BIOCHEMICAL METHODS FOR ACCELERATING PROGRESS IN TREE IMPROVEMENT

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Abstract.--The theory and practice of physiological genetic approaches to solving problems in tree improvement are discussed. The advantages of using biochemical techniques are summarized and a range of applications is described. These include measurements of genetic variability, degree of relatedness between populations, species and hybrids, breeding structure of populations, inbreeding in seed orchards, and increasing selection efficiency. Biochemicals are also proving to be useful in shortening tree breeding cycles. Some future directions in biochemical breeding of trees are outlined.

Additional keywords: Indirect selection, molecular markers, terpenes, isozymes.

Tree breeders are fortunate in having a considerable amount of natural variation as a basis for selection in most species and traits of commercial importance. On the other hand progress in tree improvement is severely constrained for two major reasons. First, phenotypic selection in the wild or even in plantations is often ineffective (Pitcher 1982) and usually inefficient because narrow sense heritabilities for complex traits are mostly low necessitating long term progeny testing. Second, both generation and rotation intervals in trees are long which, combined with the occurrence of juvenile-mature phase changes, hinder early evaluation of trait expression and accomplishment of advanced generation breeding. These two prime constraints to tree improvement have stimulated interest and research on two possible methods for alleviating them: (1) clonal propagation of improved planting stock (Libby 1977; Hanover and Dickmann 1982; Eliasson 1977; Eriksson and Lundkvist 1981; Armson, et al. 1980; Karnosky 1981; Farnum et al. 1983) and, (2) techniques for accelerating the process of genotype evaluation, selection, and breeding of superior types.

Biochemical approaches are potentially useful in both of the methods suggested for stimulating more rapid progress in tree improvement (Hanover 1974). My purpose here is to focus on ways in which biochemical techniques may be used by tree breeders to transform potentials for progress into reality. I would emphasize, however, that such techniques must always supplement and enhance but certainly not replace the standard methods of tree improvement with which we are all familiar.

PHYSIOLOGICAL GENETICS OF QUANTITATIVE TRAITS

m The quantitative traits that plant breeders are mainly concerned about, such as yield, form, hardiness, and resistance to pests, are usually controlled by many structural and regulatory genes. We refer to such traits as "complex". For convenience, breeders have typically treated such traits as simple

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or unitary in their research and improvement programs (Malmborn 1967). This practice may be appropriate in the early stages of a breeding program but as genetic gains become more difficult to achieve or perhaps even sooner, we should consider the use of component characters as bases for selection. The elucidation or "dissection" of a quantitative character into its primary components constitutes the physiological genetic approach to plant breeding. Implicit in physiological genetic studies is the enormous range of biochemical constituents and processes found in trees about which we know relatively little (Hanover 1974).

The physiological genetic approach to breeding for complex traits involves five basic procedures:

1. Identify important physiological, biochemical, morphological, or anatomical components of the complex trait.

2. Screen for genetic variation in the components and genetic correlations with the complex trait.

3. Determine heritability of the components.

4. Incorporate traits into superior genetic background through breeding.

5. Test for performance of new genotypes in appropriate environments.

It should be obvious that only the first step and part of the second are unique to physiological genetics. That is, the identification of important components and determination of their genetic correlations with the complex trait. Otherwise, the procedures are standard practices for breeders. However, application of physiological genetics to tree breeding and even plant breeding in general is virtually non-existant. This is because the concept is relatively new and the identification of components (step 1) is not an easy task but requires a multidisciplinary effort. Fortunately, biochemical studies of forest trees are now receiving considerable attention by geneticists which should contribute to step (1) above for some of our important quantitative traits. There are, however, several areas in which biochemical methods are currently employed to increase effectiveness and efficiency of tree breeding programs. One of these areas is the use of molecular markers and indirect selection (Tanksley 1983; Squillace 1976; Hanover 1974; Kriebel 1980; Bridgen and Hanover 1982).

