nitrogen, phosphorus, and carbohydrate in expanding one year-old Douglas-fir shoots. Forest Sci. 13: 352-356.

Larcher, W. 1980. Physiological plant ecology. 2nd Ed. (Translated by M.A. Biederman-Thorson) Springer-Verlag, New York. 303 pp.

Lavender, D.P. 1984. Plant physiology and nursery environment: interactions effecting seedling growth. Chapter 14 in Duryea, M.L. and T.D. Landis (Eds.). Forest Nursery Manual: production of bareroot seedlings. Martinus Nijhoff/Dr. W. Junk Publishers, Boston, Mass. 385 pp.

Levitt, J. 1956. The hardiness of plants. Acedemic Press, New York. 278 pp.

Levitt, J. 1978. An overview of freezing injury and survival, and its interrelationships to other stresses. Pp. 3-16 in Li, P.H. and A. Sakai (Eds.) Plant cold hardiness and freezing stress: mechanisms and crop implications. Academic Press, New York.

Macey, D.E. and T.J. Arnott. 1986. The effect of moderate moisture and nutrient stress on bud formation and growth of container-grown white spruce seedlings. Can. J. For. Res. 16: 949-954.

Malcolm, D.C. and B.C.Y. Freezaillah. 1975. Early frost damage on Sitka spruce seedlings and the influence of phosphorus nutrition. Forestry 48: 139-145.

McClain, K.M. and J. Elliott. 1989. Summary report on the assessment of private grower stock, Thunder Bay: assessment number 1. Nth. Forest Res. Unit, Ont. Min. Nat. Resour., Thunder Bay. 11 pp.

McClain, K.M. and J. Elliott. 1989. Summary report on the assessment of private grower stock, Thunder Bay: assessment number 2. Nth. Forest Res. Unit, Ont. Min. Nat. Resour., Thunder Bay. 10 pp.

McCreary, D.D. and M.L. Duryea. 1987. Predicting field performance of douglas-fir seedlings: comparison of root growth potential, vigor and plant moisture stress. New Forests 1: 153-169.

McFee, W.W. and E.L. Stone Jr. 1968. Ammonium and nitrate as nitrogen sources for Pinus radiata and Picea glauca. Abstr. of thesis in Dissert. Abstr. 28B(2), (439)

Morrison I.K. 1974. Mineral nutrition of conifers with special reference to nutrient status interpretation: a review of literature. Dept. Environ., Can. For. Serv., Publ. No. 1343, Ottawa 29 pp.

Nienstaedt, H. 1967. Chilling requirements of seven Picea species. Silvae Genet. 16: 65-68.

Pollard, D.F.W. 1974a. Seedling size and age as factors of morphogenesis in white spruce Picea glauca (Moench) Voss buds. Can. J. For. Res. 4: 97-100.

Pollard, D.F.W. 1974b. Bud morphogenesis of white spruce Picea glauca seedlings in a uniform environment. Can. J. Bot. 52: 1569-1571.

Pollard, D.F.W. and K.T. Logan. 1977. The effect of light intensity, photoperiod, soil moisture potential, and temperature on bud morphogenesis in Picea species. Can. J. For. Res. 7: 415-421.

Pollard, D.F.W. and K.T. Logan. 1979. The response of bud morphogenesis in black spruce and white spruce provenances to enviromental variables. Can. J. For. Res. 9: 211-217.

Radwan, M.A., G.L. Crouch and H.S. Ward. 1971. Nursery fertilization of douglas-fir seedlings with different forms of nitrogen. USDA Forest Serv., Res. Paper PNW-113. 6 pp.

Roberts, D.W.A. and J.P. Miska. 1980. Cold hardiness and winter survival of plants, 1965-1975. Commonwealth Agric. Bureau, Special Publ. No. 8. 407 pp.

Rosvall-Ahnebrink, G. 1977. (Artificial hardening of spruce and pine seedlings in plastic greenhouses.) Report No. 27, The Royal College of Forestry, Inst. For. Genetics, Can. Dept. Env. Library Serv., Translation OOENV. TR-1874. 10 pp.

Rosvall-Ahnebrink, G. 1981. Practical application of dormancy induction techniques to greenhouse grown conifers in Sweden. Pp. 163-170 in Scarratt, J.B., C. Glerum and C.A. Plexman (Eds). Proceedings of the Canadian containerized tree seedling symposium. COJFRC Sym. Proc., Toronto, Ont. O-P-10. 460 pp.

