LAKEHEAD UNIVERSITY

THE MATING SYSTEM AND POPULATION STRUCTURE IN A BLACK SPRUCE (*Picea mariana* (Mill.) B.S.P.) CLONAL SEED ORCHARD IN NORTHWESTERN ONTARIO

by

JOHN W. BARRETT (C)

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ABSTRACT

Multi-locus enzyme systems were studied in a black spruce (*Picea mariana* (Mill.) B.S.P.) clonal seed orchard in northwestern Ontario. The embryonic and megagametophytic tissues of each clone were sampled and electrophoretically analysed to examine the inheritance pattern of 8 polymorphic loci. With the exception of leucine aminopeptidase (Lap) and aconitase (Aco), allozyme segregation followed expected 1:1 ratios. The mating system is characterized by a moderate level of selfing (s=0.15) and a small effective population size. The ratio of genetically effective males to receptive females was calculated to be 0.31. Although the parental population was in Hardy-Weinberg equilibrium, the majority of the enzyme systems examined revealed a deviation from the Hardy-Weinberg equilibrium in the filial generation. Several loci exhibited heterogeneous pollen pools and there was an observed excess of heterozygotes. Indications of non-random mating and small effective population size invalidate two basic seed orchard assumptions, namely, random mating and large population size.

Table of Contents

List of Tables

I. INTRODUCTION

Forest tree improvement involves the directed control of parental stock and forest management activities towards the improvement in the overall yield and quality of forest products. Towards this goal Zobel and Talbert (1984) outline five steps required for the development of a forest tree improvement program as follows: First, the species range and geographic sources must be determined. Second, the amount, kind and causes of variability within the species must be determined. Third, packaging of desired qualities into improved individuals, such as development of trees with combinations of desired characteristics, is necessary for improvement success. Fourth, mass production of improved individuals for reforestation purposes must be obtained. Finally, a broad genetic base must be developed and maintained to fulfill the needs of advanced generations.

The implementation of these steps proceed in one of two possible directions. Traditionally, the first direction taken has been the development of programs directed towards the immediate gains which can be achieved through the use of a few genetically superior parents for production of planting stock for operational programs. A major benefit of these tree improvement programs not often recognized is the production of large and regular seed crops that are suitable for forest operations. The other, longer term approach is the development of programs directed toward future generations. There is a need to provide the broad genetic

base that is essential for continued progress over many generations. This requires the conservation of gene pools and unique genotypes as a protective move against a reduced gene base (Conkle 1979; Adams 1983; Libby 1973). The standard method of producing genetically improved seed in operational quantities is to use the seed orchard approach (Zobel and Talbert 1984; Faulkner 1975). The utility of seed orchards has been widely documented for many kinds of benefits (Faulkner 1975).

Shen *et al.* (1981) outline the assumptions necessary for seed orchard establishment:

1. Selection of the best phenotypes within a homogeneous climatic region;
2. Grafting and random plantation in a seed orchard;
3. Localization of seed orchards in sites with favourable climate and site which encourages flowering and seed ripening;
4. Isolation against contaminating pollen from outside the seed orchard;
5. No clones should dominate pollen production and fertilization;
6. The frequency of selfing should be kept to a minimum level.

Chief among these assumptions is that flowering and pollen exchange among genotypes in the orchard will be uniform and equal. Deviation from this standard would lead to non-random mating and the plethora of problems associated with

assortative breeding such as decreased yields of poor quality seed, low seedling vigour and loss of genotypes.

A common research tool presently used in forest genetic studies is starch gel electrophoresis. Use of this technique to resolve isozymes enables direct genetic evaluation of the status of forest tree genetic resources. Tree breeders and forest geneticists can utilize this valuable tool to increase their knowledge of the genetic relationships between and within species of coniferous trees and to solve problems encountered in tree breeding and genetic research (Yeh 1979). Determination of tree genotypes has enabled foresters to study such diverse problems as clonal identification (Cheliak and Pitel 1984a), establishment of forest tree mating systems (Yeh *et al.* 1983; King *et al.* 1984; Cheliak, Morgan *et al.* 1985), seed orchard efficiency (Adams 1979), determination of inheritance patterns (King and Dancik 1983) and linkage analyses (Neale *et al.* 1984).

The objective of this study was to examine the population dynamics of black spruce (*Picea mariana* (Mill.) B.S.P.) within a clonal seed orchard. Three problems were identified. It was my intention to, first, establish the mating system through electrophoretic analysis of parental and filial genotypes within the clonal seed orchard. Second, to determine the effective population size of the black spruce population, thereby determining the number of seed orchard members contributing to the next generation. Finally, to verify pollen pool homogeneity via the

estimation of pollen ratios in the filial generation.

II. LITERATURE REVIEW

A review of the population structure of boreal conifers and of seed orchard studies is necessary as background for assessing the population dynamics of a black spruce seed orchard. The term population dynamics blankets the genetic and ecological responses of an organism to its environment. For the past two decades forest geneticists have examined genetic diversity of forest species as a background for decisions about tree improvement strategies.

Population structure refers to the totality of ecological and genetic relationships among the members, individually, as well as the subdivisions, of a biological species (Jain 1979). When a population is subdivided, the amounts of genetic relatedness among the parts of the population can differ. This genetic cohesion depends primarily on the amount of genetically effective migration or gene flow that takes place among the subgroups (Hedrick 1983). A population may be considered structured if it contains localized subpopulations in which there is genetic drift, if mating is not random throughout the population, or if migration does not have equal probabilities. In other words, all evolutionary forces except mutation and selection contribute to population structure (Hedrick 1983). Relevant literature concerning conifer mating systems, estimations of inbreeding and selfing in coniferous species, the effective population size, and estimations of gene flow and pollen dispersal for the Pinaceae are reviewed.

Mating System

Mating system studies focus on the process of genetic transmission at the population level. It has been shown that plants exhibit a wide variety of mating systems including a)regular systems of inbreeding and frequently self-fertilization; b) negative assortative mating due to various kinds of incompatibility systems; and c) effective inbreeding due to the clustering of related individuals within a small neighbourhood (Clegg 1980).

Mating system assessments have been based on the analysis of floral morphology, greenhouse crossing experiments and observation of pollination behaviour (Clegg 1980). Traditionally, forest geneticists employed single gene morphological characters, such as seedling albinism to estimate the proportion of self-progeny (Mitton 1983). With the introduction of protein electrophoresis into plant population studies, biochemical markers have been used to measure the transmission of genetic products (allozymes) in numerous plant species. The use of protein polymorphisms greatly simplifies studies of mating systems because any seed bearing tree can be examined. In conifers, the maternal genotype can be observed directly from a homogenate of needle tissues, or inferred from either a sample of megagametophytes or an array of progeny. The genotypes from an array of progeny can also be used to estimate the gene frequencies in the effective pollen pool and the proportion of offspring produced by selfing. A single polymorphism can

provide the information needed for this estimation procedure (Brown and Allard 1970), or the results of several analyses can be averaged for more precise estimates. Because individuals, and not single locus genotypes, are produced by events of sexual reproduction, it is necessary to use several polymorphisms simultaneously to examine the mating system (Green *et al.* 1980; Shaw and Allard 1982; Shaw *et al.*1981; Cheliak *et al.* 1983; Neale 1983). Recent multilocus estimators have been developed to quantify mating system characteristics (Brown *et al.* 1978; Ritland and Jain 1981; Shaw *et al.* 1981). These estimators, however, are formulated specifically for angiosperms and generally for diallelic loci. These mating system estimators allow the development of mating system models to explain the mating sequence within a population. The most common model assumes random mating, providing a standard reference to which calculated mating distributions may be compared (Clegg 1980). Studies often reveal that many plant species deviate from random mating, expressing a deficiency of heterozygotes, usually as a result of inbreeding (Clegg 1980). As a result, a mixed-mating model has been developed which contains two components: random mating and self-fertilization. Clegg (1980) outlines three important assumptions specific to the mixed-mating model. First, mating events are due to random cross-fertilization (probability=t) or self-fertilization (probability=s(=1-t)). Secondly, the gene frequency distribution among the pollen available to all maternal

plants must be identical. In other words, regardless of the location of a plant in the population, the same probabilities of fertilization by various pollen types apply. Thirdly, the rate of outcrossing is independent of maternal genotype and is therefore specified by a single outcrossing parameter (t).

Shaw and Allard (1982) presented a maximum-likelihood procedure for gymnosperms, again however, only for a diallelic locus. Briefly, this method involves the identification of outcrossed progeny through comparison of multi-locus progeny genotypes with maternal genotypes, and uses the information gained from single-locus data as statistical compensation for outcrosses which are not identifiable or are ambiguous. As more loci are considered the identification of true outcross progeny becomes nearly complete and estimates of s and t become increasingly dependent on observation and less dependent on statistical compensation. One advantage of this estimator is its insensitivity to failure of assumptions that can seriously affect single-locus estimation. Another advantage is that comparisons of estimates derived from single-locus and multi-locus techniques provide a means of separating the effects of selfing from other causes of non-random pollen dispersal, thus providing additional information concerning the breeding structure of populations.

Neale (1983) developed single- and multi-locus estimators based on the maximum-likelihood function

presented by Green *et al.* (1980). The single-locus program requires data in a summarized form in which the observed number of ij th parent-gamete combinations (R_{ij}) for each parental genotypic class (M_{ij}) from the megagametophyte-embryo pairs of parent trees in the sample population are tabulated. The data are formatted into a R_{ij} matrix for each locus which is used to estimate the level of outcrossing (t_s) and probability of the most common allele (p) for the diallelic model and t_s, p, and q for the triallelic case.

The multi-locus outcrossing estimation procedure takes advantage of information at multiple loci to estimate the mating system parameter, t_m. The multi-locus genotype of a pollen gamete is compared to the genotype of the maternal parent. If the pollen gamete has an allele at any one or more loci that could not have been contributed by the maternal parent, then the progeny with that pollen gamete is classified as a definite outcross. Alternatively, if the alleles at all sampled loci in the pollen gamete could have come from the maternal parent, then the progeny may have arisen either by outcrossing to a pollen parent carrying the same alleles as the maternal parent or by self-fertilization. Matings of this type are classified as ambiguous (Neale 1983).

Cheliak *et al.* (1983) developed an iterative procedure for the maximum-likelihood estimation of mating system parameters for a mixed mating system model. The procedure

involves a two step algorithm: the expectation step and the maximization step. One of the advantages of this approach is that an explicit statement is given to determine the proportion of selfed and outcrossed embryos in phenotypically confounded classes, that is classes of observed embryos which contain both selfed and outcrossed embryos. This EM algorithm has been successfully employed in several studies of forest trees (Yeh *et al.* 1983; King *et al.* 1984; Cheliak, Morgan *et al.* 1985; Cheliak *et al.* 1985).

Inbreeding

Mating events among relatives is referred to as inbreeding. In plants, and in particular members of a seed orchard, inbreeding can be the result of fertilization by a) pollen from the ramet itself, b) pollen from other ramets of the same clone, or c) pollen from related individuals. Although the effect of inbreeding is variable in plants there is generally an increase in the homozygosity of a population. The transmission of lethal genes via inbreeding can result in a higher proportion of seedling death or lethality than expected within a randomly mating population (Lindgren 1975). One of the most common reported effects of inbreeding is the reduction in seed set and larger inbreeding depression in growth (Sorensen 1982). In a study of the family Pinaceae, the filled seed yields from self-pollination were only half as large as those from cross- and wind-pollination (Franklin 1970). Franklin (1970)

reported wide variation among the genera; *Pinus* showed the least reduction, *Larix* the most and *Picea* and *Pseudotsuga* had intermediate ratios.

Studies of inbreeding levels in forest trees can be divided into two distinct groups. The older studies dealt with the measurement of self-pollination and employed elaborate direct and indirect methods to determine pollen dispersal (Wright 1952). More recently studies have tried to measure the end product of self-pollination, that is, the level of self-fertilization that results from successful pollination. The introduction of isozyme techniques has allowed researchers to directly measure self-fertilization through the enzymatic detection of genetic markers or unique alleles. Although these two approaches are both concerned with the mating system, they measure two different mating events. The measurement of self-pollination gives an estimate of the amount of pollen that reaches the female strobili and could be potentially effective. This measurement gives no indication of the actual success or amount of realized selfed progeny. Self-fertilization is a measure of successful pollination between the pollen and egg of the same plant. Because of internal factors such as low self-embryo viability, polyembryony and pre-germination selection these numbers are lower than those found for self-pollination.

