ENZYME ELECTROPHORESIS-APPLICATION OF MOLECULAR BIOLOGY TO FOREST GENETICS RESEARCH

Peter P. Feret [1] and Gerald R. Stairs [2]

INTRODUCTION

A fundamental problem confronting the forest geneticist is to determine the distribution of genetic variation in natural populations of forest trees. The analysis of genetic variation among forest tree populations has traditionally been approached by studying quantitative aspects of inheritance and natural variation for a wide variety of physiological and morphological characters. Genetic variation among individuals within populations has been measured by clonal testing and by general and specific combining abilities. Studies of inbreeding depression and self-compatibility have further elucidated the genetic variation in forest trees. These studies have not permitted precise analysis of allelic variation because such statistical approaches are necessarily only summational estimates of polygenic expression.

Other attempts have been made to more precisely define genetic variability. Cytological investigation of forest tree angiosperm and gymnosperm chromosomes has yielded little positive information directly applicable to the analysis of intraspecific variation. The small size of angiosperm chromosomes and the conservative morphology of gymnosperm chromosomes has made intraspecific differences in cytogenetic characteristics difficult to record and of restricted value for the study of natural variation.

Efforts to isolate and analyze gene markers suitable for the comparison of gene frequencies between forest tree populations have also met with limited success. Most reported examples of phenotypes controlled by single genes are associated with lethal or semi-lethal chlorophyll deficiencies or growth forms highly detrimental to survival under natural conditions. The occurrence of these

[1]Department of Forestry and Wildlife, Virginia Polytechnic Institute, Blacksburg, Virginia.
Department of Forestry, University of Wisconsin, Madison, Wisconsin.

systems in low frequencies in natural populations, and the breeding limitations imposed by low fitness, restricts their more general use as marker genes for the study of genetic variation.

Consequently, despite the wealth of information concerning quantitative variation in forest trees, little information is available to answer a fundamental question: What is the relationship between the percentage of loci heterozygous in an individual tree and the growth performance and geographic origin of that tree? On a provenance basis, this question can be expanded to include consideration of polymorphism at single loci. In other words, in a population of forest trees, how many different alleles (isoalleles) occupy a single locus? These questions are not new to biology (Mayr, 1963). A molecular approach designed to provide answers to these questions is new (Hubby and Lewontin, 1966) and deserves the attention of forest tree improvement researchers.

DISC GEL ELECTROPHORESIS

It is a well accepted dogma that the product of a structural gene is a polypeptide composed of amino acids arranged in a colinear sequence with the nucleic acid sequence of the gene. Therefore, any alteration in the nucleotide sequence of a gene will be reflected in the amino acid composition of a protein or enzyme. Multiple molecular forms of an enzyme exhibiting identical catalytic activity but possessing slightly different amino acid sequences are known as isoenzymes. By genetic analysis of the isoenzyme composition of an individual (see Shaw, 1965), an isoenzyme phenotype can be directly associated with a heterozygous or homozygous condition at a specific locus. A survey of the isoenzymic status of several different enzymes can provide a series of gene markers useful for the precise calculation of percent heterozygosity (Hubby and Lewontin, 1966).

Isoenzyme phenotypes may be determined by analyzing enzyme mobilities through a polarized media. Davis (1964) introduced the methods of polyacrylamide disc gel electrophoresis for this purpose. Isoenzymes are separated by their ionic charge at a specific pH and by their size and shape. These physiochemical characteristics are a function of the amino acid composition of the isoenzymes in question. Once separation has occurred in the polyacrylamide gels, standard histochemical procedures may be used for the localization of enzyme activity (Smith, 1968). After staining the isoenzymes appear as "bands" and may be identified by their electrophoretic mobility relative to an appropriate standard (usually bromophenol blue or a pigment band).

ENZYME EXTRACTION

Although the techniques of polyacrylamide disc gel electrophoresis are well established, the procedures for the extraction of isoenzymes from plant tissues are highly varied. The choice of a single procedure is usually dictated by the physiochemical characteristics of the enzyme and the chemical and structural characteristics of the tissue from which it is to be isolated. Enzyme extraction from plant tissue involves tissue maceration resulting in admixture of substances that were rigidly compartmentalized in vivo. Among these substances are vacuolar acids, proteases, tannins (phenols and quinones), and cell wall fragments (Stahmann, 1963; Pririe, 1959). These materials are all capable of enzyme modification and can cause subsequent loss of enzyme activity in plant-leaf enzyme preparations.