THEORY AND OBJECTIVES OF INDIRECT SELECTION

The underlying theoretical basis for finding and using biochemical markers to accelerate tree selection and breeding resides in the concept of indirect selection. Indirect selection is simply selection applied to any character other than the one for which improvement is desired (Falconer 1960). By selecting for a "secondary" character "Y" we may make greater progress towards improving primary character "X" through the correlated response of X with Y. Following are some conditions which favor indirect selection:

1. A high genetic correlation exists between the two traits, X and Y.

_. There is less effort or cost in selecting for Y than X directly.

1. Less time is required per generation for improvement of X when selecting for Y.
2. There is a higher precision in measuring Y than X.
3. There is a better correlation between progeny means and parental means for trait Y than trait X.
4. Trait Y has a higher heritability than trait X.
5. A higher selection intensity can be applied to trait Y than to trait X.
6. Trait X can he measured in only one sex, Y in both.
7. Trait Y is a juvenile trait that can be measured earlier in the life cycle than trait X.

Calculation of the expected gains (GX) or correlated response in trait Kby selecting for trait Y is obtained by:

$$G_X = i_Y h_Y r_A \sigma_{AX} \text{ (Falconer 1960)}$$

where, i_Y = selection intensity for Y, S/σ_{PY}

h_Y = square root of the operative heritability of trait Y

r_A = genotypic correlation between X and Y

σ_{AX} = square root of the genetic variance of X

One can compare the relative efficiency of using indirect selection (G_X) instead of direct selection (R_X) by calculating the ratio of expected gains for each method:

$$\frac{G_X}{R_X} = r_A \frac{i_Y h_Y}{i_X h_X}$$

APPLICATIONS OF BIOCHEMICAL METHODS IN TREE IMPROVEMENT

Molecular Markers in Tree Breeding

There are several reasons for emphasizing development of biochemical rather than morphological markers to aid tree breeders (Tanksley 1983):

1. Genotypes of molecular loci can be determined at the whole plant, tissue, and cellular levels. Phenotypes of most morphological markers can only be distinguished at the whole plant level.

2. A relatively large number of naturally occuring alleles can be found at molecular marker loci, compared with morphological loci.

3. Usually, no deleterious effects are associated with allele substitutions for molecular markers in contrast to the case with many morphological markers.

4. Alleles of most molecular markers are codominant, allowing all possible genotypes to be distinguished in any segregating generation. Alleles at morphological marker loci are usually dominant or recessive.

5. Epistatic effects are more prevalent in morphological than in molecular marker loci which limits the number of segregating markers storable in a generation or population.

Examination of some generalized biosynthetic pathways in trees reveals the kinds of molecular markers that are available to breeders (Fig. 1). These include DNA, proteins, especially isozymes, and a wide range of secondary plant products including terpenes, phenolic substances, acetogens, alkaloids and waxes. Each of these classes of compounds has its own particular advantages and disadvantages for use in tree improvement. Of course, if the objective is to determine cause-effect rather than linkage relationships between chemicals and a quantitative trait,that would dictate which chemicals should be studied. Furthermore, it is not reasonable to search for single chemical markers closely linked to genes governing expression of a complex trait and the likelihood of establishing useful linkages between multiple chemical markers and the polygenes of interest is remote. Therefore, DNA and protein markers will initially be of most value in characterizing the breeding structures of populations and species and for estimating genetic distances between taxons rather than as marker genes for indirect selection of complex traits. Secondary plant products do offer excellent possibilities for practicing indirect selection as well as helping to solve other problems in forest genetics.

Any component character of a complex trait is a potential candidate for use in improving the primary trait by indirect selection. The components may be related to the primary trait either through tight linkage or pleiotropic (cause-effect) gene action. Thus, an array of morphological, anatomical, physiological and biochemical components are being researched by crop scientists for predicting indirect selection response in primary traits such as yield, form, quality, or pest resistance (Larsson 1982; Chen et al. 1982; Hitz et al. 1982; Bhatt et al. 1979; Tanks ley 1981). Because treebreeders are vitally concerned with developing methods for early selection and evaluation of progeny performance, research related to indirect selection in trees is beginning to receive more attention (Rudin 1976, 1979; Squillace 1976; Rockwood 1973; Harris and Borden 1982; Bridgen and Hanover 1982; Weissenberg 1976; Hanover 1975, 1980). As already indicated earlier we are concerned here only with the application of molecular or biochemical systems analysis in tree improvement.