Information on self-pollination in conifers has been provided by German workers (Fendrick 1967; Schmidt 1970;

Stern 1972; Muller 1976; all cited by Sorensen 1982) who used a method of capturing marker pollen and reported an average of 45-50% self-pollination in *Pinus sylvestris* and *Picea abies*. Sarvas (1962) estimated levels of between 22-37% self-pollination in *P. sylvestris* stands in Finland. Koski (1970) used labelled pollen and reported 7% and 18% self-pollination in two trees of *P. sylvestris* also in Finland, and Sorensen (1982) reported an average of 50-60% self-pollination in coastal Douglas-fir (*Pseudotsuga menziesii*).

Other authors have reported that one of the most drastic effects of self-pollination is the increased frequency of embryonic deaths. Fowler and Park (1983) reported that full seed yield from self-pollination averaged only 13% of the yield from unrelated cross-pollination in white spruce. Similar levels of 13% and 6% full seed following self-fertilization were reported in the same species by Mergen *et al.* (1965) and Coles and Fowler (1976). In black spruce Park and Fowler (1984) reported a mean percentage of full seed from self-pollination of 33.7% while openbag pollination and open pollination together averaged 73.4%. They estimated the mean number of embryonic lethal equivalents per zygotes to be 4.7 and the mean number of embryonic lethals to be 6.6. A lethal equivalent is defined as a group of mutant genes of such number that, if dispersed in different, randomly chosen, individuals they would cause on average one death (i.e. one lethal mutant with 100%

probability of causing death, two mutants each with 50%, etc.) (Morton *et al.* 1956). The reported embryonic lethal equivalents for black spruce are lower than those for other members of the family Pinaceae including white spruce, 9.8 (Fowler and Park 1983) and 8.7 (Coles and Fowler 1976); loblolly pine, 8.5 (Franklin 1971); Scots pine, 8.9 (Koski 1971); Norway spruce, 9.6 (Koski 1971); Douglas-fir, 10.0 (Sorensen 1969); and tamarack, 10.8 (Park and Fowler 1982).

The effect of self-pollination on seed germination is not statistically significant in either black or white spruce. It did, however, affect early seedling survival (Park and Fowler 1984; Fowler and Park 1983).

Probably the largest study on the affects of inbreeding has been the work done with red pine. It has been reported that red pine is homozygous for a large number of alleles (Fowler and Morris 1977), self-fertile, self-compatible and exhibits little or no inbreeding depression (Fowler 1965a). Natural selfing was estimated to be approximately 10% in closed stands and somewhat higher in small isolated populations (Fowler 1965b). As well, natural selfing was found to be considerably higher in the lower part of the tree crown than in the upper part (Fowler 1965b).

The incorporation of gel electrophoresis techniques into forest genetics research has enabled investigators to obtain a direct measure of self-fertilization. In a technique pioneered by Muller (1976) the rate of self-fertilization, resulting in a realized embryo, can be

estimated. This simple procedure involves the separation of the haploid megagametophytic tissue from the diploid embryonic tissue of the seed, thus enabling the identification of the maternal contribution to the seed. Analysis of an array of these seeds for each individual permits the calculation of the allelic frequency and estimation of the outcrossing levels through multi-locus mating system algorithms.

Numerous researchers both in Europe and North America have employed this procedure to determine the level of self-fertilization in conifer stands. While the levels are generally lower than those obtained for self-pollination, they are variable. Muller-Starck (1979) reported 14.7% self-fertilization in a Norway spruce tree and levels of self-fertilization between 12-14% in a West German Scots pine seed orchard (Muller-Starck 1982). Rudin and Lindgren (1977) report lower levels of 2-5% in a Swedish Scots pine seed orchard, while Shen *et al.* (1981) reported 6% self-fertilization in a Scots pine seed orchard in Sweden. All of the above European reports based their calculations on evidence of at least one unique allele or genetic marker with which they could monitor self-fertilization, and at the same time estimate pollen dispersal.

In North America, King *et al.* (1984) found a mean level of self-fertilization of 10% in a white spruce seed production area and Cheliak *et al.* (1985) reported a value of 12% in a natural population of jack pine. Both of these

studies based their estimates on the use of a mating system algorithm such as those described earlier (Cheliak *et al.* 1983; Brown *et al.* 1975). Other reported levels of inbreeding include 1.2% in a slash pine (*Pinus elliottii*) seed orchard (Adams and Joly 1980a) and 10% in a radiata pine (*Pinus radiata*) seed orchard in Australia (Moran *et al.* 1980).

In summary, the reports presented above suggest four conclusions. First, although most genera within the family Pinaceae exhibit a low degree of self-compatibility there is strong evidence of inbreeding depression. Secondly, self-pollination is higher than self-fertilization most likely due to embryo abortion and pre-germination selection (Sorensen 1982). Third, self-fertilization occurs to some degree in both natural stands and in seed orchards. Finally, the reported levels of self-fertilization in seed orchards range from 1.2% (Adams and Joly 1980a) to 14% (Muller-Starck 1982).

Effective Population Size

The effective population size is a theoretical concept devised by Wright (1931) to establish, mathematically, the number of individuals passing on their genes in a population. The effective number can be defined as the size of an idealized population, mating at random, that would have the same homozygosity increase as the observed population (Crow and Kimura 1970). The actual breeding size

is reduced to a number, equivalent to the number of individuals in the ideal population (Kimura and Crow 1963). The reason for conceptualization of an effective population number was to assess the long-term effect of random drift on the distribution of gene frequencies in a population. There are two effective numbers: 1) the inbreeding effective number, and 2) the variance effective number. Each effective number refers to a different generation in a monoecious population. The variance effective number applies to the filial generation while in the parental generation the inbreeding effective number is used (Kimura and Crow 1963). In other words, if a small population is considered, then random drift of gene frequency occurs because of the finite sampling variance in the process of gene transmission from generation to generation. This leads to an average increase in homozygosity within the population and eventually to random extinction and fixation of alleles. For example, a genetic trait which is rare in a large population may be common or absent in small populations.

Sarvas (1962) found differences in the quantity of pollen produced by a stand of Scots pine. In addition, he measured considerable differences in the number of trees producing pollen each year and often these were different trees each year. This is an example of the effective population size in which the number of individuals contributing to the next generation is smaller than the actual size of the population.

Schmidt (1970, as cited in Stern and Roche 1974) investigated production of male and female flowers in a Scots pine stand and found that 50% of the trees produce 90% of the male and female flowers. O'Reilly *et al.* (1982) examined phenological differences in a black spruce seed orchard and found that 2 of the 12 clones studied produced more than half of the total number of male strobili. The authors suggest that the clones producing the largest number of male strobili would likely contribute the bulk of the gametes to seed production. Stern and Roche (1974) state that the effective population size is important in tree breeding programs, particularly seed orchards where the random distribution of clone ramets eliminates distance isolation in natural stands.

Gene Flow

Prevailing evolutionary and ecological theories on population structure are based on the premise that breeding units are extensive and that species are assemblages of individuals which maintain a common gene pool through bonds of mating. Most gene flow, however, is restricted (Levin and Kerster 1974; Ehrlich and Raven 1969), although pollen and seeds may be collected tens or hundreds of kilometers from the source. Effects of dispersal on the breeding structure of a species is determined by the relation between the quantity of pollen or seeds produced within a population or subdivision thereof, and that which comes from a greater

distance. Studies suggest that pollen and seed are either exclusively local or highly leptokurtic (Levin and Kerster 1974; Bradshaw 1972). The level of gene exchange between populations is determined by numerous factors including size, density, and shape of the donor and recipient population, plant height and breeding system, character of surrounding vegetation, terminal velocity of pollen and seed, pollen and seed production, and characteristics of pollen and seed vectors as well as distance between populations (Levin and Kerster 1974). For sexual organisms it is the local interbreeding population and not the species that is the evolutionary unit of importance (Ehrlich and Raven 1969).

Genetic exchange in anemophilous species such as conifers is achieved by the movement of both pollen and seed, with pollen dispersing much greater distances than seed. Pollen dispersal, however, is not synonymous with gene flow; gene flow is limited to pollen that produces viable seeds that become established individuals.

Sarvas (1967) examined the time of female cone opening and closing and the limited capacity of the pollen sac in subpopulations. He concluded that between subpopulations there is a continuous flow of genes. For instance, the vast swamp forests in the northern conifer zone were mostly pollinated by subpopulations in the surrounding firm land. Langner (1953 as cited in Sarvas 1967) reported that most pollination of a given ramet is done by pollen from ramets

within a 61 m (200 ft) radius. Sluder (1970) studied effective pollination distance and flowering habit because of their importance to seed orchard management. Random breeding among clones in seed orchards is decreased by the gene flow barriers of distance and variation in flowering time and intensity. It should not be assumed that a seed orchard is one large, randomly breeding unit. Validation of this assumption would require that flowering and pollination habits of all clones in an orchard be carefully studied to determine whether or not there are serious departures from random breeding among them (Sluder 1970). Koski (1970) measured pollen disperal using labelled pollen but his conclusions differed in that he emphasized the effectiveness of pollen transmission over large distances. However, Koski's study was based solely on pollen dispersal, not on fertilization, and it is generally held that gene flow is restricted and results in population differentiation.

Recently, gene flow has been measured by the identification of unique alleles in individuals within a stand. By sampling the seeds of adjacent and more distant trees it is possible to map the successful journeys of pollen bearing the unique allele. In Scots pine the occurrence of a unique allele dropped to negligible levels within 100 meters of the source tree (Muller 1977). Loblolly pine pollen successfully moved distances in excess of 100 meters (Adams and Joly 1980a), however, Shen *et al.* (1981) concluded that most of the pollen comes from neighbours at a

short distance within a Scots pine seed orchard.

Unique alleles have also been employed in estimating the frequency of fertilizations that involve genes that originated outside of the seed orchard. Friedman and Adams (1981) sampled a loblolly pine seed orchard and estimated that 28% of the seed was fertilized by pollen coming from outside the orchard. In this study the pollen had to cross a 122 m dilution zone surrounding the orchard. Clearly, more studies are necessary for a reasonable sample of gene flow distances (Hamrick 1983). Since conifers are wind pollinated, cross-fertilized, and have wind-borne seeds they provide useful models for gene flow studies.

Seed Orchards

Traditionally, seed orchards were thought to be isolated populations of largely random mating clones producing large quantities of high quality seeds. As more and more research has been conducted into seed orchard properties, numerous questions have arisen. For example, what is the optimum number of clones that should be planted and how many ramets of each clone should be used? Several designs for clone spacing have been devised to improve the possibility of cross-fertilization (Giertych 1975).

The question of pollen dispersal has always intrigued plant biologists as the answers affect the levels of inbreeding, as well as the dilution zones around seed orchards. Probably most important are considerations as to

the usefulness of seed orchards and the possible problem of non-random mating within the orchard. Major concerns include the effects of asynchronous flowering, limited pollen dispersal, and poor orchard design and their effects on seed orchard efficiency.

The problem of achieving the optimum number of clones in a seed orchard for cross-fertilization has been examined for Scots pine. Traditionally, many clones in a seed orchard were employed under the premise that a large number was required to maintain broad genetic variation in the production population. The use of many clones was thought to reduce self-fertilization between the ramets, however, greater genetic selection and higher genetic gains can be achieved with fewer clones (Lindgren 1974). In contrast Muller-Starck's (1979) studies in cross-fertilization within a Scots pine seed orchard concluded that inbreeding can be effectively reduced by increasing the number of clones and ramets per clone. Later, Muller-Starck (1982) showed that the most effective way to reduce the presence of offspring from self-fertilization was to increase the number of clones but to decrease the number of ramets per clone.

Seed orchard location and management are important to the successful production of high quality seeds. Local topography should be carefully examined and artificial features such as wind funnels and frost pockets should be avoided. Isolation from undesirable pollen sources is important for pollen integrity.

Wright (1953) suggested that heavy pollen production is most important for diluting outside contamination effects. Orchard size is also important since contamination decreases very rapidily from the edge rows toward the centre (Werner 1975).

Silvics

Black spruce is the most important commercial tree species in Ontario (Vincent 1965). The tall straight trunk and narrow canopy yield a greater volume per unit of stand area than other species and the long, tough fibres of the wood make black spruce the premier species for high quality paper.