Scarratt, J.B. 1986. An evaluation of commercial soluble fertilizers for culture of jack pine container stock. Grt. Lakes For. Cent., Can. For. Serv. Inf. Rep. O-X-377. 21 pp.

Swan, H.S.D. 1960. The mineral nutrition of Canadian pulpwood species. I. The influence of nitrogen, phosphorus, potassium and magnesium deficiencies on the growth and development of white spruce, black spruce, jack pine and western hemlock seedlings grown in a controlled environment. Pulp and Paper Inst. Can., Tech. Rep. No. 168. (cited in McFee and Stone 1968).

Timmer, V.R. and A.D. Munson. 1989. Nutrient loading of containerized seedlings improves early outplanting performances. Abstr. on P. 9 in Smith, G.K.M. and D. Bates (Chair.) 1989. Forest Research Marketplace. Proc. of a Res. Exhibition sponsered by the Ont. Min. Nat. Resour. and For. Canada, OFRC Symposium Proc. O-P-18.

Timmer, V.R. and W.J. Martin 1982. Monitering nutrient status of containerized seedlings. Pp. 48-58 in Nurserymen's meeting Thunder Bay, Ontario, June 7-11, 1982. Ont. Min. Nat. Resour., Forest Resour. Br., 104 pp.

Timmis, R. 1974. Effect of nutrient stress on growth, bud set and hardiness in douglas-fir seedlings. Pp. 187-193 in Tinus, R.W., W.I. Stain and W.E. Balmer (Eds). Proceedings of the North American containerized forest tree seedling symposium. Gt. Plains Agric. Council Publ. No. 68, 458 pp.

Timmis, R. 1976. Methods of screening tree seedlings for frost hardiness. Pp. 421-435 in Cannell M.G.R. and F.T. Last. Tree physiology and yield improvement.

Tinus, R.W. 1981. Environmental control of seedling physiology. Pp. 75-82 in Scarratt, J.B., C. Glerum and C.A. Plexman (Eds). Proceedings of the Canadian containerized tree seedling symposium. COJFRC Sym. Proc., Toronto, Ont. O-P-10. 460 pp.

Tinus, R.W. and S.E. McDonald. 1979. How to grow tree seedlings in containers in greenhouses. USDA, Forest Serv., Gen. Tech. Rep. RM-60. 256 pp.

van den Driessche, R. 1971. Response of conifer seedlings to nitrate and ammonium sources of nitrogen. Plant and Soil 34: 421-439.

van den Driessche, R. 1980. Effects of nitrogen and phosphorus fertilization on douglas-fir nursery growth and survival after outplanting. Can. J. For. Res. 10: 65-70.

Waggaman, Wm.H. 1969. Phosphoric acid, phosphates and phosphatic fertilizers. 2nd ed., Hafner publishing company, New York. 683 pp.

Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science 169: 1269-1278.

APPENDICES

APPENDIX 1

CENTRAL COMPOSITE EXPERIMENTAL DESIGNS

A general discussion of the central composite design can be found in many general texts on experimental design (e.g., Anderson and MacLean 1974, Khuri and Cornell 1987). The layout for a 2-factor design is illustrated in Figure A1.1. My design is analogous but in 3 dimensions instead of 2.

The central composite design has 3 parts: a 2-levelled factorial (2^k), an extra point at the centre of the entire design and 2k star points (where k = the number of controlled factors). There are 2 star points on the axis of each design factor at a scaled distance from the centre point (Khuri and Cornell 1987).