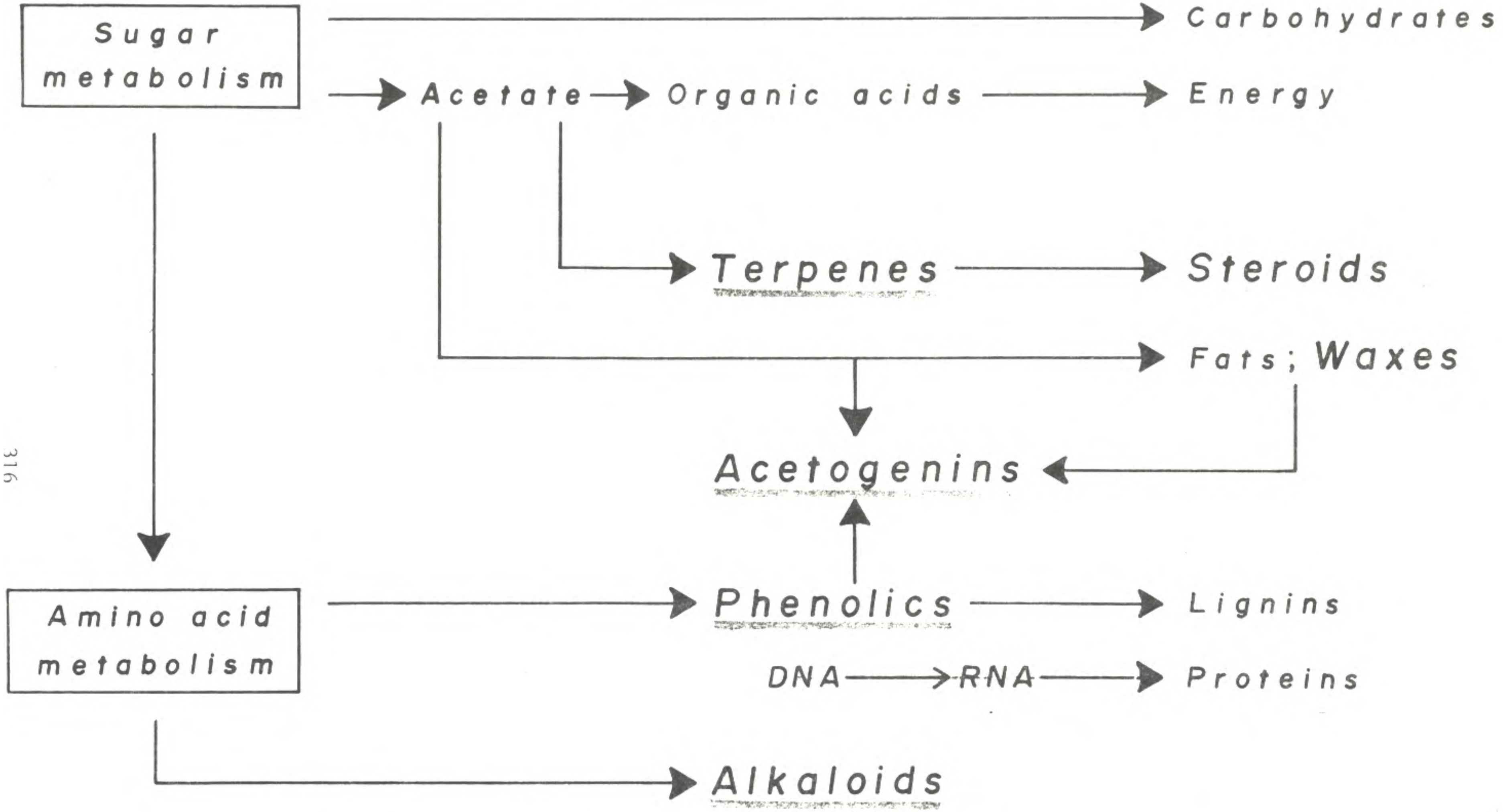

Fig. 1. Generalized biosynthetic pathways showing primary and secondary (lightly underlined) chemical classes which may be used as molecular markers for indirect selection.