Black spruce is considered a hardy forest species that can grow on organic and mineral soils of varying depth and moisture levels, although nutrient availability and tree height are influenced by site quality (OMNR 1974). Cone production can occur after 10-20 years and reaches an optimum production age at 50-150 years (OMNR 1977). Spruce conelets are fully developed and pollen is shed in early to mid-June in northwestern Ontario (O'Reilly 1981; OMNR 1984). Cones are 0.5 to 1.5 inches long and are often concentrated in the upper reaches of the crown with cone maturation occurring in early September (Fowells 1965). Cones of black spruce are mature when they turn purple and remain firm and unopened. Black spruce is considered a dependable seeding species because seed crops seldom fail completely and heavy

seed production occurs about every four years (Heinselman 1957; Vincent 1965). The seeds of black spruce are small (2 mm), 404,000 seed per pound (917,080 per kg), and tend to fall close to the parent tree (Vincent 1965; Fowells 1965). Black spruce seed dispersal studies in Minnesota (LeBarron, 1939) suggested that seed deposition was over rather short distances and effective from 2 to 3 tree heights.

It has been noted by several authors that black spruce cones are persistent and semi-serotinous (Fowells 1965; Vincent 1965). The semi-serotinous habit, plus fairly consistent new seed crops mean that a seed supply is almost constantly present in stands that are 40 or more years old (LeBarron 1948).

Millar (1939) noted that a large portion of the black spruce stands in Northwestern Ontario are of fire origin and he suggested that the retention of the seed within semi-serotinous cones, coupled with slow dissemination over 2-3 years, have been important factors in maintaining the wide distribution of the species. It has been noted that the seed within densely packed cone clusters are often uninjured after a fire. The heat opens the cones causing heavy seedfall, which may account for the dense stands of even-aged black spruce (LeBarron 1939; Lutz 1956, Millar 1939, 1940).

Generally, the establishment and growth of seedlings is possible under varied conditions, although it is optimal in open conditions with a light overstory to provide protection

from occasional frost (Fowells 1965). Lack of moisture and heavy slash are barriers to reproduction and seedling competitors include tamarack, aspen and jack pine. Black spruce is susceptible to flooding, fire and windstorms but is relatively free from insect and disease problems (Fowells 1965).

The most common method of vegetative reproduction in natural stands is by layering whereby pendant lower branches are buried in moss or duff resulting in the formation of adventitious roots. The end result is a rooted branchlet which becomes an independent tree (Heinselman 1957).

Genetics

Morgenstern (1969a,b) correlated physiological and morphological variation in black spruce provenances with ecological factors. He proposed that if the sub-population component of variance was larger than the population and family component, the variation would be considered ecotypic. He examined six characters related to germination and drought resistance and found that the family variance, expressed as a percentage of the total variation was largest; for seven additional phenological and morphological characters, Morgenstern (1969b) found that the population variance was largest. The variance of the subpopulation was small and never exceeded either the family or population variance. Therefore, based on Morgenstern's criteria variation in black spruce appears to be essentially clinal.

This interpretation was consistent with earlier greenhouse experiments (Fowler 1966) in which no ecotypic variation was found. In contrast, Khalil (1975) noted ecotypic variation for all the morphological and physiological variables he studied in Newfoundland. Morgenstern (1978) states, however, that these findings could be related to the fact that Newfoundland's climate does not follow simple north-south gradients.

Morgenstern's (1978) later studies on a provenance test of range-wide seed sources suggested that ecological regions could be differentiated for black spruce based on timing of growth characteristics and seedling height. For example, the boreal group flushed first, formed buds earliest and remained shortest in total height. Association with climatic and geographic variables suggested that photoperiod and temperature were the major selective factors and that the variation pattern was clinal in a north-south direction. Furthermore, significant variation existed between stands within ecological regions. Supporting evidence for this pattern of clinal variation has been reported elsewhere (Morgenstern 1969b; Dietrichson 1969; Fowler 1966), as well as evidence suggesting a more irregular discontinuous (ecotypic) variation (Morgenstern 1972; Khalil 1975).

Although range-wide clinal differentiation is present in black spruce (Morgenstern 1978), ecotypic differentiation corresponding to upland and lowland conditions within a single locality has not been conclusively demonstrated. In

fact Fowler and Mullin (1977) examined seedling survival, and growth rate of seedlings from upland and lowland stands and concluded that there was no indication of ecotypic differentiation at the local level. Parker *et al.* (1983) found no significant differences between the two origins either morphologically or chemically (flavonoids). O'Reilly *et al.* (in press) found that isozyme data served to distinguish 70% of the sampled black spruce trees from upland and lowland stands across northern Ontario.

Self-fertility of individual black spruce trees is highly variable; however, compared with many conifers, average self-fertility of the species is moderately high (47%) (Park and Fowler 1984). Morgenstern (1972) reported average inbreeding coefficients (F) of 0.08 and 0.03 for southern and northern Ontario populations respectively. O'Reilly *et al.* (1982) studied the effect of pollination period and strobili number on random mating in a black spruce clonal seed orchard and found that the difference in the timing of male and female pollination events were not restrictive. Generally, the black spruce clones producing the largest number of male strobili however, would contribute the bulk of the gametes to orchard seed production.

III. MATERIALS AND METHODS

Site Location and Description

The study site chosen was the Matawin Clonal Seed Orchard established by the Ontario Ministry of Natural Resources in 1966 and located in the Fort William Management Unit in the Thunder Bay Forest District, site region 4W (43° 23'; 89° 80'). The site is approximately 80 km west of Thunder Bay in Northwestern Ontario. The seed orchard was established on a ten hectare clearcut surrounded by a mature even-aged stand of jack pine to minimize pollen contamination. Plus-trees, based on phenotypic characteristics outlined by Morgenstern *et al.* (1975) were selected throughout the 3W seed zone as parental stock for clonal propagation (Appendix A). The orchard is split into two subunits, one for white spruce and one for black spruce, each consisting of 18 blocks. Only the black spruce portion of the seed orchard containing 61 clones was included in this study. Each block was approximately 0.2 hectares (0.5 acres) in size and all blocks contained 12 clones represented by 12 ramets each. Within the orchard each block was designed in a random fashion but constrained by the fact that no two adjacent ramets would be of the same clone. Blocks were designated by the year of ramet planting with the first block planted in 1966 and the last in 1972. The black spruce plus-trees were all cone bearing trees that exceeded 40 years of age at the time of scion collection.

Scions were grafted onto white spruce rootstock, and outplanted two years after graft establishment at a 3.6 m x 3.6 m spacing.

Sampling

In December 1983 and January 1984 the Matawin clonal seed orchard was mapped to verify the layout design and to establish a sampling procedure. Each ramet was labelled with either a metal or plastic identification tag at the time of grafting. All tags were checked to verify correspondence with initial plantation maps. Maps were developed on the location of:

1. correctly identified living ramets,
2. living members in which identification tags were not located,
3. dead ramets still retaining their ID tags,
4. those members which were dead and for which no tags were found.

These completed maps are presented in Appendix B. The mapping of the orchard had two benefits. First, it allowed for evaluation of mortality over the whole orchard including individual clonal survival frequencies which may assist in answering inconsistencies in mating events. Secondly, it permitted identification of suitable ramets for population dynamics measurements.

Once the mapping was complete, sampling of cones from selected ramets of each clone was undertaken in February and

March of 1984. The criteria for specific ramet selection was based on the following list of requirements: First, single representatives, in the form of a ramet, were chosen for each of the 61 clones. Second, where possible, sampling was directed at those specific ramets used in earlier studies (O'Reilly 1981) for the purpose of comparing the results. Third, sampling was restricted to those ramets which had sufficient closed cones at the time of collection. Finally, for clones which had not been previously studied representatives were obtained from those areas of the orchard that exhibited the highest survival rate. In some cases a lack of sufficient cones or the occurrence of open cones forced the selection of an alternative ramet from the same block. An average of six mature cones were collected per clone.

Seed Extraction

The method used to extract seed was adapted from Safford (1974) as follows: Cones were air dried in paper bags for several hours after collection and then placed in mason jars of cold water. The cones were then soaked for 3-4 hours to induce cone saturation. They were then dried at room temperature for approximately 20 hours. The cones were then placed in a kiln and heated to 57 degrees celsius over a 3-4 hour period. This heat was maintained for another 8 hours. The bags were removed and the cones were vigourously shaken to extract the seed, which was then collected,

dewinged and stored at 4 degrees celsius.

Electrophoretic Analysis

The seeds were transported in a cooler to the isozyme laboratory at the Petawawa National Forestry Institute in late April 1984 for electrophoretic analysis. Between 35-50 black spruce seeds per clone were placed on moist filter paper (Whatman #4) in petrie dishes, labelled and placed in a growth chamber to induce germination. The seeds were exposed to temperatures of 30 degrees and 24 degrees celsius for 8 and 16 hours respectively to initiate germination. When the radicle was between 3-10 mm long the seeds were prepared for analysis by removing the seed coat and then microsurgically separating the embryo from the surrounding megagametophyte. The paired tissues were placed in individual 0.5 ml conical, polystyrene sample cups and homogenized with a motorized teflon grinding head using 30 μl of seed extraction buffer (Cheliak and Pitel 1984b). Homogenization of the tissues allows the introduction of the enzyme into a buffered extract solution.

Starch Gel Preparation

The following is a detailed description of the electrophoretic protocol employed at the Petawawa National Forestry Institute (Cheliak and Pitel 1984b).

Molds for the gels are formed by four plexiglass strips (26 x 260 mm) formed into a rectangular arrangement and

secured to a plexiglass plate (177 x 260 x 12 mm) with paper clamps. The whole arrangement is leveled with a small spirit level. Starch gels, 12.5 % w/v, were prepared from two brands of starch, electrostarch and Connaught starch, in a 1:1 ratio. The starch is placed in 1000 ml Erlenmeyer flasks with side evacuation sleeves. Buffer concentrations are then prepared (Cheliak and Pitel 1984b), diluted to the correct volume and placed in the refrigerator.

Approximately 1/5 of the buffer is added to the dry starch to make a suspended solution, free of lumps. The remaining buffer is heated in a microwave oven to boiling. Approximately 1/2 of this heated buffer is added initially to the starch suspension and swirled vigorously. The starch suspension becomes quite viscous. The remaining buffer is quickly added and again swirled vigorously. This starch solution is then returned to the heat until it begins to boil throughout.

A vaccuum is then applied to the solution until only large bubbles are left. The solution is poured into the gel molds and allowed to set. After about 20 minutes a plastic film covering is placed over the gel to prevent dehydration. The molds are then placed in the refrigerator. The gels are subsequently ready to use after they have been trimmed of their excess starch and have cooled to about 4 degrees celsius.

To set the origin for the placement of the wicks, a vertical cut is made through the gel approximately 2.5 cm

from the intended cathodal end. A sample wick is removed from the homogenate cups, wiped lightly with paper towel to remove excess homogenate and then placed against the anodal side of the cut in the gel. Marker dyes are loaded into either end slot for tracking purposes.

After the gels have been loaded they are ready for electrophoresis. Bridge wicks, which allow electric current to pass through the gel, are saturated with electrode buffer and applied to the gel surface. Power is then applied to the system.

The gels are started at half of the total running voltage until the tracker dyes have migrated about 3-5 mm. The sample wicks are then removed and full running voltage is applied. To ensure that the gels are kept cool, a tray of ice water is placed on top of the gel slab. Electrophoresis is continued until the buffer front has migrated approximately 8 cm whereupon the power is shut off.

To slice the gels, plexiglass guides (20 x 260 x 1 mm) and nylon invisible thread are used. A weight, usually a staining tray partially filled with water is placed on top of the gel to prevent it from slipping on the base plate. Guides are then placed next to the gel and the line is drawn through the gel towards the person slicing. After reaching the top, the top slice is discarded and the gel is marked in the top left hand corner to indicate its identity. The slices are then laid in shallow chemical pans and stained for the following enzymes according to the buffer systems

and recipes reported in Cheliak and Pitel (1984b): acid phosphatase (Aph); aconitase (Aco); aldolase (Ald); aspartate aminotransferase (Aat); glutamate dehydrogenase (Gdh); isocitrate dehydrogenase (Idh); leucine aminopeptidase (Lap); malate dehydrogenase (Mdh); menadione reductase (Mdr); phosphoglucose isomerase (Pgi); phosphoglucomutase (Pgm); and 6-phosphogluconate dehydrogenase (6-Pgd). The recipes for the various histochemical stains are presented in Appendix B. After the stains have been added, the gels are incubated at 37 degrees celsius for a half hour, the excess stain is rinsed off and a negative fixer is added to ensure that the phenotypes do not fade. For a more detailed description of this procedure see Cheliak and Pitel (1984b).