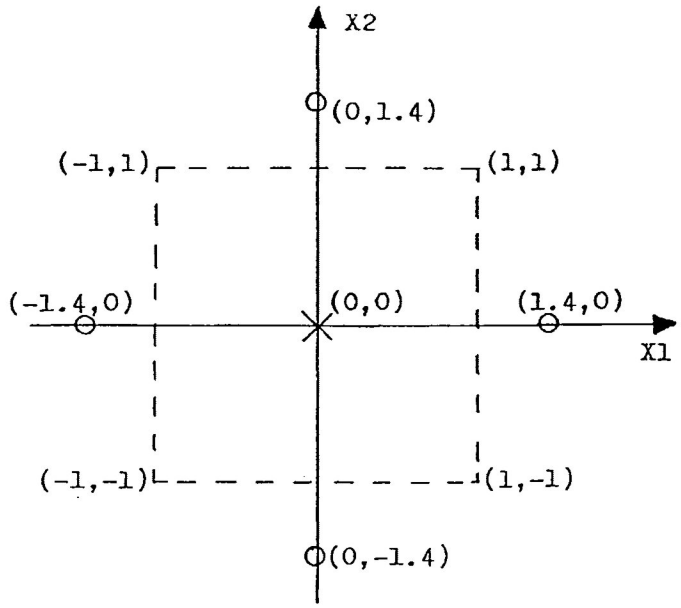

Figure A1.1 Central composite design with two factors (x1 and x2) (O-star point).

Central composite designs differ in

a. the number of controlled factors (I had 3)
b. the number of replications at centre-point (I used 3)
c. the number of replications at factorial-points (1)
d. the number of replications at star-points (2)
e. the scaled distance that the star points lie from the centre point (I used 1.414 units relative to the factorial spacing)

These choices resulted in a design that was both rotatable and orthogonal. The motives behind my choices are technical, but interested readers will find a full discussion of this aspect of the subject in Draper (1982) and in Khuri and Cornell (1987). The central composite design was used to allow the response surface to be described by a second-order regression model.

APPENDIX 2

ONE-WAY UNIVARIATE ANALYSIS OF VARIANCE

Table A2.1 shows the results of the one-way univariate analysis of variance for 8 response variables.

Table A2.1. Results of univariate analysis of variance for 8 response variables.

Response variable	Source of variation	df	MS	F	Pr(F)
BD	treatment	14	0.0009	0.58	0.85
	error	24	0.0016		
SR	treatment	14	0.3711	1.02	0.47
	error	24	0.3637		
RDW	treatment	14	0.0002	1.16	0.36
	error	24	0.0001		
DW1	treatment	14	2.2866	0.67	0.78
	error	24	3.4079		
DW2	treatment	14	8.2304	1.56	0.16
	error	24	5.2802		
DW3	treatment	14	2.7211	0.54	0.88
	error	24	5.0052		
CT2	treatment	14	20.1667	0.68	0.77
	error	24	29.6793		
CT3	treatment	14	59.0310	3.29	0.005 **
	error	24	17.9516		

APPENDIX 3

FACTORIAL ANALYSIS OF VARIANCE OF VARIABLE CT3

The analysis of variance of CT3, the critical temperature response from the third test date, for the factorial part of the design is presented in Table A3.1. Pure error was measured on the replicated star- and centre-points. Otherwise, only the factorial part of the composite design is included in this analysis.

Table A3.1. Results of the analysis of variance for the critical temperature on the third test date.

Source of Variation	df	MS	F	Pr(F)
Grower	2	17.36	<1	
restriction error	0			
MP	1	2.67	<1	
PN	1	52.57	2.93	0.10
MP by PN	1	72.79	4.06	0.055 *
PS	1	2.16	<1	
MP by PS	1	57.09	3.18	0.087
PN by PS	1	6.35	<1	
MP by PN by PS	1	0.22	<1	
Grower-by-treatment interaction	14	34.86	1.94	0.079
Total	23			
Pure error (from centre- and star-points)	24	17.95		

APPENDIX 4

RESPONSE SURFACE ANALYSIS OF VARIABLE CT3′

The Fitted Response Surface

For purposes of the response surface analysis, the 15 treatment combinations in Table 2 were coded as in Table A4.1.

Table A4.1. Codes used in the analysis for the 15 treatment combinations.

Treatment number	Controlled factors			Coded Variables		
	$NH_4H_2PO_4$	KNO_3	K_2SO_4	MP	PN	PS
1	24.30	23.25	22.28	0	0	0
2	7.11	6.81	6.52	-1	-1	-1
3	7.11	6.81	38.03	-1	-1	1
4	7.11	39.69	6.52	-1	1	-1
5	7.11	39.69	38.03	-1	1	1
6	41.49	6.81	6.52	1	-1	-1
7	41.49	6.81	38.03	1	-1	1
8	41.49	39.69	6.52	1	1	-1
9	41.49	39.69	38.03	1	1	1
10	0.00	23.25	22.28	-1.41	0	0
11	48.60	23.25	22.28	1.41	0	0
12	24.30	0.00	22.28	0	-1.41	0
13	24.30	46.50	22.28	0	1.41	0
14	24.30	23.25	0.00	0	0	-1.41
15	24.30	23.25	44.55	0	0	1.41

A second-order regression model was fitted to the response data for variable CT3′, the adjusted critical temperature response for the latest cold hardiness test date. The adjustment removed the effect of grower.