Applications to Selection and Breeding

Biochemical markers are of direct benefit to tree breeders when they serve as correlated traits for practicing indirect selection. One of the best examples of this use is in tomato where Rick and Robes (1974) discovered a remarkably tight linkage between an acid phosphatase locus (Aps-1) and the gene for resistance to root-knot nematodes (Mi) (Medina-Filho 1960). Seed companies now use this marker to: (1) identify resistant plants at a much earlier stage, (2) distinguish resistant and susceptible plants with ease and accuracy, and (3) distinguish between homozygous and heterozygous plants without progeny testing for nematode susceptibility (Rick 1982).

In spite of this elegant example in tomato of how indirect selection may be accomplished with molecular markers, our chances of finding such simple systems in other plants and trees are slight. As mentioned earlier this is because most of our important traits are polygenic rather than monogenic so identification of multiple markers would be required. Nevertheless, the principle of indirect selection using molecular markers is sound and I would like to suggest a strategy for developing and employing indirect selection for quantitative traits in trees. The proposed strategy is summarized in Figure 2.

In searching for either or both linkage and pleiotropic relationships between important quantitative traits and chemicals literally thousands of chemicals can be rapidly and accurately screened. This is due to the availability of powerful analytical tools such as gas and liquid chromatography and electrophoresis. Combined with computerized multivariate analytical techniques relationships (correlations) between chemicals and complex traits can be established. Those correlations that are consistent and meet the other requirements outlined earlier for secondary traits can be used in multivariate analyses such as discriminate or factor analysis to construct an index. Index selection comprising a series of molecular markers would then be the basis for obtaining more rapid improvement in the primary trait of interest. Although no data are available to illustrate the utility of molecular marker indices for selection, we are working towards this end with terpenes, aromatic compounds, and isozymes in the spruces, firs and pines.

Once molecular marker index selection techniques are developed they should be important adjuncts to all aspects of selection and advanced generat breeding for metric characters.

Applications to Other Problems in Forest Genetics

Chemical markers are now widely used in solving problems or providing basic information for tree breeders. Time will not allow any detailed consid of these uses but I would like to summarize them to briefly indicate the principles underlying each use. Table 1 lists the major applications for chemical markers and some selected examples from the literature.

From this admittedly incomplete summary it is apparent that interest in chemical markers in trees is high. There are innumerable examples of published and unpublished research on chemical markers that are inconclusive or incomplete at this time in terms of providing data of specific practical use. However, as biochemical methods continue to be explored, expanded and refined, we can expect substantial benefits to forest genetics in the near future from both the basic and applied aspects.