Gel Interpretation

Gel phenotype interpretation involved scoring the stained banding patterns based on their similarity in mobility. The method used allowed for the most common allele to be denoted as 1. Faster alleles were designated with odd numbers and slower alleles with even. This notation was used for the megagametophytic haploid tissue. The null alleles at the Lap locus were designated as zeros. Since the embryo is a diploid tissue, it was scored with a two character notation. Homozygotes for a particular allele were usually scored as either 11, 22, 33. Heterozygotes, embryos having different alleles at one or more loci were scored as 12, 13,

23, etc.

Data Analysis

The genetic data obtained from electrophoresis is in the form of the histochemically stained bands representing enzyme (allozyme) protein phenotypes. Calculation of the allelic frequencies are used to test the inheritance of the isozymes to verify the segregation according to Mendelian expectations. It is necessary to verify that the particular isozymes found in the study segregate as single gene units and are not the result of environmental control or of multi-locus origin. The chi-square test was used to statistically compare the observed segregation of gametes in heterozygous maternal trees to the expected 1:1 ratio as predicted by Mendelian inheritance ratios. Also, the allelic frequencies per locus were compared against each other and against each generation to check for homogeneity.

The allelic frequencies are also used to test for Hardy-Weinberg equilibrium. The G-statistic (Sokal and Rohlf 1981) was used to test each locus separately for the "goodness of fit" of their observed frequencies to expected values. This principle also could be tested against both parental and filial genotypes. Statistically significant differences within either generation would indicate non-equilibrium caused by mutation, selection, non-random mating or genetic drift.

Another measure of deviation from the Hardy-Weinberg equilibrium in the whole population is Wright's F-statistic, F_{it}. F-statistics were developed as a system for describing the properties of hierarchically subdivided natural populations. F_{it} is defined as the correlation between gametes that unite to produce the individual, relative to the gametes of the total population (Wright 1965). The F-statistics are derived from Wright's F coefficient, or fixation index, where (1-F)=P (the panmictic index) gives the amount of heterozygosis relative to that expected under random mating (Wright 1951, Jain and Workman 1967). As a fixation index, F may vary between 1.0 and -1.0, where negative values of F represent a higher level of heterozygosis than expected on the basis of Hardy-Weinberg proportions (Jain and Workman 1967). F_{it} is positive if there is any systematic subdivision, whether into demes or into inbred groups, but it may be negative if there is no systematic subdivision and there is prevailing avoidance of consanguine mating (Wright 1965). The F_{it} coefficient used in this study follows Nei's 1977 expression in which F-statistics are defined as functions of observed and expected heterozygosities.

$$F_{it} = \frac{\bar{H}_t - \bar{H}_o}{\bar{H}_t}$$

where $\bar{H}o$ is the average observed heterozygosity over loci and $\bar{H}_t$ is the average expected heterozygosity over loci.

Calculations for effective population size were formulated according to Yasuda (1969) who developed an extension of Wright's tenet for assessing random genetic drift in a small population. The change in allelic frequencies between two generations is expressed as $(\delta p)=x-p$, where x is the arc-sine transformed value for the frequency of allele 1 in the parental generation and p is the arc-sine transformation of the frequency of allele 1 in the filial generation. The value δp is expected to be zero with a variance ($V\delta p=pq/2N$). To normalize the variance and make it independent of the allelic frequencies the data are transformed by

$$p=\sin^2\theta$$

$$\text{or } \theta=\sin^{-1}\sqrt{p}$$

letting $\delta\theta=\theta m-\theta f$, the variance $\delta\theta$ is

$$V\delta\theta=1/n\Sigma(\delta\theta)^2$$

where n is the number of independent alleles studied, m is the transformed mature population and f is the transformed filial population. The effective population number (Ne) is $Ne=1/8V\delta\theta$. This method is based on allelic frequencies and measures directly the random genetic drift. The number of effective males (Nm) within the population can be calculated by $Nm=(Ne*N)/(4*N-Ne)$; where N is the actual population size. The number of receptive females is thus $Nf=Ne-Nm$.

Cheliak *et al.* (1983) developed an iterative procedure for the maximum-likelihood estimation of mating system parameters for a mixed mating system model. The

maximum-likelihood estimate of the outcrossed pollen pool frequency of allele i is the sum of the outcrossed embryos containing pollen allele i (..X. ^{i}) divided by the total number of outcrossed embryos (..X:). Similarily, the maximum-likelihood estimate of the selfing rate (s) is the sum of the numbers of selfed embryos (..X..) divided by the total number of embryos (..X.. + ..X:). The subscripts on the left are the maternal genotype and the subscripts on the right denote embryo genotypes. The inner right hand subscript denotes the maternal gamete. The super-script or outer right-handed subscript denotes the paternal contribution for outcrossed and selfed embryos respectively. Estimates of the numbers in these various classes of embryos are obtained from the expectation step (Cheliak *et al.* 1983). The number of outcrossed embryos containing pollen allele i is calculated as follows:

$$..X.^{i} = {}_{ii}X_{i}^{i} + \Sigma\left({}_{ij}X_{i}^{i} + {}_{ij}X_{j}^{i} + {}_{ji}X_{i}^{i} + {}_{ji}X_{j}^{i} + \Sigma\left[{}_{jj}X_{j}^{i}\right) + \Sigma\left({}_{j\ell}X_{j}^{i} + {}_{j\ell}X_{\ell}^{i}\right)\right]$$

and the number of selfed embryos as:

$$..X..=\Sigma\left[{}_{ii}X_{ii} + \Sigma\left({}_{ij}X_{ii} + {}_{ij}X_{jj} + {}_{ij}X_{ij}\right)\right]$$

This approach calculates a value representing the outcrossing rate based on inference of the pollen alleles and estimating the probabilities of the embryo genotype

deriving the maximum likelihood from an outcrossed or selfed mating.

Neale (1983) employs a multi-locus estimator similar to that of Green *et al.* (1980) in which the maximum-likelihood equation for the estimation of tm is given as:

$$Lt=\pi(1-Gi*tm)^{(Ni-Ri)}*(Gi*tm)^{Ri}$$

where Ri is the number of detectable outcross pollen gametes observed among the total (Ni) progeny sampled from the ith maternal parent, and Gi is the conditional probability of detecting an outcrossed pollen gamete in progeny of the i th maternal parent, given that an outcross has occurred.

Both estimates are based on a mixed mating model and the following assumptions are applicable to both estimators.

1) Each mating event is the result of either a random outcross (with probability t) or a self-fertilization (with probability s), further all embryos, regardless of mating event, have equal fitness.

2) The probability of an outcross is independent of the genotype of the maternal parent.

3) The frequency of alleles in the pollen pool is homogeneous over the array of mature plants sampled.

A simple test for paternity was established through the comparison of observed alleles contributed to the progeny versus the expected number of gametes based on the maternal allelic frequencies. The pollen pool was determined via inference from the observed embryonic genotypes. For contrast, the allelic frequencies in the parental population were determined, and these frequencies were then used to calculate the expected pollen contribution within the filial generation. A chi-square test was used to test the similarity between the observed pollen gametes and the expected paternal contribution. This approach was employed for each locus separately.

To assess homogeneity of the pollen pool reaching each female, the number of alleles inferred from electrophoretic analysis was summed and compared with a chi-square test to the numbers of expected pollen alleles, based on the allelic frequencies calculated for the maternal plant.

IV. RESULTS

Description of Loci and Inheritance

Allelic frequencies for 15 loci were determined from maternal genotypes of 59 clones (Table 1). Of the 61 clones originally planted, one clone (#542) suffered 100% mortality and another (#384) yielded only non-germinating seeds. Maternal genotype assignments were based on 12 megagametophytes scored per clone. Of these 15 loci, five exhibited poor resolution with inconsistent activity and poor clarity (Gdh, Aat-2, Mdr, Pgm and Idh). Poor resolution in the usually polymorphic systems of Idh and Pgm hampered the unique genotyping of all the clones represented in the seed orchard. Thus, of the 59 clones sampled, exactly 40 unique multi-locus genotypes were determined. There were 7 genotypes shared amongst the remaining 19 clones.

Estimation of the frequencies of all alleles in the progeny and the maternal trees for the 10 loci consistently assayed are presented in Table 2. The allelic frequencies in the filial generation were based on resolution of between 41 seeds in Mdh to 750 seeds in Pgi and Lap. This range is the result of variation in the resolution of the progeny stains. Maternal allelic frequencies were based on resolution of megagametophytes of 59 maternal trees at 10 loci. Significant allelic heterogeneity existed between maternal and progeny generations for Aat-1, 6-Pgd and Lap (Table 2). The maternal genotypes exhibited an average heterozygosity

TABLE 1 Maternal Genotypes For Black Spruce Clones in the Matawin Clonal Seed Orchard

Locus	Clone Number																														
	283	284	285	288	290	291	303	304	354	355	356	357	358	367	369	370	373	374	383	385	386	387	392	393	451	452	453	454	487	489	
6Pg-1	11	11	12	11	13	22	11	13	11	11	33	12	13	11	11	11	12	11	11	13		11	12		11	13	11	11	11	11	
6Pg-2	11	11	11	13	11	11	11	11	11	11	11	11	11	13	11	13	11	11	11	11	11	11	11	11	11	12	11	11	11	13	
Pgi-2	13	13	11	13	11	13	11	11	11	11	11	13	11	11	11	12	13	11	12	11	11	11	11	11	11	11	11	13	11	11	
Aat-1	11	11	11	11	11	11	11	11	13	13	11	11	11	11	11	11	13	11	11	11	11	11	11	11	11	11	11	11	11	11	
Mdh	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	
Lap-1	10	10		10	10	10	10	10	10		10	00		10	10	10	10	10	00	10	10				10	10	10	10		00	
Ald	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	
Aco	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	33	11	11	11	11	
Aat-3	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	
Aph	11		11	11	11	13	11	12	11	11	11	11	11	11		11	11	11	12	11	13	11	12	11	13		11	11	13	11	
Pgm													12																		
Mdr											12																				

Locus	Clone Number																														
	490	491	492	493	507	533	543	544	545	546	547	551	552	555	556	562	563	564	565	566	567	568	600	601	602	609	611	622	628		
6Pg-1	11	11	13		22	11	11	13	11	13	11	11	11	11	11	11	13	11	13		11	13	11	11	13	11	13	11	12		
6Pg-2	11	11	11	11	11	11	12	11	11	13	11	11	11	11	11	11	13	11	11	11	11	11	11	11	11	11	11	11	11		
Pgi-2	11	13	11	11	13	13	13	11	11	11	23	13	11	11	13	11	11	13	11	11	11	13	11	11	13	11	15	11	11		
Aat-1	11	12	13	13	11	11	11	11	13	11	13	11	11	11	11	11	11	11	11	12	11	11	11	11	12	13	11	11	11		
Mdh	11	11	11	11	11	11	11	11	11	11	11	11	13	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11		
Lap-1	10	10		10	10	10	10	10			10	10	10	10			10	00			10		10	10		10		10	10		
Ald	11	11	12	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11		
Aco	11	11	11	11	11	11	11	11	11	11	11	13	11	11	11	13	11	11	11	13	11	11	11	11	11	11	11	11	13		
Aat-3	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11		
Aph	11	11		12	11	11	11	11	11	11	11	11	13	12	11	11		13	13	11		12	11	11	11	11	11	11	11		
Pgm																							12								
Mdr	12														12																

Table 2. Allelic Frequencies and Heterogeneity Values For Each Locus.

Locus	Allele	Maternal	Progeny	G-test
Aat-1	1	.917	.909	G=7.682*
	2	.025	.004	df=2
	3	.058	.046	
	H	.157	.168	
Aat-3	1	.992		
	2	.008		
	H	.016		
Pgi-2	1	.819	.798	G=7.102 ns
	2	.033	.003	df=3
	3	.139	.189	
	4	.008	.006	
	H	.308	.328	
6 Pg-1	1	.781	.666	G=20.812***
	2	.079	.294	df=3
	3	.140	.039	
	H	.364	.468	
6 Pg-2	1	.933	.947	G=0.212 ns
	2	.017	.012	df=2
	3	.050	.041	
	H	.126	.101	
Aco	1	.950	.942	G=1.032 ns
	2	.050	.058	df=1
	H	.095	.109	
Ald	1	.992	.998	G=0.562 ns
	2	.008	.002	df=1
	H	.016	.003	
Mdh	1	.992	.951	G=1.772 ns
	2	.008	.049	df=1
	H	.163	.098	
Lap	1	.602	.721	G=5.5704*
	N	.398	.279	df=1
	H	.479	.402	
Aph	1	.879		
	2	.056		
	3	.065		
	H	.219		

H = Heterozygosity
ns = not significant * 0.05 ** 0.01 *** 0.001

value of 0.15 while the progeny had a calculated heterozygosity value somewhat higher (0.19). The filial generation also had a greater number of alleles per locus: 2.6 versus the 2.0 expressed in the maternal generation.