Table A4.2 reports the coefficients for the full second-order model and some associated statistics. The fitted model had a multiple R^2 of 0.302.

Table A4.2. Regression coefficients and associated statistics for the second-order analysis of variable CT3′.

Variable	Coefficient	Standard error	t-value[a]	
Intercept	0.1798	1.4991	0.1200	
MP	-0.9966	0.7419	-1.3433	
PN	1.6068	0.7419	2.1695	*
PS	0.5354	0.7419	0.7218	
MP^2	-1.2266	0.9033	-1.358	
PN^2	2.1505	0.9033	2.3808	*
PS^2	-1.185	0.9033	-1.3119	
MP*PN	1.7415	1.0439	1.6683	*
MP*PS	1.5424	1.0439	1.4775	
PN*PS	0.5144	1.0439	0.4927	

[a] The critical value for $t_{(0.05,59\ df)}$ is 1.67.

Canonical Analysis of the Response Surface

Using the second-order model a point was located on a region of the response surface that was flat: the slope of the response surface was zero when taken in all directions from this point. This point was called the stationary point (x_o). The coordinates of x_o were; -0.6, -0.1, -0.2. A new set of axes (W_1, W_2, W_3) was then created using x_o as the origin. The original axes (MP, PN, PS) used the centre point as an origin. The W_i axes define the response surface and are called the principle axes:

$$W_1 = 0.751MP - 0.116PN - 0.650PS + 0.316$$
$$W_2 = -0.609MP + 0.258PN - 0.750PS - 0.480$$
$$W_3 = 0.255MP + 0.959PN + 0.123PS + 0.280$$

A canonical form of the predicted response equation was then developed:

$$CT3' = 0.505 - 2.029W_1^2 - 0.647W_2^2 + 2.415W_3^2$$

The coefficients in the equation for CT3′ were not all negative or all positive, therefore the stationary point (x_o) was not a minimum or a maximum point on the response surface. The stationary point was at a minimax point on the response surface, meaning that the response increases when moving away from x_o along the W_3 axis and the response decreases when moving away from x_o along the W_1 and W_2 axes. The magnitude of the individual coefficients showed how quickly the surface

height changed along the W_i axes, moving away from x_o (Khuri and Cornell 1987).

I wanted to find the minimum critical temperature on the response surface. To do this, I moved along the W_1 axis which has the largest negative coefficient (-2.029), in the direction of decreasing critical temperature (Figure 3), stopping at the edge of the experimental region. The predicted minimum value of -38.8°C was interpolated. Further experiments outside of my experimental region in the direction of the W_1 axis are needed to discover whether the critical temperature decreases further. The canonical analysis of a response surface is explained in detail by Khuri and Cornell (1987).

APPENDIX 5

LIST OF DATA USED IN THE ANALYSIS

CODES USED FOR DATA IN TABLE A5.1

Tr - treatment number
Gr - grower number
MP - monoammonium phosphate
PN - potassium nitrate
PS - potassium sulphate
Rep - replicate number
NP - number of primordia
BD - bud diameter
S/R - shoot/root ratio
RDW - root dry weight
DWP1 - dry weight percent on 1st test date
DWP2 - dry weight percent on 2nd test date
DWP3 - dry weight percent on 3rd test date
CT2 - critical temperature for 2nd test date
CT3 - critical temperature for 3rd test date

