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Indirect Selection for Resistance to Fusiform Rust in Loblolly Pine

Epäsuora valinta Cronartium fusiforme-kestävyyden lisäämiseksi Loblolly-männyllä (Pinus taeda L.)

Kim von Weissenberg

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INDIRECT SELECTION FOR RESISTANCE TO FUSIFORM RUST IN LOBLOLLY PINE

KIM VON WEISSENBERG

SELOSTE

EPÄSUORA VALINTA CRONARTIUM FUSIFORME-KESTÄVYYDEN LISÄÄMISEKSI LOBLOLLY-MÄNNYLLÄ (PINUS TAEDA L.)

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PREFACE

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Raleigh, August 1971

Kim v. Weissenberg

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Suonenjoki, May 1972

K. v. W.

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INTRODUCTION

Fusiform rust caused by *Cronartium fusiforme* HEDGC. and HUNT *ex* CUMM. is the most important disease of loblolly and slash pines (*Pinus taeda* L. and *Pinus elliottii* ENGELM var. *elliottii*) in the southern United States (HEPTING and JEMISON, 1958; POWERS, 1969). Selection and breeding of genetically resistant trees is the most promising method of control for the disease in plantations. This is true because (i) silvicultural and chemical methods of control are either inefficient or economically unfeasible, and (ii) moderate to substantial genetic variation and control of resistance to the disease exists in both loblolly and slash pines, (HENRY and BERKAW, 1956; BARBER, 1964; JEWELL and MALLETT, 1964; GODDARD and ARNOLD, 1966; WELLS and WAKELEY, 1966; KINLOCH and STONECYPHER, 1969; BLAIR, 1970; DINUS, 1972).

Several tree improvement programs in the southern United States have made resistance to fusiform rust a major breeding goal. In these programs the predicted gain in resistance from each succeeding cycle of mass selection will depend on the magnitude of each of three factors: (i) the extent of genetic control of resistance, (ii) the phenotypic variation of rust resistance in the base population, and (iii) the intensity of selection that is applied in each cycle of selection (FALCONER, 1967, p. 193). If the magnitude of the first two factors is estimated accurately they may be considered inherent characteristics of the base population. Thus, they are not readily amenable to manipulation by tree breeders. The intensity of selection, however, depends only on the proportion of the base population selected for breeding in each cycle of selection and therefore can be increased by (i) testing for rust-resistance as large numbers of individuals in the base population as possible and (ii) decreasing the proportion of individuals selected for breeding. If the selected proportion is very small, continuous gain from subsequent cycles of selection will be endangered due to decreased variation in resistance. In order to avoid decreasing both the selected proportion and the intensity of selection, the procedures of selection must be rapid and inexpensive enough so that a large portion of the base population can be tested.

At present, selection of phenotypically preselected breeding material is dependent mainly on field tests for resistance of progeny; but the results frequently are not consistent (HENRY and JEWELL, 1963; KINLOCH and KELMAN, 1965; LAFARGE and KRAUS, 1967). Difficulties inherent in these field tests include: (i) variation in the amount of inoculum from year to year, (ii) possible variation in the virulence of the pathogen between years and test-localities, (iii) variation in microclimatic conditions that influence the amount of infection, (iv) variation in soil factors that may influence susceptibility, and (v) the substantial costs and considerable time required for completion of satisfactory field tests (LEWIS and COWLING, personal communication). For these five reasons, in spite of several years of intensive field testing, only a limited number of clones with satisfactory data on resistance of their offspring have been identified; also only a relatively low selection intensity has been achieved. In addition, the population of plants available for subsequent breeding for both disease resistance and other desirable characteristics continues to be limited.

The need for more efficient methods of selection has prompted intensive research to develop and utilize artificial inoculation techniques suitable for large scale testing of breeding material (JEWELL, 1960; JEWELL and MALLETT, 1964; GODDARD and ARNOLD, 1966; SNOW, 1968; SCHMIDT, 1972; MILLER, 1970; DINUS, 1972; DWINELL, 1972). Some of these techniques have increased the capacity of selection procedures (GODDARD and STRICKLAND, 1970). Considering the practically unlimited size of the base population of loblolly and slash pines, however, available procedures still will allow for testing of only a very small part of the host populations.