The calculated allelic frequencies are useful in separating polymorphic loci from the monomorphic loci. Hartl (1981) suggests that a monomorphic locus is one in which its most common allele is greater than 0.99. Based on this criterion it is clear that of the 12 enzyme systems studied, 4 loci (Gdh, Ald, Mdh, and Aat-3) were monomorphic in the parental and filial generations. Gdh, for example, was observed to be polymorphic in only one of 720 seeds. The slow allele was clearly a variant, however, activity was not strong and both the clarity and consistency were poor for this system.

A similar situation occurred for Pgm and Idh. Pgm stains of seed proteins were faint and it was difficult to unambiguously assign genotypes. There appears to be only one zone of activity, and variants were positively identified in only two clones. This is unusual since Pgm traditionally has 2 loci and is highly polymorphic in most conifers (Adams and Joly 1980b; Guries and Ledig 1978; Neale *et al.* 1984). One polymorphic locus has been reported for black spruce (Boyle and Morgenstern, in press), white spruce (King and Dancik 1983) and tamarack (Cheliak and Pitel, in press).

In several systems, there was good observed resolution of the megagametophytes but no or very poor resolution of

the embryos (Mdh, Aph, Aat-3 and Gdh).

Verification of the segregation ratios for each polymorphic locus are presented in Table 3. The following are brief descriptions of the inheritance patterns observed in black spruce.

Aspartate aminotransferase (Aat)

Three areas of activity were observed for this system. The most active was the fastest zone (Aat-1) in which both megagametophytes and embryos were resolved. Resolution of Aat-2 was poor and it migrated close to Aat-1. Resolution of Aat-3 was good for megagametophytes only showing a pattern of 3 bands. This triple-banded phenotype has been recorded in loblolly pine (Adams and Joly 1980b). Segregation ratios indicated agreement with single gene inheritance.

Acid phosphatase (Aph)

This particular system did not resolve well and only the megagametophytes were clear. Embryo band patterns did not resolve clearly enough to reveal the diploid expression or structure of the Aph enzyme. Megagametophytes expressed a double banded phenotype for the three active allozymes.

Table 3. Observed Allozyme Segregation in Megagametophytes of Heterozygous Maternal Trees and Goodness of Fit to the Expected 1:1 Ratios.

Enzyme Locus	Allelic Designation		Observed Number		Deviation		Heterogeneity	
	F	S	F	Total	$X^2(1)$	P	$X^2(df)$	P
Aat-1	1	2	18	36	0.00	>0.90	4.67(2)	0.05-0.10
	1	3	58	97	3.72	0.05-0.10	10.44(8)	0.10-0.25
Aat-3	1	2	6	12	0.00	>0.90	---	--
Aco	1	2	15	28	0.14	0.50-0.75	2.00(2)	0.90-0.95
Ald	1	2	11	12	8.33	0.01	---	--
Lap	1	N	259	473	4.28	0.05	58.45(47)	0.10-0.25
Mdh	1	2	3	12	3.00	0.05-0.10	---	--
Mdr	1	2	14	24	0.67	0.25-0.50	3.56(2)	0.10-0.25
Pgi-2	1	2	19	23	9.78	0.01	9.78(1)	0.01
	1	3	81	159	0.06	0.75-0.90	17.17(15)	0.10-0.25
	1	4	5	12	0.33	0.50-0.75	---	--
	2	3	7	12	0.37	0.50-0.75	---	--
6Pg-1	1	2	25	40	2.50	0.10-0.25	4.60(3)	0.10-0.25
	1	3	70	119	3.70	0.05-0.10	17.31(12)	0.10-0.25
6Pg-2	1	3	31	44	7.36	0.01	8.84(3)	0.05

F = common allele
S = variant allele

Aconitase (Aco)

Aconitase displayed a single locus with a single fast variant. This variant was rare, occurring in four of the 60 clones and it did not segregrate according to expected ratios.

Aldolase (Ald)

A single slow variant in aldolase occurred in only one of the 59 clones. As seen in Table 3, segregation deviated significantly from expectation. Strong selection against the variant allele is a posible explanation for this segregation distortion. The small sample size, however, suggests that a more thorough assessment of this segregation anomaly is warranted.

Leucine aminopeptidase (Lap)

Two polymorphic zones were detected, however only the upper locus (Lap-1) was consistently scorable. Two megagametophyte variants, including one null allele, were resolved. A null allele for this locus has been reported in numerous other species including Scots pine (Rudin 1977), pitch pine (Guries and Ledig 1978), ponderosa pine (O'Malley *et al.*1979), Norway spruce (Lundkvist 1974), loblolly pine (Adams and Joly 1980b), eastern white pine (Eckert *et al.*

1981), white spruce (King and Dancik 1983), and eastern larch (Cheliak and Pitel 1985). A consistent segregation distortion was also noted for Lap-1 and has been reported to occur in Scots pine, Norway spruce and loblolly pine. The consistency of the null allele deficiency in the present data set suggests that selection may be acting against the null allele itself or a closely associated gene within the block of loci that it marks.

Phosphglucose isomerase (Pgi)

There are two reported zones of Pgi activity in spruce (King and Dancik 1983). In this study only Pgi-2 was resolved consistently for both megagametophytes and embryos with a total of 4 alleles scored in the megagametophytes. As seen in Table 3, observed segregation of these four alleles fit the expected ratios in all cases except that of the heterozygous clones for the alleles 1 and 2. Adams and Joly (1980b) reported segregation distortion in some allelic combinations and suggested linkage between other loci as a possible causal factor altering segregation ratios. The effect of pollen competition might be considered as a possible causal influence in the present results since the sampled clones are open pollinated. This possibility cannot be examined without controlled pollinations.

6-Phosphogluconic dehydrogenase (6-Pgd)

Three variants were scored in both 6Pgd-1 and 6Pgd-2 Although numerous (1-3) loci have been reported for 6-Pgd in conifers, including 3 loci for white spruce (King and Dancik 1983), there was no evidence of a third locus in black spruce. Inheritance for 6Pgd-2 indicated a deficiency in allele 3. Adams and Joly (1980b) reported a single zone of activity for 6-Pgd in loblolly pine with a segregation distortion resulting from a deficiency of the third of six alleles resolved. The consistency of this deficiency among clones led them to suggest a mechanism involving selection against this allele or a closely linked gene or genes. The present results indicate heterogeneity among clones for this segregation distortion. The underlying mechanisms involving selection, as well as modification by environmental conditions and sampling error due to small sample sizes, are all possible factors contributing to segregation distortion for this locus.

Malate dehydrogenase (Mdh)

Only one area of activity was scored for malate dehydrogenase with variation occurring in only one of the 59 clones with segregation following expected ratios.

Menadione reductase (Mdr)

This system exhibited three areas of activity of which only the fastest was of sufficient clarity and consistency to score. Variants existed in only 2 clones and showed normal segregation patterns.

Mating System Analysis

The log likelihood G statistic (Sokal and Rohlf 1981) was used to test the goodness of fit of the observed genotypes to the expected genotypes under Hardy-Weinberg equilibrium for each system. Table 4 presents these equilibrium values for the parental and filial generations. Clearly, the loci of the maternal trees appear, excepting Lap, to be in equilibrium whereas the progeny deviate significantly from equilibrium.

The calculated F-statistics comparing the individual to the total black spruce population are presented in Table 5. Of note is the inconsistency in direction of deviation between generations. Table 6 represents the estimated levels of outcrossing based on single-locus and multi-locus parameters. The values were derived from two separate estimation algorithms. The single-locus estimates are a product of the algorithm developed by Cheliak

Table 4. Log-Likelihood G Test for Hardy-Weinberg Equilibrium

Locus	Maternal	(df)	Progeny	(df)
Aat-1	0.910	3	10.673	3*
Pgi-2	3.514	6	36.704	6***
6 Pg-1	6.991	3	65.565	3***
6 Pg-2	0.572	3	04.231	3
Aco	2.674	1	50.023	1***
Ald	0.008	1	00.003	1
Mdh	0.008	1	00.205	1
Lap	5.856	1*	134.292	1***
Aph	1.785	3		
Aat-3	0.008	1		

* 0.05
** 0.01
*** 0.001

Table 5. The F(it) Values For a Black Spruce Clonal Seed Orchard.

Locus	Maternal	Progeny
Aat-1	-0.070	0.057
Pgi-2	-0.119	-0.136
6 Pg-1	0.086	-0.175
6 Pg-2	-0.057	-0.046
Aco	0.298	0.525
Ald	-0.008	-0.002
Mdh	-0.008	-0.051
Lap	-0.308	0.436
Aph	-0.099	
Aat-3	-0.008	
Mean	-0.029	0.076
Mean excluding Aco, Ald, Lap*	-0.034	-0.070

* Exclusion due to segregation distortion

et al. (1983) and are pooled to get a mean outcrossed value. Single-locus estimators were also calculated with Neale's (1983) program and are used to summarize the multi-locus level of outcrossing. This multi-locus value takes into account all 5 loci and gives one level of outcrossing for the whole orchard.

The effective population size at the Matawin clonal seed orchard was calculated to be composed of 17 individuals. Within this effective population number it was determined that there were 4 effective males contributing pollen to 13 receptive females based on the allelic frequencies of the realized embryos from that mating season.

Evaluation of pollen pool differences indicate significant differences between observed pollen and the expected frequencies for several loci (Table 7). Pgi-2, Aat-1 and 6Pg-1 deviated significantly from expectations 99.9% of the time.

Table 6. Estimate of the Outcrossing Rate (t) Using the EM Algorithm and Neale's Single- and Multi-locus Estimator.

Locus	EM	Neale's Single-locus Estimation	Multi-locus Estimation
Aat-1	0.826	0.910	0.837
Pgi-2	0.941	0.955	
6 Pg-1	0.843	1.076	
6 Pg-2	1.000	1.087	
Mdr	0.904	0.682	
Ald	1.000		
Mean	0.919	0.942	

Table 7. Differences in the Pollen Pool Evaluated by Chi-square.

Locus	X^2
Aat-1	35.59***
Pgi-2	42.50***
6 Pg-1	1262.02***
6 Pg-2	4.96 ns
Aco	2.00 ns
Ald	3.23 ns

ns not significant
* 0.05
** 0.01
*** 0.001

V. DISCUSSION

The results of this study indicate a substantial level of outcrossing in the Matawin clonal seed orchard. The major evidence for this is the single-locus and multi-locus outcrossing values. Neale's multi-locus estimator calculated a seed orchard value of 0.84, while the mean of Neale's single-locus estimate was 0.94 and the mean single-locus EM value was 0.92. Differences in the three outcrossing values requires an explanation as to the variation in these estimates. The differences are of two types, the first being variation between single-locus estimates and the second being differences between the single-locus and multi-locus estimates.

Part of the variation in single-locus estimates is explained by the dimensions of the EM algorithm which prevents t_s from exceeding 1.0. In contrast, the Neale algorithm will exceed 1.0 ($t_s > 1.0$) and this may be attributed to violation of the mixed mating model assumptions and to random error due to statistical inefficiency of the estimator (Shaw 1980). If the values for 6 Pg-1 and 6 Pg-2, obtained by the Neale estimator, are prevented from exceeding 1.0 then the mean outcrossing value becomes 0.91 which is in much closer agreement with the EM algorithm estimate.

Variation between individual single-locus estimates calculated by the two estimators may be the result of differences in the number of alleles per locus each will

accept. The EM algorithm is dimensioned to accept up to 4 alleles per locus as long as there are at least two different genotypic classes of maternal genotypes. The Neale method is a diallelic model which has been extended for the triallelic case. If more than 3 alleles are observed, the two most common cases are preserved and the remaining alleles are collapsed into a common class. Some loci, such as Pgi-2 and 6 Pg-1 which had 4 and 3 alleles respectively, had to be collapsed to a diallelic system for acceptance by the Neale estimator.