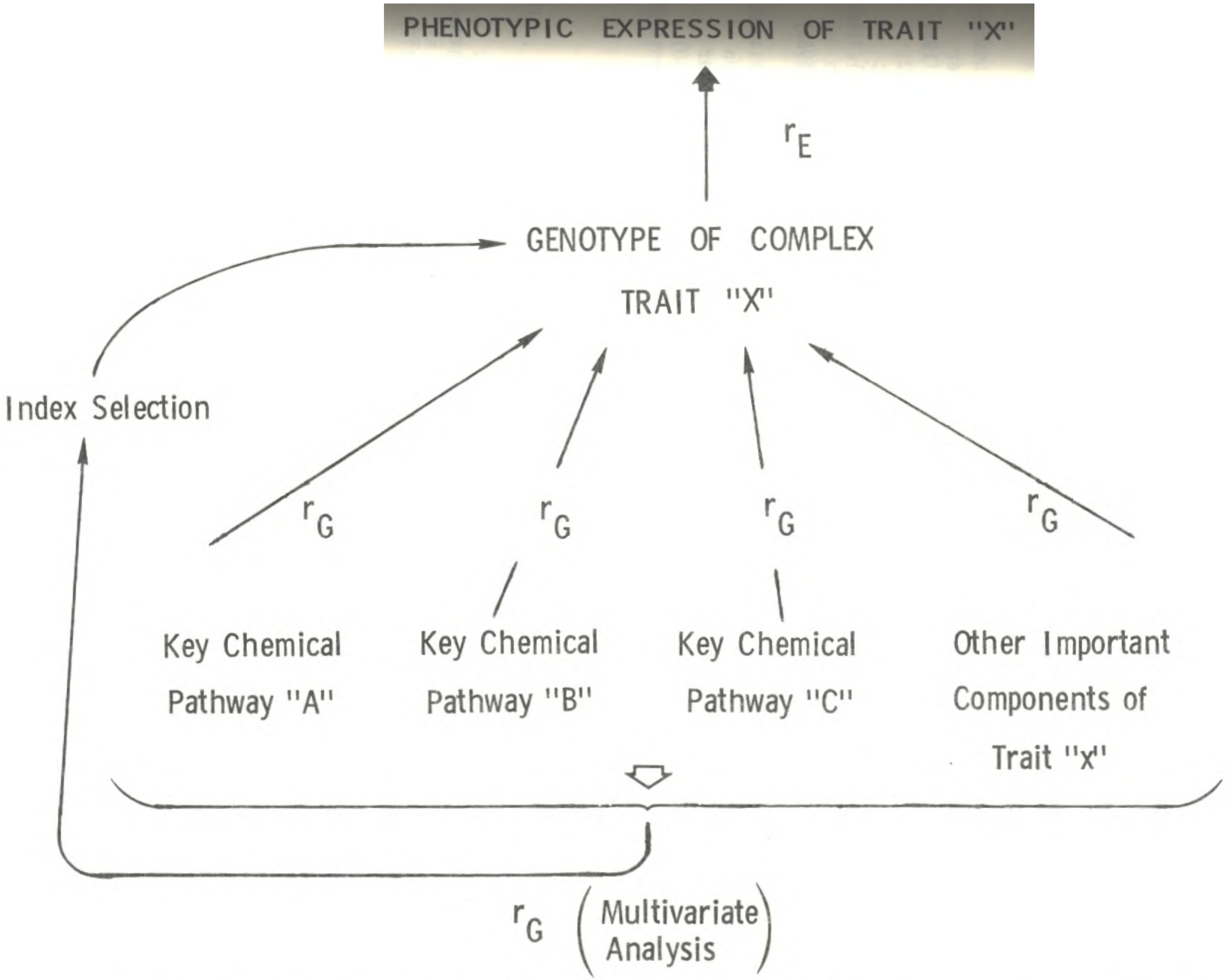

Fig. 2. Application of biochemical and morphological markers in combination with multivariate analysis and index selection for improvement of a complex trait.

Table 1. Applications of chemical markers to forest genetics problems.