The presence of high concentrations of tannins in plant tissues (Harborn, 1964) has been the principle cause of plant protein and enzyme instability in

aqueous homogenates. The ability of tannins to react and denature or partially denature proteins and enzymes has stimulated investigation of the mechanisms involved. These mechanisms and their relevance to plant protein and enzyme extraction have been reviewed by Loomis and Battile (1966). They suggested that phenols and quinones can combine with proteins by: (1) reversible hydrogen bonding between a phenolic hydroxyl group and the carboxyl group of the peptide linkage; and (2) oxidation of phenol compounds to quinones and subsequent covalent bonding of these compounds to sulfhydryl, free amino or imino groups of a protein. The importance of these reactions is significant in that they not only can cause loss of enzyme activity, but can also alter the electrophoretic mobility of proteins, thus causing artifacts.

In view of the above considerations, it is strongly recommended that general protein electrophoresis not be used for genetic analysis. Enzyme assay in electrophoretic gels requires that the macromolecules in question have catalytic activity, a strong indication that the enzymes have been maintained in their native biological state throughout extraction and electrophoretic separation. This insurance guards against possible recording of artifact enzyme species. Conversely, the staining of general protein separated by gel electrophoresis provides no assurance that the molecules accepting the stain have maintained their biological integrity during extraction and separation, since partially denatured protein will stain as intact protein. In addition to the possibility that partial denaturation can cause artifacts, electrophoretic separation of plant proteins commonly permits identification of fifteen or twenty proteins (Hall et al., 1969). Under these conditions, genetic analysis could be misleading.

To protect enzymes from the adverse effects of tanning, a variety of chemical reagents have been successfully applied. Sodium hydrosulfite (Gell, Hawks, and Wright, 1960), ascorbate (Macko, Honald, and Stahmann, 1967), cysteine, 2-mercaptobenzothiazol, mercaptoethanol, and polyvinylpyrrolidone (PVP) (Pierpoint, 1966; Anderson, 1968) have been used to prevent phenolic oxidation or to reduce oxidized phenols as they are formed. Tween 80 (Goldstein and Swan, 1965), dithiothreitol (Cleland, 1964), sucrose, and glycerol (Simpson and Kauzman, 1953) have also been suggested as enzyme protective reagents. The broad differences in chemical structure between phenols and proteins has also permitted separation of these compounds by chemical and physical methods, thus preventing protein-tannin interactions (Loomis, 1969).

With care and judicious use of the above-listed reagents, the technique of isoenzyme electrophoresis for the elucidation of gene markers is highly reliable and has broad applicability. There are, however, other factors that may confound genetic analysis.

MODIFICATION OF ENZYME EXPRESSION

The theoretical assumption that isoenzyme variation reflects only allelic variation may be invalid. Physiological modification of isoenzyme gel profiles is known to occur during growth and development (Macko et al., 1967). McCown et al. (1969) have shown seasonal variation in isoenzyme profiles of several plant species. Water stress has been observed to affect peroxidase and dehydrogenase profiles in T. aestivum (Stutte and Todd, 1969) and hormonal levels had an effect on isoenzyme profiles of mature Pisum stem tissue (Okerse, Seigel, and Galston, 1965) and Avena (Stuber and Levings, 1969).

In addition to normal physiological modification of isoenzyme profiles, phytopathogens affect plant isoenzyme profiles. This has been shown to occur in

P. vulgaris infected with U. phaseoli (Staples and Stahmann, 1964) and southern bean mosaic virus (SBMV) (Farkas and Stahmann, 1966); and in T. aestivum infected with P. recondita (Johnson, Brannaman, and Zsheile, 1968).

Genetic factors other than the alleles directly controlling enzyme synthesis may also affect isoenzyme variation. Enzymes composed of two or more polypeptides may exhibit electrophoretic profiles modified by interallelic interactions (Allen, 1965). Schwartz (1960) demonstrated that the expression of phosphatase isoenzymes in maize was controlled by a regulator element.