Presently, the EM algorithm does not calculate a multi-locus estimate and this is a major disadvantage to using this algorithm. The Neale multi-locus estimator required that some of the families be discarded because of the models inefficiencies with low sample numbers. Therefore an arbitrary number of 8 germinates per clone was established for analysis. This resulted in a multi-locus estimate based on 39 families instead of the 59 that were sampled. As well, the analysis was collapsed to just 5 loci after Aco, Lap, Mdh, and Gdh were removed because of either a lack of allelic variation or insufficient data.

Multi-locus estimates are theoretically less sensitive than single-locus estimates to violation of assumptions of the mixed mating model (Shaw *et al.* 1981). In particular, multi-locus estimators are more robust to violation of the assumption of pollen pool homogeneity. This is because the multi-locus procedure can more powerfully discriminate

between outcrosses and true selfs. If there are related matings (other than selfs) in the population, the single-locus procedure will tend to underestimate t_s (Neale and Adams in press). It is felt that because of variation in the single-locus estimates, as well as the theoretical robustness of multi-locus estimate, multi-locus estimates of t_m are considered more accurate and should be favoured over single-locus estimates, especially in predominantly outcrossing species (Neale and Adams in press).

Preliminary results from black spruce natural stands in Alberta suggest a multi-locus outcrossing level of 0.70 (Sproule: personal communication). Calculated estimates for other tree species range from 0.90 (King *et al.* 1984) to 0.98 (Cheliak *et al.* 1985) in white spruce, 0.88 for jack pine (Cheliak, Morgan *et al.* 1985) and 0.85 in *Eucalyptus citriodora* (Yeh *et al.* 1983). All of the above results were obtained through the EM algorithm (Cheliak *et al.* 1983) which calculates single-locus values and permits a calculated mean value to be derived.

Since relatedness may be common in natural forest stands, most tree improvement programs select only one tree per stand for use in operational seed orchards (Zobel and Talbert 1984). Although it is understood that many degrees of relatedness can occur, little is known of the effects of sibling, cousin or other types of related matings in natural forest stands. Work done by Franklin (1971), Orr-Ewing (1976), Libby *et al.* (1981) show that matings between close

relatives have some adverse results like reduced seed set, and should be avoided. Zobel and Talbert (1984) state that within first generation clonal seed orchards, like the Matawin, it is often adequate to separate ramets of a clone at a sufficient distance to avoid significant amounts of inbreeding. Therefore, the design of the clonal seed orchard avoids the problems of mating among relatives which may occur in natural stands. Since selfing is also minimized by the design of the orchard, then the level of outcrossing is understandably substantial.

Other problems, however, are the small effective population size (Ne) and the excess of heterozygotes over expected equilibrium values, both of which warrant closer examination. The calculated size of 17 effective individuals is much smaller than the actual population size of 60 monoecious clones. The four effective males represent only 7% of the actual number of males with the 13 receptive females representing 22% of total females. Together there are 17 effective genotypes from a possible 120 genomes indicating that a minimum 14% of the population is involved in the production of the progeny. This estimate (Ne) is consistent with Wright's (1978) statement that the effective population size in any generation is theoretically only one-tenth of the actual size due to gross differences in the reproductive success among individuals. These results also support O'Reilly's (1981) belief that two of the 12 clones he studied appeared to be producing 50% of the pollen for

one orchard. He examined flowering, however, and did not consider the number of realized embryos after the fertilization event. The present results extend O'Reilly's findings from the pollination stage to successful fertilization events.

The observed excess of heterozygotes found in this study is interesting in two respects. First, it would appear that the black spruce within the seed orchard do not adhere to the "heterozygote paradox" which occurs when known outcrossers exhibit deficiencies of expected heterozygotes (Table 8) and inbreeders yield an unexpected excess of heterozygotes (Brown 1979). Second, there are more heterozygotes in the filial generation than in the parental generation. This resulted in Hardy-Weinberg disequilibrium in the progeny.

The F-statistic F_{it}, as stated earlier, is used as a measure of deviation from Hardy-Weinberg proportions. F_{it} values give the degree of deviation with the negative sign indicating an excess of heterozygotes and the positive sign, a deficiency of heterozygotes. In this study both the parental (-0.03) and filial (-0.07) generation exhibited negative values which is what might be expected from a known outcrosser like black spruce. Numerous other studies on North American conifers, however, have found that the "heterozygote paradox" applies. The analysis most commonly involves the calculation of F_{it} values and these are presented in Table 8. It is of some interest to note that

most of the forest species that reportedly exhibit an excess of homozygotes are from natural stands, while this study population is an artificial one. This anomaly may be an artifact of consanguineous mating within natural stands resulting in more homozygotes than expected. In other words because a clonal seed orchard is designed to avoid such interrelated mating occurrences, an excess of homozygotes would not be expected and in fact, was not obtained.

The F_{it} values for each generation indicate a shift in the randomness of gametic recombination. The F_{it} of -0.03 for the parental generation shows a slight excess of heterozygotes. However, this excess increases in the next generation to -0.07. The occurrence of a greater number of heterozygotes in the progeny indicates that inbreeding is not being expressed. This difference in F_{it} values is not of any applied importance, however the theoretical implications are interesting because based on seed orchard assumptions, the progeny, should be the product of random mating events and express neither a negative or positive F_{it} value.

The observed deviation from a Hardy-Weinberg equilibrium was observed in only one of the ten systems studied for the maternal plants, but it occurred in 5 of the 8 loci studied for the progeny. The disequilibrium in the Lap locus of the maternals and progeny is probably due to the expression of a null allele which poses the technical problem of appearing as a phenotypic homozygote when in fact

Table 8. Comparison of F_{it} Values in Forest Genetics.

Species	Values	Authors
Pitch pine	0.034	Guries and Ledig 1982
Lodgepole pine	0.043	Knowles 1984
	0.043	Dancik and Yeh 1983
Black spruce	-0.009	O'Reilly, Parker and Cheliak (in press)
	-0.03(maternal) -0.07(progeny)	present study
Balsam fir	0.013	Neale 1983
Jack pine	0.119	Dancik and Yeh 1983

it may be a genetic heterozygote (Brown 1979). The fact that many of the loci in the progeny are exhibiting disequilibrium demands an explanation. Of the evolutionary forces acting on this system, it is felt that drift and non-random mating most likely contribute to the genetic anomalies.

Plant populations breed under one of several mating schemes, including the random mating system and variations of that approach, as well as several non-random mating systems. In a random mating scheme, it is assumed that each member of the population has an equal chance to produce offspring and that any female gamete is equally likely to be fertilized by any male gamete (Dorman 1976). These conditions are rarely met in plant breeding and so in

practice there is some modification to the system.

In terms of seed orchards, random mating may occur amongst clones within the limitations imposed by flowering characteristics and self-compatability, but these plus-trees are selected mainly on criteria for growth form. Therefore their gamete production would be expected to be representative of a natural stand, where there is a great variation in gamete production. Therefore, there is great variation in gamete production in seed orchard members. It appears that seed orchards definitely do not meet the first requirement and only partially meet the second.

In this present study it was found that a heterogeneous pollen pool exists in the Matawin seed orchard thereby violating the assumption of pollen pool homogeneity within a seed orchard. Three of the six polymorphic loci analyzed in this study had significant differences (0.001) in their pollen pools. This suggests that problems exist in the basic underlying assumptions within this seed orchard.

This study suggests a situation where genotypic assortative mating leads to an excess of heterozygotes. This could occur in one of several fashions. First, heterozygote excesses may be the product of sampling accidents or genetic drift which can be appreciable in small populations. The rate of decay of heterozygosity is 1/2Ne per generation, where Ne stands for the effective size (Wright 1969). The effective population size can be smaller that the actual breeding size which in turn may be smaller than the total

number of individuals in the population. Aside from random fluctuations associated with small population size, gene fixation is not characteristic of populations under random mating conditions, with or without selection. The utility of random mating in breeding is therefore greatest for special purposes such as preserving desirable alleles which might be lost by chance under mating systems which increase homozygosity.

Second, female receptivity on a tree may be different in time than the male phenology on the same tree. This may allow males on genotypically different trees, whose flowering coincides with the receptivity of the females, to undergo a form of non-random mating, negative assortative mating (Cheliak, Morgan *et al.* 1985). O'Reilly *et al.* (1982) examined male and female pollination events within this same seed orchard and found that differences in the timing of each event was inconsequential for individual clones. Although there were statistically significant differences measured between clones in timing of pollen release and female receptivity, the events coincided within most clones.

A third possibility is male gamete competitiveness, which if mediated by maternal effects can result in gametes of dissimilar genotypes being favoured and allowing an excess of heterozygotes to occur (Cheliak, Morgan *et al.* 1985). Since controlled crosses were not conducted in the present study, the role of this factor cannot be directly assessed.

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Fourth, differences in allelic frequency between male and female gamete pools will result in excesses of heterozygotes after correcting for the mating system (Robertson 1965; Workman 1969). The extent to which this is occurring in monoecious plant populations is difficult to assess because differential contribution and gametic incorporation is confounded with classical migration. Robertson (1965) showed that it was not valid to expect Hardy-Weinberg frequencies in a population if there were a small number of parents for the individuals in the population. The calculated effective population size in the Matawin suggests just such a restricted parental population. Based on the gene frequency of heterozygotes on average the progeny population will exceed Hardy-Weinberg expectations by a proportion 1/2Ne. The small effective population size calculated for the seed orchard makes this population susceptible to Hardy-Weinberg incongruencies.

The above factors, individually or in combination, could be responsible for the observed heterozygote excesses in the filial generation. It would be necessary to test these factors with controlled crosses, flowering and phenological studies and analyses of the gamete pools. Heterozygote excesses have also been reported in other mature conifers including jack pine (Cheliak, Morgan *et al.* 1985), ponderosa pine (Linhart *et al.* 1981; O'Malley *et al.* 1979) and lodgepole pine (Yeh and Layton 1979).

All the evidence points to the violation of at least two seed orchard assumptions: random mating, and large population size. The outcome of these problems on the phenotypic expression of the progeny is unclear. However, the seed produced comes from fewer parents than either a natural stand or a randomly mating seed orchard.

Applications of this study to operational forestry can be approached in two directions. First, the use of isozyme analysis for characterization of the seed orchard population is necessary in established orchards to determine the mating system, level of outcrossing and the effective population size. This allows the seed orchard manager to monitor the mating success and efficiency of the orchard. Hattemar *et al.* (1982) have shown that seed orchards with small effective population sizes risk the possibility of losing alleles through genetic drift. Muller-Starck and Ziehe (1984) examined gametic fitness values and found that clones within a Scots pine seed orchard were sexually asymmetrical, so much so that one clone made exclusive female contributions in one flowering period. Both of these problems were elucidated through isozyme mating system studies.

Inconsistencies which are found in seed orchard matings after isozyme characterization will allow the seed orchard manager to modify the orchard to maintain its genetic strengths. For example, rougeing of poorer clones would eliminate problematic clones.

The second use of this type of study concerns seed orchard establishment. Mating system characterization of potential plus-trees before final selection allows tree breeders to select ortets occurring in natural populations which are predominantly outcrossers and not heavy self-pollinators. It will allow for the study of the population in terms of Hardy-Weinberg expectations and this information can be readily updated over the years of seed orchard production to allow a lucid picture of reproductive changes from a natural to closed population structure.

VI. CONCLUSIONS

1. Inbreeding within the Matawin seed orchard is inconsequential and appears to be less than that which occurs in natural populations.

2. Although inbreeding is not a major problem, mating schemes do not fit desired random mating systems. Non-random mating is a problem in the Matawin seed orchard and may explain why the progeny are not in Hardy-Weinberg equilibrium.

3. Isozyme analysis is a useful tool for population characterization by enabling estimation of mating systems, level of outcrossing and inbreeding, and determination of the effective population size.

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A. APPENDIX A

Additional Information Concerning The Original Ortets.