Application	Example
Early selection of a chemical trait which itself is of primary interest	Sap sugar in sugar maple (Kriebel 1960) Oleoresin composition in slash pine (Squillace 1977) Leaf surface wax for foliage color in blue spruce (Hanover 1975)
Assessment of population variability	Isoyzmes (Conkle 1972; Guries and Ledig 1977, 1982; Lundkvist and Rudin 1977; etc.) Terpenes (Hanover 1974)
Assessment of geographic variability	Terpenes (Tobolski and Hanover 1971; Squillace and Wells 1981; Wilkinson and Hanover 1972; Bernard-Dagan *et al*. 1971; Gausel and Squillace 1976) Isozymes (Weber and Stettler 1981; Wheeler and Guries 1982)
Identification of clones, seed sources, and natural or artificial hybrids; seed identification	Terpenes (Rottink and Hanover 1972; Hanover and Wilkinson 1969; Bongarten and Hanover 1982; Squillace *et al*. 1980) Phenolics (Hoff 1968; Hanover and Wilkinson 1970: Isozymes (Bergmann 1972) DNA (Miksche and Hotta 1977)
Estimation of outcrossing and inbreeding rates in natural populations and seed orchards	Terpenes (Squillace and Kraus 1963) Isozymes (Adams and Joly 1980; Rudin and Lindgren 1977; Shaw and Allard 1982; Mitton *et al*, 1977; Adams 1981; Muller 1977)
Measurement of genetic relatedness among species, races and individuals; speciation	DNR, RNA (Kriebel 1982; Miksche and Hotta 1973) Terpenes (Squillace 1982; Hanover 1975) Isozymes (Copes and Beckwith 1977)
Identification of haploid-derived plants from anther culture and of parasexual hybrids	None
Indirect selection using molecular markers correlated with a primary trait	Terpenes (Rockwood 1973, 1974; Bridgen and Hanover 1982; Weisse 1973, 1976; Hanover and Furniss 1966) Isozymes (Ryu 1982)

LITERATURE CITED

Ams, W.T. 1981. Applying isozyme analyses in tree breeding programs. U.S.D.A., Forest Service Gen. Tech. Rep. PSW-48, p. 60-64.

Adams, W.T. and R.J. Joly. 1980. Allozyme studies in loblolly pine seed orchards: Clonal variation and frequency of progeny due to self-fertilization. Silvae Genetica 29:1-4.

Amson, K.A., M. Fung, W.R. Bunting. 1980. Operational rooting of black spruce cuttings. J. Forestry 78:341-343.

Bermann, Fritz. 1972. Experiments on possibilities for genetic certification of forest seed. IN Proc., IUFRO and SABRO Joint Meeting, Tokyo, Japan. A-6(V):1-5.

Bernard-Dagan, C., C. Fillon, G. Pauly, Ph. Baradat, and G. Illy. 1971. Les terpenes du Pin maritime: aspects biologiques et genetiques. I. Variabilite de la composition monoterpenique dans individu, entre individus et entre provenances. Ann. Sci. Forest. 28:223-258.

Matt, K.C., P.P. Vaishnav, Y.D. Singh and J.J. Chinoy. 1979. Nitrate reductase activity: a biochemical criterion of hybrid vigour in *Sorghum bicolor* (L.) Moench. Ann. Bot. 44:495-502.

Bongarten, B. and J.W. Hanover. 1982. Hybridization among white, red, and blue spruces and the white x blue spruce hybrid. Forest Science 28:129-134.

Bridgen, M.R. and J.W. Hanover. 1982. Indirect selection for pest resistance using terpenoid compounds. IN: Heybroek, H.M., B.R. Stephan, & K. von Weissenberg (Eds). Resistance to diseases and pests in forest trees. p. 161-168. Pudoc, Wageningen.

Chen, Hwei-Hwang, Zheng-Yan Shen, and P.H. Li. 1982. Adaptability of crop plants to high temperature stress. Crop Sci. 22:719-725.

Conkle, M. Thompson. 1972. Analyzing genetic diversity in conifers -- isozyme resolution by starch gel electrophoresis. U.S.D.A. Forest Service Res. Note PSW 264, 5 p.

Copes, D.L. and R.C. Beckwith. 1977. Isoenzyme identification of *Picea glauca*, P. *sitchensis*, and P. *lutzii* populations. Bot. Gaz. 134:512-521.

Eliasson, Lennart (Ed.) 1977. Vegetative propagation of forest trees - physiology and practice. Proc. Symposium in Uppsala, Sweden, Feb. 16-17, 1977, Swedish University of Agricultural Sciences. 159 pp.

Eriksson, asta and Kenneth Lundkvist (Eds.). 1981. Symposium on clonal forestry, Uppsala, Sweden. April 8-9, 1981. Swedish University of 'Agricultural Sciences, 131 pp.

Falconer, D.S. 1960. Introduction to quantitative genetics. Ronald Press, N.Y. 365 pp.