From this brief review, it becomes obvious that extreme caution is necessary when applying isoenzyme analysis to the study of forest tree genetics. If isoenzyme phenotypes can be shown to segregate according to a simple Mendelian hypothesis, then this molecular approach requires no a priori assumptions about gene action or physiological stability of the tissues used. In this sense, the theoretical approach of enzyme electrophoresis, based upon concepts derived from molecular biology, is in reality a classical approach to the study of genetics.

As an example of the kind of isoenzyme variation that may be expected from an analysis of single diploid individuals of a forest tree species, the following results of a peroxidase isoenzyme analysis in Ulmus pumila L. (Siberian elm) is presented.

PEROXIDASE ISOENZYME VARIATION IN U. PUMILA

Enzyme extraction - Leaves from seven U. pumila seed sources (see Table 1) were collected June 23 and stored at -20° C until used. 0.7 g of leaf tissue was homogenized in 3 ml 0.1 M Tris-HC1 (pH 8.0) buffer containing 0.5 M sucrose, 6 mM cysteine, 6mM ascorbate, and 1% (v/v) tween 80 at 4° C. Tissue homogenates were centrifuged at 0° C for 10 min at 12,000 x G followed by centrifugation of the 12,000 x G supernatent for 1 hr at 40,000 x G. The 40,000 x G supernatent was decanted into 5 x 75 mm test tubes and stored at -20° C until electrophoresed.

Table 1.--Seed sources used in the analysis of U. pumila peroxidase isoenzyme variation

Seed Source Number	Seed Source
417	Commercial; Schumacker, Massachusetts
418	Commercial; Shenandoah, Iowa
270	Commercial; Yankton, South Dakota
196	Stalingrad, USSR
198	Kara-Kalpak, USSR
206	Rostov, USSR
217	Ukrainskayah, USSR

Electrophoresis - Discontinuous disc gel electrophoresic apparatus was designed with slight modification according to Desborough (1966). Glass tubes (8 mm OD x 75 mm) were used for polyacrylamide gel support. Polyacrylamide gel formulation was slightly modified from Davis (1964). The 7.5% small pore running gel (60 mm in length) was crosslinked by 0.2% (w/v) Bis and buffered at pH 8.9 with 0.38 M Tris-HC1. Polymerization at room temperature was initiated using 0.0575% (v/v) Temed and catalyzed with 0.07% (w/v) ammonium persulfate. The 3.7% large pore spacer gel (6 mm in length) was crosslinked by 0.83% (w/v) Bis and buffered with 0.11 M Tris-HC1 (pH 6.7). Photopolymerization of the large pore gel was performed at room temperature using 0.1% (v/v) Temed and 2.22 x 10^{-5}% (w/v) riboflavin as the catalyst. The large pore gel also contained 7.8% (w/v) sucrose to prevent gel distortion.

Enzyme staining - After electrophoresis of the tissue homogenates, gels were removed from the glass support tubes and stained for peroxidase activity. Gels were placed in petri dishes and covered with 0.02 M benzidine dihydrochloride in 1.5 M acetic acid containing 0.15% (v/v) H_2O_2 (Macko et al., 1967). After 35 seconds, gels were removed from staining solution and placed in 7% acetic acid for recording.

Isoenzyme patterns were diagramatically sketched and electrophoretic mobilities relative to the pigment band (see Fig. 1) were calculated for band identification. (Pigment compounds in the enzyme extracts exhibited a constant electrophoretic mobility for all samples.) Rf (x 100) values were calculated from the ratio of the distance between the leading edge of the enzyme band and the top of the small pore gel to the distance of pigment band migration. Rf reproducibility was ± 3.5%. Coelectrophoresis of mixed homogenates possessing dissimilar enzyme profiles was routinely used to verify isoenzyme identification.