Clone Number	Base Map Location	Year of Collection	Present District
283	492871	1958	Nipigon
284	492871	1958	Nipigon
285	492871	1958	Nipigon
288	494884	1958	Thunder Bay
290	494884	1958	Thunder Bay
291	494884	1958	Thunder Bay
303	488894	1959	Thunder Bay
304	488894	1959	Thunder Bay
354	497853	1959	Nipigon
355	497853	1959	Nipigon
356	496861	1959	Nipigon
357	496863	1959	Nipigon
358	495863	1959	Nipigon
367	495863	1959	Nipigon
369	493874	1959	Nipigon
370	493874	1959	Nipigon
373	494874	1959	Nipigon
374	494874	1959	Nipigon
383	498861	1960	Nipigon
384	498861	1960	Nipigon
385	498861	1960	Nipigon
386	498861	1960	Nipigon
387	498861	1960	Nipigon
392	497861	1960	Nipigon
393	497861	1960	Nipigon
451	492871	1960	Nipigon
452	492871	1960	Nipigon
453	492871	1960	Nipigon
487	493884	1961	Thunder Bay
490	494881	1961	Nipigon
491	493884	1961	Nipigon
492	493884	1961	Nipigon
493	496874	1961	Nipigon
507	496891	1961	Thunder Bay
533	494874	1963	Nipigon
543	502863	1964	Nipigon
544	497871	1964	Nipigon
545	494874	1964	Nipigon
546	494874	1964	Nipigon
547	494874	1964	Nipigon
551	495863	1965	Geraldton
552	495863	1965	Geraldton
555	498861	1965	Geraldton
556	498861	1965	Geraldton
562	494861	1965	Terrace Bay
563	494861	1965	Terrace Bay

564	494861	1965	Terrace Bay
565	498861	1965	Geraldton
566	498861	1965	Geraldton
567	498861	1965	Geraldton
568	498861	1965	Geraldton
600	496874	1965	Geraldton
601	496874	1965	Geraldton
602	496874	1965	Geraldton
609	495873	1965	Nipigon
611	495873	1965	Nipigon
622	496861	1965	Terrace Bay
628	493882	1965	Thunder Bay

B. APPENDIX B

Matawin Clonal Seed Orchard Maps

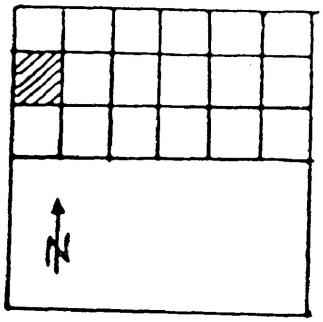
Legend

1	indicates correctly identified living ramets
2	indicates living members in which identification tags were not located
3	indicates those members which were dead and for which no tags were found
4	indicates dead ramets still retaining their ID tag
5	indicates dead ramets which had been removed from the orchard
9	indicates ramets whose identification tag did not correspond to the seed orchard map

288	374
290	383
291	384
303	386
304	387
373	393

1966 A

MATTAWIN SEED ORCHARD INVENTORY



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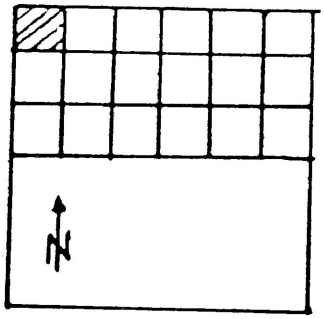
1966 B

MATTAWIN SEED ORCHARD INVENTORY



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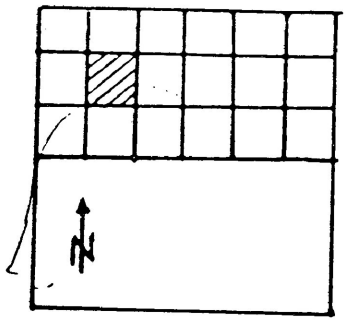
1967 A

MATTAWIN SEED ORCHARD INVENTORY



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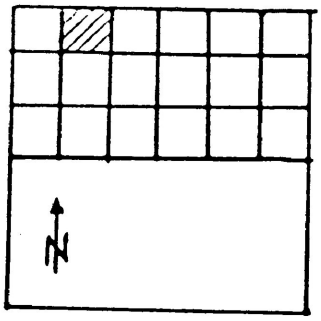
1967 B

MATTAWIN SEED ORCHARD INVENTORY



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304	507
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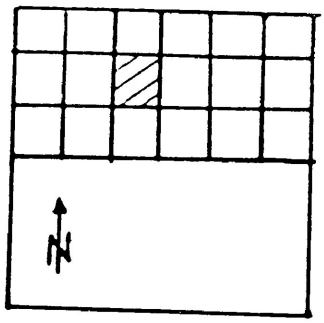
367	546

1968 A

MATTAWIN SEED ORCHARD INVENTORY



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5 454	5 304	5 451	5 507	3 285	5 533	1 354	4 355	3 451	5 454	5 283	5 285
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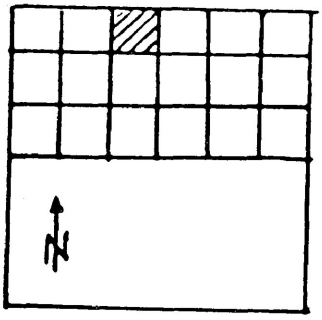
5 507	5 307	1 451	1 544	5 283	1 546	1 533	1 451	5 546	5 354	4 355	4 283

283	454
288	507
304	533
355	543
367	544
451	545

1968 B

MATTAWIN SEED ORCHARD INVENTORY



3 283	5 507	1 355	5 288	5 454	2 533	1 545	5 355	5 544	5 545	2 451	5 367
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5 304	5 544	1 533	4 507	5 543	5 451	5 367	5 544	5 507	2 355	5 288	5 507
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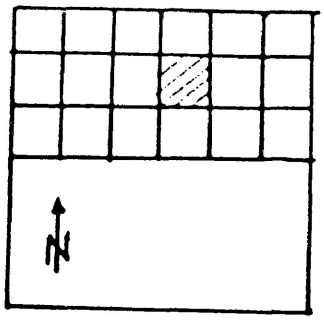
5 288	5 543	5 367	5 507	1 355	5 451	5 304	5 544	5 533	4 288	5 544	5 304
5 533	5 451	5 454	5 544	1 283	5 545	5 543	5 454	1 545	4 355	5 367	1 283

290	452
304	453
357	454
358	507
367	533
451	544

1968 C

MATTAWIN SEED ORCHARD INVENTORY



1 290	5 453	5 358	3 304	5 452	1 454	2 544	5 358	5 533	1 544	5 451	4 367
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5 357	5 533	1 454	5 453	5 507	5 451	3 367	5 533	5 453	4 358	5 304	5 453
1 358	1 544	4 367	1 452	3 304	5 358	5 454	5 290	5 544	4 451	5 290	5 533
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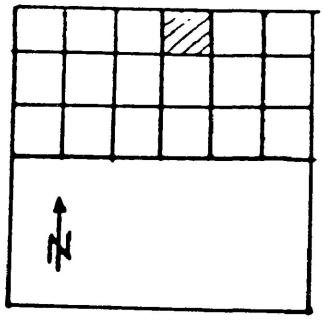
5 367	4 453	3 507	1 304	5 290	2 357	5 533	5 454	5 451	5 507	5 544	4 317
5 357	5 544	5 454	5 452	5 507	5 454	5 367	5 304	4 453	4 290	3 304	2 533
1 451	5 358	4 533	2 290	5 357	5 451	5 358	5 533	1 367	5 544	5 368	1 452
1 452	4 367	5 544	5 451	5 453	1 544	5 290	5 357	5 507	5 454	3 451	5 507
1 453	2 357	2 452	5 454	5 304	5 507	5 358	5 367	5 452	5 453	1 290	5 304
5 304	5 507	5 367	2 453	5 358	5 451	1 357	5 533	5 454	1 304	1 533	5 357
5 454	3 451	2 452	1 533	5 290	5 544	4 507	1 452	1 544	5 358	5 367	5 290

283	451
285	543
288	544
291	545
354	546
356	547

1968 D

MATTAWIN SEED ORCHARD INVENTORY



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1 288	5 546	5 544	5 543	5 545	5 356	4 354	5 546	5 543	2 291	1 285	5 543
1 291	5 547	5 354	5 451	5 285	2 291	5 544	4 283	5 547	1 856	5 283	5 546
1 283	5 544	5 546	5 288	2 547	5 543	5 356	5 545	5 451	5 288	5 291	5 451
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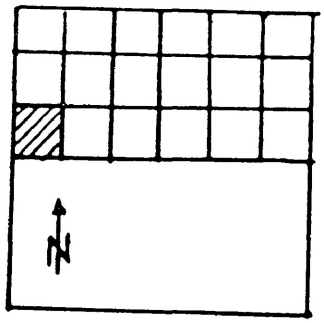
5 288	5 547	4 544	4 451	5 545	2 544	1 354	5 285	5 543	5 283	5 285	1 546
5 356	4 291	5 546	2 283	5 288	5 356	5 291	1 546	2 354	5 547	5 291	5 451
4 451	5 354	5 547	5 356	5 543	1 547	2 283	1 288	5 545	5 544	5 356	5 545
5 543	1 288	5 451	5 544	1 285	1 545	5 291	5 354	1 451	5 543	5 283	5 285
4 285	4 545	5 354	5 543	5 291	2 356	1 288	5 546	5 544	5 285	1 546	1 288
5 544	5 356	5 451	5 546	5 283	5 547	1 545	1 451	5 547	5 291	5 354	4 283

288	533
290	551
291	565
304	566
451	567
459	568

1969 A

MATTAWIN SEED ORCHARD INVENTORY

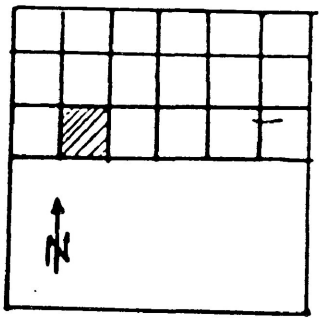


288 2	551 1	304 1	290 1	533 5	565 1	568 5	304 1	567 1	568 5	453 1	451 5
290 2	566 5	453 5	451 5	291 1	551 2	288 5	290 1	533 5	291 2	566 5	565 1
291 2	567 1	565 5	551 1	566 1	453 5	451 1	567 1	551 5	304 5	290 5	551 2
304 5	568 1	451 5	533 5	290 1	304 1	565 5	288 1	568 4	453 1	288 2	567 1
288 2	565 1	567 1	291 2	568 1	551 1	453 1	566 5	533 2	291 1	304 5	533 5
451 5	551 5	566 1	290 1	288 1	291 1	567 1	565 1	453 5	566 5	568 1	451 4
291 2	568 1	565 5	533 5	566 4	565 1	451 1	290 1	551 1	288 1	290 1	567 1
453 5	304 5	567 1	288 1	291 1	453 2	304 1	567 1	451 1	568 3	304 5	533 1
533 1	451 1	568 5	453 5	551 1	568 1	288 1	291 5	566 1	565 1	453 9	566 1
551 1	291 5	533 2	565 1	290 1	566 1	304 1	451 4	533 2	551 1	288 1	290 3
290 5	566 5	451 4	551 1	304 1	453 5	291 5	567 1	565 1	290 1	567 5	291 1
565 1	453 5	533 5	567 1	288 3	568 1	566 5	533 1	568 1	304 1	451 5	288 1

288	552
290	565
291	566
304	568
545	556
551	564

1969 B

MATTAWIN SEED ORCHARD INVENTORY

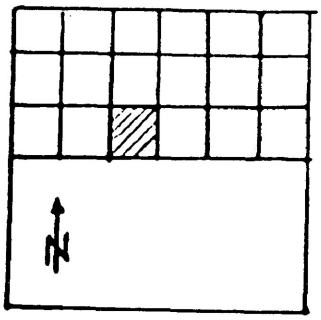


1 288	1 565	1 304	2 290	1 552	1 566	5 564	5 304	1 556	1 564	1 551	1 545
1 290	2 568	1 551	1 545	1 291	5 565	2 288	2 290	1 552	1 291	5 568	1 566
1 291	2 556	1 566	1 565	4 568	1 551	5 545	1 556	2 565	1 304	1 290	5 565
1 304	2 564	5 545	1 552	1 290	1 304	5 566	4 288	1 564	1 551	1 288	5 556
1 288	1 566	1 556	1 291	1 564	1 565	4 551	3 568	1 552	1 291	1 304	4 552
5 545	5 565	5 568	1 290	1 288	4 291	5 556	2 566	1 551	1 568	1 564	4 545
1 291	1 564	1 566	3 552	1 568	2 566	5 545	1 290	5 565	1 288	4 290	5 556
1 551	2 304	1 556	1 288	1 291	1 551	1 304	2 556	1 545	5 564	5 304	5 552
5 552	1 545	1 564	4 551	4 565	1 564	3 288	2 291	5 568	5 566	4 551	2 568
1 565	1 291	1 552	1 566	3 290	3 568	5 304	5 545	5 552	1 565	2 288	5 290
1 290	5 568	1 545	1 565	1 304	1 551	2 291	5 556	1 566	1 290	5 556	1 291
4 566	1 551	2 552	1 556	1 288	2 564	5 568	1 552	1 564	1 304	3 545	5 288