Table A5.1. List Of Data

Tr	Gr	MP	PN	PS	REP	NP	BD	S/R	RDW	DWP1	DWP2	DWP3	CT2	CT3
1	1	0	0	0	1	99	0.98	5.5	0.080	30.9	37.9	33.9	-30.5	-31.7
1	1	0	0	0	2	106	1.02	5.6	0.088	36.5	37.7	36.2	-36.3	-27.8
1	1	0	0	0	3	101	1.01	4.4	0.102	36.2	37.6	38.6	-29.1	-28.0
2	1	-1	-1	-1	1	100	1.01	4.9	0.081	33.3	35.7	37.3	-30.7	-29.7
3	1	-1	-1	1	1	87	0.93	5.8	0.087	30.5	41.0	34.5	-32.4	-36.7
4	1	-1	1	-1	1	108	1.05	5.8	0.103	36.6	36.6	35.1	-27.5	-28.0
5	1	-1	1	1	1	112	1.06	4.3	0.115	33.8	37.5	37.9	-23.1	-32.0
6	1	1	-1	-1	1	109	1.01	4.5	0.088	35.6	39.1	35.1	-31.8	-39.4
7	1	1	-1	1	1	100	1.01	6.0	0.101	33.4	35.0	33.9	-19.2	-38.4
8	1	1	1	-1	1	110	1.04	5.7	0.100	32.9	42.9	35.0	-30.0	-26.0
9	1	1	1	1	1	99	1.01	7.6	0.072	37.6	35.3	36.2	-22.5	-22.6
10	1	-1.4	0	0	1	99	1.03	5.3	0.105	39.9	38.8	35.8	-29.2	-34.8
10	1	-1.4	0	0	2	101	1.01	5.3	0.101	32.8	37.6	37.8	-31.2	-43.9
11	1	1.4	0	0	1	96	0.98	6.0	0.101	39.2	39.8	34.6	-28.4	-49.8
11	1	1.4	0	0	2	108	1.00	5.1	0.117	38.3	39.4	39.1	-27.5	-37.9
12	1	0	-1.4	0	1	93	1.01	5.8	0.092	39.1	39.8	37.7	-28.4	-32.9
12	1	0	-1.4	0	2	107	1.04	5.9	0.074	36.9	41.5	36.9	-29.6	-33.8
13	1	0	1.4	0	1	110	1.04	6.5	0.088	32.5	37.9	38.4	-21.1	-27.5
13	1	0	1.4	0	2	108	1.08	5.0	0.091	36.4	37.2	37.3	-10.6	-27.1
14	1	0	0	-1.4	1	104	1.05	5.4	0.088	35.2	38.2	41.3	-29.7	-43.5
14	1	0	0	-1.4	2	102	1.04	5.9	0.101	36.3	39.3	36.2	-29.7	-38.9
15	1	0	0	1.4	1	100	1.00	6.0	0.096	34.4	46.1	36.0	-30.0	-27.3
15	1	0	0	1.4	2	91	0.95	6.6	0.085	33.9	41.7	38.9	-30.5	-39.3

Table A5.1 (continued)