Peroxidase zymograms were arbitrarily divided into three gel regions (see Fig. 1). Region I (Rf 0-20) contained peroxidases with weak and indistinct activity. Because of nonreproducibility, these were not analyzed. Peroxidases in Region II (Rf 20-55) displayed high activity, were reproducible, and exhibited qualitative variation. Gel Region III (Rf 55 to the pigment band) always

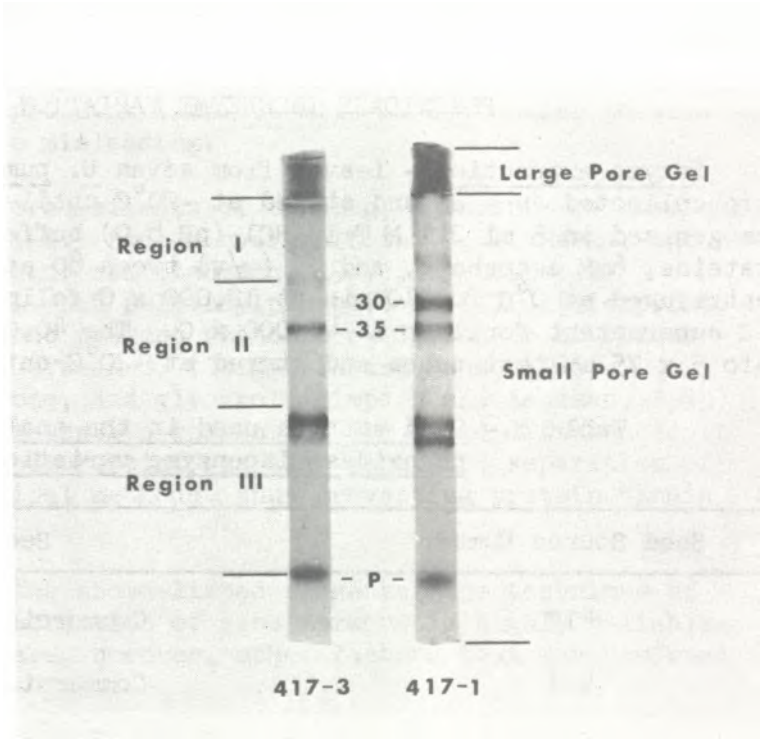

Figure 1.--Zymograms of two trees from seed source 417 illustrating the polyacrylamide gel Regions I, II, and III. Peroxidase phenotypes 30 and 3035 are shown. Each isoenzyme is labeled with its corresponding Rf value and the pigment band is designated by (P).

contained two electrophoretically distinguishable enzymes. Although some quantitative differences in activity were noted, no qualitative variation was observed.

Results - The results of peroxidase isoenzyme analysis of U. pumila seed sources are presented in Table 2.

Table 2.--Frequency of peroxidase isoenzyme combinations in U. pumila

Isoenzyme Pattern	Seed Source Number						
	217	206	198	196	270	418	417
	Number of Individuals						
30	1	2	1	3	2	2	-
33	-	2	-	2	-	3	1
35	-	1	-	4	6	17	10
38	6	-	3	-	-	-	-
4o	-	3	-	-	-	1	4
42	2	2	1	3	-	-	2
48	2	-	-	-	-	-	-
30, 33	-	-	-	-	2	-	-
30, 35	-	1	-	2	11	4	3
3o, 38	-	-	1	-	-	-	-
30, 42	-	-	-	-	-	-	-
33, 35	-	-	-	-	2	-	2
33, 40	-	1	-	-	-	1	2
33, 42	-	-	-	1	-	-	-
35, 40	-	-	-	-	-	-	2
35, 42	-	-	-	1	-	-	-
38, 42	2	-	5	-	-	-	-
40, 42	-	1	-	-	-	-	-
42, 45	1	-	-	-	-	-	-
42, 48	1	-	-	-	-	-	-
None	7	12	2	7	-	4	-
Number of Samples	22	25	13	23	23	32	26

A total of 21 different peroxidase patterns were recorded and eight different peroxidases were observed. These results are summarized in Table 3.