Farnum, Peter, Roger Timmis, and J.L. Kulp. Biotechnology of forest yield. Science 219:694-702.

Gansel, R. and A.E. Squillace. 1976. Geographic variation of monoterpenes in cortical oleoresin of slash pine. Silvae Genet. 25:150-154.

Guries, R.P. and F.T. Ledig. 1977. Analysis of population structure from allozyme frequencies. IN: Proc. 14th So. Forest Tree Improv. Conf., Gainesville, Fla. p. 246-253.

Curies, R.P. and F.T. Ledig. 1982. Genetic diversity and population structure in pitch pine (Pinus rigida Mill). Evolution 36:387-402.

Hanover, *J.W.* 1974. Biochemical analysis of tree speciation. IN: Proc. Third North Amer. Forest Biol. Workshop. Fort Collins, Co. pp. 106-131.

Hanover, J.W. 1975. Comparative physiology of eastern and western white pines: oleoresin composition and visiosity. For. Sci. 21:214-221.

Hanover, J.W. 1975. Genetics of blue spruce. U.S.D.A. Forest Service Res. Paper WO-28, 12 p.

Hanover, J.W. 1975. Physiology of tree resistance to insects. Ann. Rev. Entomol. 20:75-95.

Hanover, J.W. 1980. Breeding forest trees resistant to insects. IN: Maxwell F.G. and P.R. Jennings (Eds.) Breeding plants resistant to insects p. 486-511. Wiley-Interscience, N.Y. 683 p.

Hanover, J.W. and D.I. Dickmann. 1982. Systems for production of improved stock for intensive culture. Proc. 7th North Amer. Forest Biol. Workshop, Lexington, Ky.

Hanover, J.W. and M.M. Furniss. 1966. Monoterpene concentration in Douglas-fir in relation to geographic location and resistance to attack by the Douglas-fir beetle. U.S.D.A., Forest Service Res. Paper NC-6, p. 23-28.

Hanover, *J.W.* and R.C. Wilkinson. 1969. A new hybrid between blue spruce and white spruce. Can. J. Bot. 47:1693-1700.

Hanover, J.W. and R.C. Wilkinson. 1970. Chemical evidence for introgressive hybridization in Picea. Silvae Genetica 19:17-22.

Hitz, W.D., J.A.R. Ladyman, and A.D. Hanson. 1982. Betaine synthesis and accumulation in barley during field water stress. Crop. Sci. 22:47-54,

Hoff, R.J. 1968. Chemical verification of the hybrid of Pinus monticola and Pinus flexibis. Forest Sci. 14:119-121.

Karnosky, David F. 1981. Potential for forest tree improvement via tissue culture. Bio. Science 31:114-120.

Kriebel, Howard B. 1960. Selection and testing for sugar yield in Acer saccharum L. Proc. Fifth World Forestry Congress, Seattle, WA.

Kriebel, Howard B. 1980. Research approaches to forest genetics at the DNA level. Proc. Sixth North Amer. Forest Biol. Workshop. Edmonton, 49-76.

Larsson, S. 1982. A simple, rapid and non-destructive screening method useful for drought resistance breeding in oats (Avena sativa L.) Z. Pflanzenzuchtg. 89:206-211.

Libby, W.J. 1977. Rooted cuttings in production forests. IN Proc. 15th Southern Forest Tree Improvement Conference, Gainesville, Florida, p. 13.

Lundkvist, K. and Dag Rudin. 1977. Genetic variation in eleven populations of Picea ahies as determined by isozyme analysis. Hereditas 85:67-74.

Malmborn, Sven O. 1967. Some aspects of studies of complex characters in the field of genetics. Hereditas 56:109-112.

Medina-Filho, H.P. 1980. Linkage of Aps-1, Mi and other markers on chromosome 6. Rep. Tomato Genetics Coop. 30:26-28.

Miksche, J.P. and Y. Hotta. 1973. DNR base composition and repetitious DNR in several conifers. Chromosoma 41-29-36.