Tr	Gr	MP	PN	PS	REP	NP	BD	S/R	RDW	DWP1	DWP2	DWP3	CT2	CT3
1	2	0	0	0	1	90	0.95	6.2	0.067	34.4	37.3	38.8	-31.0	-34.1
1	2	0	0	0	2	85	0.95	3.9	0.100	34.0	34.3	35.0	-34.0	-32.8
1	2	0	0	0	3	97	1.02	4.2	0.113	36.6	35.8	36.8	-31.7	-31.4
2	2	-1	-1	-1	1	97	0.98	5.1	0.081	34.4	35.7	32.9	-27.9	-24.7
3	2	-1	-1	1	1	82	0.91	6.8	0.069	34.9	34.6	34.3	-19.3	-20.4
4	2	-1	1	-1	1	79	0.87	4.1	0.099	32.5	34.2	35.1	-18.7	-24.1
5	2	-1	1	1	1	97	1.00	4.4	0.105	35.3	36.6	37.5	-30.1	-30.1
6	2	1	-1	-1	1	85	0.90	5.2	0.082	33.6	36.2	38.6	-33.5	-38.3
7	2	1	-1	1	1	90	0.94	4.3	0.099	32.8	36.5	36.9	-31.4	-29.8
8	2	1	1	-1	1	87	0.93	4.5	0.087	36.7	35.6	35.1	-30.5	-33.7
9	2	1	1	1	1	88	0.93	4.5	0.076	33.3	37.2	33.4	-30.1	-31.7
10	2	-1.4	0	0	1	90	0.95	4.9	0.082	35.0	35.8	36.8	-30.6	-29.2
10	2	-1.4	0	0	2	76	0.83	4.6	0.098	35.7	36.5	34.9	-13.4	-21.8
11	2	1.4	0	0	1	83	0.92	4.8	0.082	33.4	36.2	30.5	-28.2	-34.6
11	2	1.4	0	0	2	86	0.91	4.7	0.100	34.1	35.4	36.4	-20.4	-30.3
12	2	0	-1.4	0	1	84	0.88	4.9	0.096	32.8	37.9	32.7	-29.8	-30.7
12	2	0	-1.4	0	2	86	0.92	4.7	0.073	33.0	36.7	36.8	-28.8	-28.9
13	2	0	1.4	0	1	93	0.93	5.8	0.068	36.8	36.6	36.8	-31.9	-17.1
13	2	0	1.4	0	2	89	0.92	4.4	0.082	35.4	35.1	32.4	-30.6	-24.7
14	2	0	0	-1.4	1	79	0.87	5.5	0.071	34.9	34.3	36.9	-32.3	-34.1
14	2	0	0	-1.4	2	94	0.96	4.8	0.084	35.6	36.8	34.9	-10.7	-32.0
15	2	0	0	1.4	1	80	0.83	5.1	0.084	36.7	35.3	39.3	-30.6	-37.0
15	2	0	0	1.4	2	91	0.94	4.7	0.098	35.8	36.1	34.3	-32.0	-38.7
1	3	0	0	0	1	140	1.15	4.3	0.092	31.0	34.4	32.2	-21.2	-29.7
1	3	0	0	0	2	126	1.09	4.5	0.087	31.7	31.6	34.4	-29.7	-27.9
1	3	0	0	0	3	148	1.15	4.6	0.082	34.9	31.8	31.3	-19.8	-18.5
2	3	-1	-1	-1	1	136	1.13	4.2	0.095	33.5	34.2	31.9	-19.9	-28.5
3	3	-1	-1	1	1	137	1.12	4.0	0.093	31.9	37.9	34.5	-29.4	-35.9
4	3	-1	1	-1	1	148	1.15	4.2	0.091	33.9	35.4	36.1	-24.0	-35.0
5	3	-1	1	1	1	120	1.08	5.2	0.79	32.1	35.3	34.7	-22.3	-29.9
6	3	1	-1	-1	1	135	1.11	5.7	0.068	32.8	38.5	34.4	-25.1	-26.3
7	3	1	-1	1	1	115	1.10	4.1	0.82	34.8	35.6	34.5	-24.8	-28.5
8	3	1	1	-1	1	125	1.05	5.2	0.080	34.5	35.9	34.7	-33.3	-28.7
9	3	1	1	1	1	143	1.10	5.4	0.080	31.2	34.5	31.0	-24.0	-19.4
10	3	-1.4	0	0	1	143	1.15	4.7	0.083	33.9	35.5	33.7	-26.2	-34.9
10	3	-1.4	0	0	2	131	1.10	5.4	0.077	34.0	34.7	35.5	-25.4	-29.6
11	3	1.4	0	0	1	132	1.10	4.6	0.079	33.1	34.6	34.5	-16.6	-36.4
11	3	1.4	0	0	2	122	1.09	5.1	0.075	31.0	34.4	32.4	-26.9	-33.2
12	3	0	-1.4	0	1	121	1.10	5.0	0.066	35.9	33.8	35.0	-28.4	-32.3
12	3	0	-1.4	0	2	130	1.10	4.4	0.089	33.6	35.6	34.4	-20.3	-24.3
13	3	0	1.4	0	1	136	1.07	4.2	0.097	32.9	37.0	35.2	-27.0	-28.7
13	3	0	1.4	0	2	139	1.12	5.0	0.072	31.0	37.6	33.9	-28.6	-28.6
14	3	0	0	-1.4	1	130	1.11	4.6	0.081	35.4	34.0	32.9	-29.0	-36.5
14	3	0	0	-1.4	2	142	1.11	4.8	0.079	32.9	35.7	35.8	-20.6	-29.2
15	3	0	0	1.4	1	130	1.10	4.9	0.088	33.3	35.5	35.2	-26.2	-30.9
15	3	0	0	1.4	2	151	1.23	4.0	0.085	34.7	34.7	31.9	-19.3	-28.0