Table 3.--Frequency of peroxidase isoenzymes in U. pumila irrespective of isoenzyme combination

Seed Source	Number of Samples	Peroxidase Isoenzyme								
		30	33	35	38	40	42	45	48	None
217	22	1	-	-	8	-	6	1	3	7
206	25	3	3	2	-	5	3	-	-	12
198	13	2	-	-	9	-	6	-	-	2
196	23	5	3	7	-	-	5	-	-	7
270	23	15	4	19	-	-	-	-	-	-
418	32	6	4	21	-	2	-	-	-	4
417	26	3	5	17	-	8	2	-	-	-

Approximately 67% of the individuals in the Russian seed sources (217, 206, 198, 196) exhibited peroxidase activity in gel Region II. In contrast, 95% of the individuals in commercial seed sources (270, 418, 417) exhibited peroxidase activity in gel Region II.

Of the eight isoenzymes observed, three were seed-source specific and five exhibited varying frequencies in most seed sources (see Table 3). Isoenzymes 45 and 48 were found only in seed source 217, while only seed sources 217 and 198 contained individuals with an isoenzyme at Rf 38. Among the four Russian seed sources, only 206 contained isoenzyme 40,and isoenzyme 35 was infrequent. Commercial U. pumila seed sources 270, 417, and 418 contained a high frequency of individuals with isoenzyme 35. Isoenzyme 30 was common to all seed sources analyzed.

Preliminary analysis of F_1 and Si U. pumila families indicated that peroxidase phenotypes 30, 3035, 35, and the phenotype "None" (complete absence of peroxidases in gel Region II) were controlled by three alleles: (30), (35), and (-) (Feret, 1970). Alleles (30) and (35) coded for peroxidases with Rf's of 30 and 35 while the (-) allele was inactive. Brown and Allard (1969) also demonstrated single gene control of peroxidase isoenzymes. Analysis of the U. pumila seed sources indicated that a number of additional isoenzymes and isoenzyme combinations are possible in the species. Assuming that each isoenzyme is the phenotypic expression of a single gene and all genes are allelic, a total of nine alleles are represented in the seed sources: (3^0), (33), (35), (38), (40), (42), (45), (48), and (-). The assumption of allelism is supported by the seed-source data. In no individual were there more than two peroxidases in gel Region II. The maximum number of alleles in any given seed source was six, and one seed source (270) contained only three.

The causal factors responsible for the peroxidase variation between and within seed sources remains obscure. This experiment was only designed to elucidate qualitative peroxidase variation in the seed sources. If there is an adaptive

significance in the peroxidase polymorphism observed, its nature can only be discussed speculatively. The elucidation of an isoenzyme's adaptive significance requires knowledge of the enzyme systems metabolic function; unfortunately, the specific metabolic role of plant peroxidases has not been elucidated. In addition, to assign an adaptive significance to a particular enzyme or combination of isoenzymes, data must be obtained on the effects changing environments have on the enzyme's rate of synthesis and degradation, its catalytic characteristics, and its interrelationship with other enzymatic systems. In higher plants, these problems have not been critically evaluated. Further analysis under controlled environmental conditions, utilizing larger sample sizes and F1 or S1 families will be required to substantiate the true significance of the peroxidase variation observed.

SUMMARY

Genetic variation in forest trees has traditionally been investigated using techniques of quantitative genetics. Allelic variation has not been intensively studied because of a general absence of useful gene markers. The technique of enzyme electrophoresis allows analysis of the enzyme products of an allele; hence, a promising method is available for the study of allelic variation in forest trees. The broad application of the technique for genetic analysis may be restricted by a number of factors including: allelic interactions, genic regulators, environmental alteration of enzyme expression and extraction artifacts.

An analysis of peroxidase isoenzymes in U. pumila seed sources indicates that peroxidase expression is controlled by a polymorphic locus with nine alleles. Genetic experiments will be required to validate this interpretation.

LITERATURE CITED

Allen, S. L. 1965. Genetic control of enzymes in Tetrahymena. Brookhaven Symp. in Biol. No. 18:27-51.

Anderson, J. W. 1968. Extraction of enzymes and subcellular organelles from plant tissues. Phytochem. 7:1973-1988.

Brown, A. D. H., and R. W. Allard. 1969. Further isoenzyme differences among bred parents of a reciprocal recurrent selection population of maize. Crop Sci. 9:643-644.

Cleland, W. W. 1964. Dithiothreitol, a new protective reagent for SH groups. Biochem. 3:480-482.