Mitton, J.B., Y.B. Linhart, J.C. Hamrick, and J.S. Beckman. 1977. Observations on the genetic structure and mating system of ponderosa pine in the Colorado front range. Theor. Appl. Genetics 51:5-13.

Muller, G. 1977. Cross-fertilization in a conifer stand inferred from enzyme gene-markers in seeds. Silvae Genetica 26:223-226.

Pitcher, John A. 1982. Phenotype selection and half-sib family performance in black cherry. Forest Sci. 28:251-256.

Rick, C.M. 1982. Isozymes in plant breeding. Calif. Agr. 36:28.

Rick, C.M. and J.F. Fobes. 1974. Association of an allozyme with nematode resistance. Rep. Tomato Genet. Coop. No. 24:25.

Rockwood, Donald L. 1973. Monoterpene - fusiform rust relationships in Loblolly pine. Phytopathology 63:551-553.

Rockwood, D.L. Cortical monoterpenes and fusiform rust resistance relationships in slash pine. Phytopathology 64:976-979.

Rottink, B.A. and J.W. Hanover. 1972. Identification of blue spruce cultivars by analysis of cortical oleoresin monoterpenes. Phytochem. 11:3255-3257.

Rudin, D. 1976. Biochemical genetics and selection - application of isozymes in tree breeding. Proc. IUFRO Meeting on Advanced Generation Breeding, Bordeaux, France. p. 145-164.

Rudin, Dag (Ed.) 1979. Proc. Conf. on Biochemical genetics of forest trees. Swedish Univ. of Agricultural Sci., Umea, Sweden. 201 pp.

Rudin, D. and D. Lindgren. 1977. Isozyme studies in seed orchards. Stud Forest Suec 139:23 p.

Ryu, Jang Bal. 1982. Relations between isozyme variables and height growth of Pinus strobus L. Korean Inst. For. Genet. Res. Rpt. 18, p. 17-27.

Shaw, D.V. and R.W. Allard. 1982. Estimation of outcrossing rates in Douglas-fir using isozyme markers. Theor. Appl. Genet. 62:113-120.

Squillace, A.E. 1976. Biochemical genetics and selection - composition of volatile terpenes. Proc. IUFRO Meeting on Advanced Generation Breeding, Bordeaux, France. pp. 167-178.

Squillace, A.E. 1977. Use of monoterpene composition in forest genetics research with slash pine. Proc. 14th South. For. Tree Improv. Conf.: 227-238.

Squillace, A.E., H.T. Schroeder, and H.T. Battacharyya. 1980. Identification of seed origin of slash pine plantations. Silvae Genetica 29:152-154.

Tanksley, Steven D. 1983. Molecular markers in plant breeding. Plant and Molecular Biology Reporter 1:3-8.

Tanksley, S.D., H. Medina-Filho and C.M. Rick. 1981. The effect of isozyme selection on metric characters in an interspecific backcross of tomato-basis of an early screening procedure. Theor. Appl. Genet. 60:291-296.

Tobolski, J.J. and J.W. Hanover. 1971. Genetic variation in the monoterpenes of Scotch pine. Forest. Sci. 17:293-299.

Weber, J.C. and R.F. Stettler. 1981. Isoenzyme variation among ten populations of Populus trichocarpa Torr. et Gray in the Pacific Northwest. Silvae Genetica 30:82-87.

Weissenberg, Kim von. 1973. Indirect selection for resistance to fusiform rust in loblolly pine. Acta Forestalia Fennica 134:1-46.

Weissenberg, K. von. 1976. Indirect selection for improvement of desired traits. IN: J.P. Miksche (Ed.) Modern methods in forest genetics. Springer-Verlag, 288 p.

Wheeler, N.C. and R.P. Curies. 1982. Population structure, genic diversity, and morphological variation in _Pinus contorta_ Dougl. Can. J. For. Res. 12:595-606.

Wilkinson, R.C. and J.W. Hanover. 1972. Geographical variation in the monoterpene composition of red spruce. Phytochem. 11:2007-2010.