288	556
290	562
304	563
542	564
551	565
555	566

1970 A

MATTAWIN SEED ORCHARD INVENTORY



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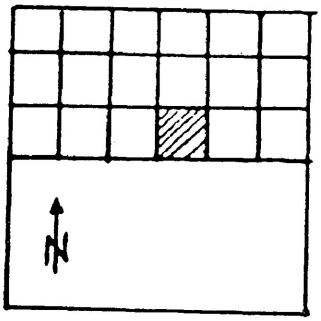
5 304	1 565	5 563	1 562	5 564	4 555	1 551	5 565	5 562	5 542	5 291	5 562
5 542	4 566	5 551	5 556	5 291	5 542	5 563	5 288	5 566	5 555	5 288	5 565
1 288	1 563	1 565	5 304	1 566	5 562	1 555	4 564	5 556	2 304	5 542	5 556
1 551	5 562	3 564	2 291	1 288	5 304	5 565	5 563	1 555	4 564	1 566	1 551
1 304	5 566	1 563	5 556	5 564	1 563	1 551	1 291	5 562	1 288	1 291	4 565
5 555	5 542	3 565	5 288	1 304	1 555	5 542	3 565	3 551	1 566	5 542	5 556
5 556	2 551	5 566	5 555	5 562	2 566	1 288	1 304	1 564	2 563	1 555	1 564
5 562	5 304	3 556	5 563	5 291	5 564	5 542	1 551	2 556	5 562	5 288	5 291
5 291	3 564	1 551	5 562	3 542	5 555	1 304	5 565	1 563	5 291	5 565	3 304
5 563	5 555	5 556	5 565	5 288	1 566	1 564	5 556	5 566	5 542	1 551	1 288

288
551
556
564
565
566

1970 B

MATTAWIN SEED ORCHARD INVENTORY



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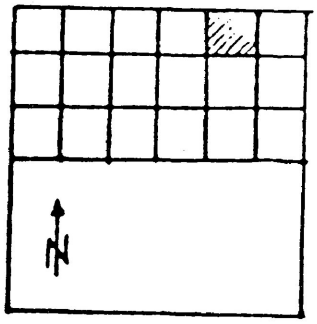
1 551		5 566	5 565	1 556		5 288	3 551		2 556		
5 556					5 566	5 565			2 564	3 551	
1 564		5 565		3 551	1 564		2 288		3 566	1 288	
1 288			5 556			1 566			5 556	3 564	
5 565			1 551	1 288	5 556			1 566			5 565
5 556						5 565	1 551		1 288	1 551	
1 566	1 564		1 288	1 556	1 566	5 564		5 565		5 564	
	1 565		5 566			1 288	1 556			1 566	
	1 556			1 551		5 564	5 565			3 288	1 551
1 551		5 565		5 564	4 566	5 556			2 551		5 556
	1 566			2 288					3 564	5 565	1 288

288	556
489	562
490	563
491	564
551	565
555	567

1971 A

MATTAWIN SEED ORCHARD INVENTORY

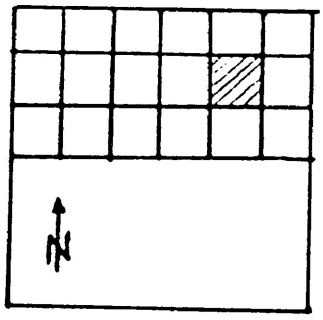


1 288	5 562	5 491	1 489	1 556	5 563	1 567	1 491	1 565	5 567	5 555	1 551
1 489	5 564	1 555	1 551	2 490	1 562	1 288	5 489	1 556	1 490	5 564	5 563
5 490	4 565	5 563	1 562	5 564	5 555	1 551	5 565	5 562	5 491	1 489	1 562
1 491	1 567	1 551	5 556	2 489	5 491	1 563	5 288	5 567	1 555	5 288	4 565
4 288	5 563	1 565	1 490	1 567	5 562	2 555	1 564	5 556	5 490	1 491	1 556
1 551	5 562	1 564	5 489	1 288	5 490	3 565	1 563	1 555	5 564	5 567	1 551
1 490	5 567	1 563	1 556	1 564	1 563	1 551	5 489	5 562	1 288	5 489	1 565
5 555	1 491	5 565	5 288	5 490	5 555	1 491	5 565	1 551	1 567	1 491	5 556
1 556	5 551	5 567	1 555	5 562	1 567	1 288	5 490	4 564	5 563	5 555	5 564
5 562	5 490	5 556	1 563	1 489	4 564	1 491	5 551	5 556	5 562	1 288	5 489
5 489	5 564	5 551	5 562	1 491	5 555	1 490	5 565	5 563	5 489	1 565	1 490
1 563	1 555	5 556	4 565	3 288	5 567	5 564	5 556	5 567	5 491	2 551	1 288

489	552
490	555
491	556
492	562
493	565
551	567

1971 B

MATTAWIN SEED ORCHARD INVENTORY

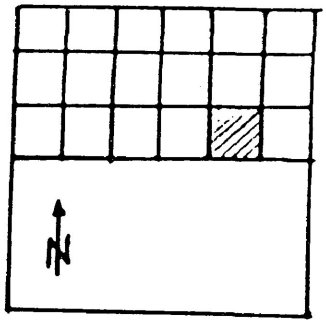


5 489	5 555	5 492	5 490	5 552	2 556	5 567	4 492	5 565	5 567	4 551	5 493
2 490	5 562	1 551	1 493	5 491	5 555	5 489	5 490	1 552	5 491	1 562	5 556
5 491	5 565	5 556	5 555	5 562	1 551	4 493	1 565	1 555	1 492	5 490	5 555
1 492	5 567	5 493	5 552	5 490	5 492	1 555	5 489	4 567	4 551	5 489	5 565
2 489	5 556	2 565	5 491	5 567	5 555	5 551	1 562	1 552	5 491	5 492	5 552
5 493	5 555	5 562	5 490	5 489	5 491	1 565	5 556	5 551	5 562	5 567	4 493
1 491	4 567	5 556	3 552	1 562	1 556	5 493	1 490	4 555	5 489	1 490	5 565
5 551	5 492	5 565	5 489	5 491	2 551	5 492	5 565	1 493	5 567	4 492	5 552
5 552	1 493	1 567	1 551	5 555	1 567	5 489	5 491	1 562	5 556	5 551	5 562
1 555	1 491	3 552	5 556	2 490	2 562	1 492	4 493	5 552	1 555	5 489	5 490
1 490	2 562	5 493	5 555	5 492	5 551	5 491	4 565	1 556	5 490	5 565	5 491
5 556	5 551	3 552	4 565	5 489	5 567	1 562	4 552	5 567	5 492	5 493	5 489

489	567
490	568
491	600
492	601
493	602
555	609

1971 C

MATTAWIN SEED ORCHARD INVENTORY



4 489	1 568	5 492	5 490	2 567	1 600	5 609	5 482	1 602	5 609	1 555	1 493
2 490	4 601	2 555	1 493	5 491	5 568	2 489	2 490	5 567	5 491	2 601	1 600
2 491	2 602	5 600	1 568	5 601	1 555	5 493	5 602	3 568	2 492	1 490	1 568
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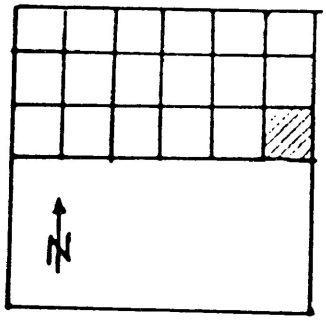
2 489	1 600	2 602	5 491	3 609	2 568	1 555	1 601	5 567	5 491	1 492	5 567
2 493	1 568	5 601	5 490	1 489	5 491	1 602	5 600	1 555	1 601	1 609	5 493
2 491	2 609	5 600	1 567	5 601	1 600	5 493	5 490	5 568	5 489	5 490	5 602
2 555	2 492	5 602	5 489	5 491	5 555	5 492	4 602	5 493	1 609	2 492	2 567
2 567	2 493	2 609	2 555	5 568	5 609	1 489	2 491	4 601	5 600	2 555	2 601
2 5 68	2 491	2 567	2 600	5 490	5 601	5 492	4 493	5 567	2 568	5 489	5 490
2 490	2 601	2 493	2 568	5 492	4 555	5 491	5 602	1 600	1 490	5 602	5 491
2 600	2 555	2 567	1 602	5 489	5 609	1 601	1 567	2 609	5 492	1 493	5 489

490	556
491	566
492	568
551	609
552	611
555	622

1971 D

MATTAWIN SEED ORCHARD INVENTORY



1 490	5 566	4 551	5 491	1 556	5 568	1 622	1 551	5 611	5 622	5 555	5 552
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1 492	5 611	1 568	1 566	1 609	1 555	5 552	1 611	1 566	5 551	5 491	5 566
1 551	1 622	5 552	5 556	2 491	5 551	1 568	1 490	1 622	1 555	1 490	5 611
5 490	5 568	1 611	1 492	1 622	5 566	1 555	1 609	5 556	5 492	2 551	5 556
5 552	1 566	2 609	2 491	2 490	5 492	5 611	5 568	1 555	1 609	1 622	5 552
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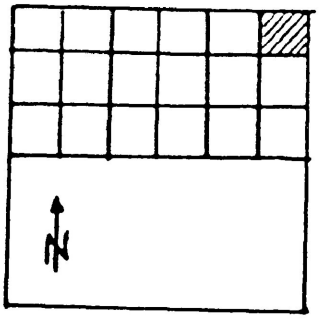
5 555	1 551	1 611	1 490	1 492	5 555	5 551	5 611	5 552	1 622	5 551	5 556
5 556	5 552	5 622	5 555	5 566	1 622	1 490	5 492	1 609	1 568	1 555	5 609
5 566	5 492	5 556	1 568	5 491	1 609	1 551	5 552	5 556	1 566	1 490	5 491
5 491	1 609	1 552	5 566	1 551	1 555	5 492	1 611	1 568	5 491	5 611	4 492
5 568	1 555	5 556	1 611	1 490	5 622	5 609	5 556	5 622	1 551	5 552	5 490

487	600
489	601
490	609
493	611
543	622
568	628

1972 A

MATTAWIN SEED ORCHARD INVENTORY



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489 5	611 1	568 1	543 1	490 5	601 1	487 5	489 5	600 1	490 1	611 1	609 1
490 3	622 5	609 1	601 1	611 5	568 5	543 5	622 5	601 5	493 5	489 5	601 5
493 1	628 5	543 5	600 5	489 5	493 5	609 5	487 5	628 5	568 5	487 5	622 5
487 1	609 4	622 5	490 1	628 5	601 5	568 5	611 5	600 4	490 5	493 1	600 5
543 5	601 1	611 1	489 1	487 1	490 5	622 5	609 5	568 5	611 1	628 5	543 5
490 2	628 5	609 1	600 1	611 1	609 5	543 1	489 5	601 5	487 5	489 1	622 1
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600 1	543 5	628 5	568 1	601 1	628 5	487 1	490 1	611 1	609 5	568 1	611 5
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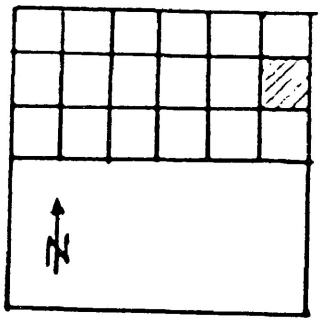
609 1	568 1	600 1	622 5	487 1	628 1	611 5	600 1	628 5	493 4	543 5	487 1

291	622
490	628
491	
564	
568	
611	

1972 B

MATTAWIN SEED ORCHARD INVENTORY



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5 491			5 622			1 568	1 490	5 628	5 291	5 490	
1 611	5 564		5 291	5 491	5 611	5 564		5 568		5 564	5 622
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5 490		1 568	5 628	5 564	5 611	5 491			1 490		5 491
	5 611	1 622		5 291			1 622		5 564	5 568	1 291