Davis, B. J. 1964. Disc electrophoresis. II. Methods and application to human serum proteins. Ann. N. Y. Acad. Sci. 121:404.

Desborough, S. 1967. Disc electrophoresis of tuber proteins and esterases from Solanum species and cultivars. Ph.D. Thesis. Univ. of Wisconsin.

Farkas, G. L., and M. A. Stahmann. 1966. On the nature of changes in peroxidase isoenzymes in bean leaves infected with southern bean mosaic virus. Phytopath. 56:669-677.

Feret, P. P. 1970. Manuscript in preparation.

Gell, P. G. H., J. G. Hawks, and S. T. C. Wright. 1960. The application of

1

Proc. Roy. Soc. B. 151:364-383.

Goldstein, J. L., and T. Swan. 1965. The inhibition of enzymes by tannins. Phytochem. 4:185-192.

Hall, T. C., B. H. McCown, S. Desborough, R. C. McLeester, and G. E. Beck. 1969. A comparative investigation of isoenzyme fractions separated from plant tissues. Phytochem. 8:385-391.

Harborn, J. B. 1964. Phenolic glycosides and their natural distribution. pp. 129-169. In Biochemistry of Phenolic Compounds. J. B. Harborn (ed.), Academic Press, London..

Hubby, J. L., and R. C. Lewontin. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in Drosophila pseudoobscura. Genetics 54:577-594.

Johnson, L. B., B. L. Brannaman, and F. P. Zsheile. 1968. Protein and enzyme changes in wheat following infection with Puccinia recondita. Phytopath. 58: 578-583.

Loomis, W. D. 1969. Removal of phenolic compounds during the isolation of plant enzymes. pp. 55-563. In Methods of Enzymology. J. M. Lowenstein (ed.), Vol. 13.

Loomis, W. D., and J. Battile. 1966. Plant phenolic compounds and the isolation of plant enzymes. Phytochem. 5:423-438.

Macko, V., G. R. Honald, and M. A. Stahmann. 1967. Soluble proteins and multiple enzyme forms in early growth of wheat. Phytochem. 6:465-471.

Mayr, F. 1963. Animal Species and Evolution. Harvard University Press, Cambridge.

McCown, B. H., R. C. McLeester, G. E. Beck, and T. C. Hall. 1969. Environment-induced changes in peroxidase zymograms in the stems of deciduous and evergreen plants. Cryobiology 5:410-412.

Ockerse, P., B. Siegel, and A. W. Galston. 1965. Hormone induced repression of a peroxidase isoenzyme. Sci. 151:452-453.

Pierpoint, W. S. 1966. The enzymic oxidation of chlorogenic acid and some reactions of the guinone produced. Biochem. J. 98:567-580.

Pririe, N. W. 1959. Leaf proteins. Ann. Rev. Pl. Physiol. 10:33-52.

Schwartz, D. 1960. Electrophoretic and immunochemical studies with endosperm proteins of maize mutants. Genetics 45:1419-1427.

Shaw, C. R. 1965. Electrophoretic variation in enzymes. Sci. 149:936-9[1]13.

Simpson, R. B., and W. Kauzman. 1953. The genetics of protein denaturation. I. The behavior of the optical rotation of ovalbumin in Urea solutions. J. Amer. Chem. Soc. 75:5139-5152.

Smith, I. 1968. Chromatographic and electrophoretic techniques. Vol. II. Zone electrophoresis. Pitman Press, G. B.

Stahmann, M. A. 1963. Plant proteins. Ann. Rev. Pl. Physiol. 13:137-158.

Staples, R. C., and M. A. Stahmann. 1964. Changes in proteins and several enzymes in susceptible bean leaves after infection by bean rust fungus. Phytopath. 54:760-764.

Stuber, C. W., and C. S. Levings III. 1969. Auxin induction and repression of peroxidase isoenzymes in oats (Avena sativa L.). Crop Sci. 9:415-416.

Stutte, C. A., and G. W. Todd. 1969. Some enzyme and protein changes associated with water stress in wheat leaves. Crop Sci. 9:510-512.

NOTE: Research submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree in plant breeding and plant genetics at the University of Wisconsin. Appreciation is extended to Dr. E. B. Smalley for providing the U. pumila seed-source collections.

DIG - Is there any evidence or any mention of the filtering effect of trees in taking particulate matter out of the air?

DOOLITTLE - I don't know, Tom. People that are supposed to be knowledgeable in this field tell me that this takes place. This is another one of those areas where we need some good solid information as to just how much--some quantitative data. I can't give you any more answer than that I'm afraid.

MERRILL - Is there any evidence of noise pollution having an effect on the growth of trees?

DOOLITTLE - Could be. I have heard of such things. I don't know of any specific research on that. Perhaps someone else here could comment.

TRIP - There was quite an extensive study done on the effect of noise on growth of wheat. I don't know any tree species that was worked on. It was found at wheat tillered better, say about 30% to 40% better, at noise levels that you would find uncomfortable.

DOOLITTLE - You understand that in my paper I was talking about (1) the effect of noise on people, and (2) vegetation as something that could be used to alleviate such noise problems.

MERRILL - I was thinking specifically of jet port problems.

SMITH - Putting seed production areas beside boiler factories, perhaps.

LARSSON - Regarding soil pollution and its effects on trees, We received a letter the other day from a maple syrup producer regarding absorption of salt by tapped trees which in turn impregnated the sap and polluted the syrup and maple sugar. Has anyone here any information on this subject?

ANONYMOUS - In regards to particle pollution--don't you think the trees can be used not only directly as a filter, but also as a condensing unit to get rid of air currents that would carry particles into the upper atmosphere. You might comment on this.

DOOLITTLE - This has been mentioned. As a matter of fact, I saw a reference on this subject the other day.

IP - Does the fact that larch is deciduous make it slightly more resistant to air pollution than say spruce?

OK - The British seem to think so. It is pretty easy to hurt a larch tree when the foliage is out. But I have no first-hand knowledge about its resistance to air pollution. The larches being in leaf only six months out of the year, the presumption might be that they would be at least that much less susceptible.

DORN - Have you found any differences in susceptibility for sawfly--not only between Japanese and European, but within each species?

COOK - This spring I had nearly complete defoliation of 35 Siberians growing along the highway. A wildling also was roughly handled. Elsewhere, and on many species and races, defoliation was light and spotty. If I were to give you a rule-of-thumb, I would say that the Japanese are less susceptible than the European. But, when you have a major sawfly epidemic, it really does not matter--the bugs get them all!

HEIT - I know this is a forestry conference, but do you think you could comment on the ornamental uses of larch. Why isn't it used more? Do you have any reasons for that?

COOK - The only reason I know, Claude, why the larches are not more used for ornamentals is that the nurserymen find them hard to handle. One year they are this big (4-6 inches), the second year they are two feet tall, and the third year they are head-high. They have not been a very popular tree. People think of ornamentals in terms of being green--pine, spruce, or Douglas-fir.

HEIT - Many times, they desire fast-growing ornamentals, and as you state, larch ---- is just that.

COOK - I think it is a wonderful tree, but you must be careful where you put it--don't put it on subsoil fill on some city lot. I do not know why it is not more popular--perhaps a failure in selling. I once asked a friend of mine who ran a garden magazine if he would accept a story on how good larch was for ornamentals, and he said, "If I do, where could the people get the trees?" I said, "They can't." His reply, "I don't want your article."

LARSSON - Do you grow any mixtures, or are they all monocultures?

COOK - In any mixture that I have ever seen, one of two things happens; either the larch smothers the other species, or the other tree gets the better of the larch and kills it by shading. Do not mix Norway or white spruce with larch; it will cause a lot of trouble because the surface roots of the spruce intercept much of the summer rainfall, which is normally deficient. If you plant Norway spruce with larch in alternate rows, and the site be a good one for larch, the larch will simply outgrow and smother the spruce. The same is true of white pine. I have several examples where I intruded hybrid larch into plantations of Norway spruce and red pine. Larch simply grows away from the others--that's all!

ANONYMOUS - How about wood quantity and quality? What is the comparison with hardwoods at 20, 25, 30 years? Do you have any idea?

COOK - At the risk of offending some of my friends here, let me say this: In the management of a coniferous plantation--larch or anything else--"the only good hardwood is a dead hardwood." I have a number of nice hardwoods that I have left in my plantations, and I have lived to regret every last living one of them! I don't think there is any possibility of mixing hardwoods and larch. The only one I know that worked out all right was a plantation of alternate rows of Japanese and European larch where, instead of getting a good strain of European, I got a high alpine source that would not grow. It ended up with a Japanese larch plantation spaced 6x 20 feet, with a frill of hardwoods between the rows. I tolerated the hardwoods only long enough for them to prune the Japanese larch.

ANONYMOUS - Have you done any work with native larch?

COOK - Yes, I have done quite a little work with the native larch. In general, tamarack will grow anywhere from 50 to 80% as fast in height as European or Japanese, but only half as fast in diameter. Native larch is particularly susceptible to a whole lot of things--case-bearer, red squirrel, porcupine--even more so than European or Japanese.

LIN - If you were given a chance to start everything all over again, considering conditions in the Northeast, which one would you favor--Japanese, European, Dunkeld, or any of these mixes?

COOK - Oscar, you can't favor Dunkeld because you simply cannot get any Dunkeld seed. It's a collector's item. I think I would favor Japanese on any site that has enough moisture to adequately support it. If the site be a little on the dry side, perhaps you'would want to use European. But if you do use European, be darn sure your seed is from a good source. Don't get seed from high alpine sources, don't get--well, I had better not start in on that! Eli and I have been through this a lot and we think we know the minus side--the things NOT to do. We would tell you that, if you can buy it, get European larch seed from the Weinerwald, which is in the hill country just north of Vienna. Ask Eli how good that is--he has seen it.

SCHREINER - Dave, why don't you tell this audience to buy your book if they are interested in larch.

ANONYMOUS - What is the name of the book and the publisher? I want to buy one.

COOK - I'm the publisher. The title is "Planted Larch in New York," but it is not quite as restrictive in its thinking as the title would indicate. See me later!

SCHREINER - I wouldn't be surprised if he had a few in his pocket.

SMITH - The publishing house is at 12 McPherson Terrace, Albany, New York 12206. Name of the publishing house is David B. Cook. We'll send you a bill for the commercial later! Price is $2.00.

COOK - For that, I'll give you an autographed copy!

LEDIG - I would like to direct this question to Mr. Feret. What did you use in your extracting solution?

FERET - I've used sucrose and Tween 80. Both are fairly effective.

LEDIG - How about polyvinylpyrrolidone?

FERET - I've tried it, and it works very well on conifers. It's not needed for this material. It does an excellent job on spruce, though.

CECH - You mentioned spruce--what other conifers have you used?

FERET - I've worked quite extensively in spruce and also on balsam fir, but not intensively.

CECH - You didn't get into pines at all then?

FERET - Yes, some very interesting information in pines. Instead of just having two bands, there are 13, 14, and 15 for each individual. The variation is very complex. I've analyzed full-sib material, and there's no simple genetic hypothesis. Incidentally, in corn, Brown and Allard also demonstrated that peroxidases were controlled by single alleles.

RESOLUTIONS

The Conference, by unanimous vote, adopted the following resolutions submitted by the Resolutions Committee, David B. Cook, Chairman.

WHEREAS Yale University and its School of Forestry have provided outstanding facilities and accomodations, and

WHEREAS Ted Childs has given us a most enlightening and entertaining view of Great Mountain Forest and its operations, and

WHEREAS the Connecticut weather has cooperated most graciously; therefore,

BE IT RESOLVED that we hereby record our appreciation to all of these for making this, our 18th Northeastern Forest Tree Improvement Conference, an outstanding event.

WHEREAS James M. Carlaw, Assistant Regional Manager for International Paper Company, has served as our Chairman for the past two years, and

WHEREAS he has brought to us progressive and able leadership, and

WHEREAS, as a result of his efforts, the interest of the Forest Industries in the Northeast has been effectively rekindled; therefore,

BE IT RESOLVED that the Conference extend to our retiring Chairman its sincere thanks for a job well done.