Recognizing the limitations in present direct selection methods, attention was given

to the possibilities of using indirect selection — selection by means of some trait (here called a marker trait) correlated with but different from the desired trait itself. The desired trait — sustained resistance to fusiform rust in loblolly pine — most likely is, within each mechanism of resistance, conditioned by a variety of complex physiological responses to infection. In tree breeding programs and genetic studies, resistance has been defined usually as a single trait easy to measure, such as percentage of noninfected plants per family or clone, c-score, or number of galls per individual plant (STONECYPHER, 1966; KINLOCH and STONECYPHER, 1969; BLAIR, 1970). In the present study resistance also has been defined as a single trait and measured as percentage of noninfected plants per clone or family. Possibly discovered marker traits will be correlated with resistance defined and measured in this manner.

The objectives of this study were to: (i) examine the theory of indirect selection and its applications in breeding programs, (ii) review earlier efforts to use indirect selection in breeding forest trees for disease resistance in general and for resistance to *Cronartium fusiforme* in loblolly pine in particular, (iii) develop a method for evaluation of environmental influences on the stability of relative rust resistance of genotypes tested in several environments, and (iv) conduct a preliminary search for chemical markers of resistance to fusiform rust in loblolly pine in the hope that possibly discovered phenotypic correlations with resistance may suggest genotypic correlations strong enough to justify further attempts to use indirect selection in breeding for rust-resistance.

INDIRECT SELECTION IN BREEDING PROGRAMS

Indirect selection is defined as a selection procedure by which improvement of a desired trait X is achieved by selecting the breeding material for another trait Y which is genetically correlated with the desired trait. The gain that can be expected from indirect selection, assuming that resistance is a quantitative rather than a qualitative trait, depends on four factors (FALCONER, 1967, p. 320):

$$G_x = i_y h_y \sigma_{ax} r_a$$

where G_x = gain in the desired trait X by indirect selection for a marker trait Y

i_y = selection intensity achieved when selecting for the marker trait

h_y = square root of the heritability of the marker trait

σ_{ax} = standard deviation of the additive genetic variance of the desired trait

r_a = genetic correlation between the desired trait and the marker trait

Genetic correlation between two traits occurs when: (i) two different genes controlling the traits are located closely together on the same chromosome (linkage), (ii) one gene effects both traits (pleiotropy), or (iii) linkage and pleiotropy occur simultaneously. If the relationship between two traits is dependent on linkage, and assuming no epistasis between the genes, the genetic correlation between the two traits will be decreased by repeated cycles of selection due to breaking up of the linkage block during the breeding process (FALCONER, 1967, p. 320; MILLER and RAWLINGS, 1967a); if the relationship is due to pleiotropy, however, the genetic correlation will remain unchanged over several cycles of selection (FALCONER, 1967). For this reason, the most useful markers probably will be traits that have a high genetic correlation predominantly due to pleiotropy.

Indirect selection is likely to be superior to direct selection when there are large technical difficulties in selecting for the desired trait directly. Such difficulties include large errors in measurement of the desired trait and very expensive as well as time consuming methods of measurement. Since such difficulties occur frequently in breeding programs, the possibilities of using indirect selection have been explored both in plant and animal breeding. An elegant example of successful indirect selection is the improvement of the nutritional quality of corn endosperm (*Zea mays* L.) by selecting for opaque and floury endosperm. These traits are closely correlated with the amounts of lysine and tryptophane in the seed — two amino acids that are essential for humans and many animals (MERTZ, 1968). Another interesting example of indirect selection is breeding for resistance of tobacco to the root-knot nematode, *Meloidogyne incognita* KOFOID and WHITE, by using susceptibility to potato virus Y as a marker trait (HENDERSON and TROUTMAN, 1963; LAPRADE and HENDERSON, 1968).

Table 1 contains some examples of successful or suggested applications of indirect selection in crop, animal, and forest-tree breeding. Most suggested applications are based on observed phenotypic correlations. Only a few genetic correlations have been estimated. Pleiotropy or close linkage was suggested in only one case.

Table 1. Examples of successful or suggested applications of indirect selection

Organism	Desired trait	Marker trait	Remarks	References
Triticum aestivum L. Wheat	Rust resistance, hardiness, yield	Globulin fractions	Suggested; consistent ranking of five varieties	NELSON and BIRKELAND, 1929
Allium cepa L. Onion	Resistance to *Colletotrichum circinans* (BERK.) VOGL.	Red outer Scales[1]	Used; homozygotes with red scales are resistant, heterozygotes with cream colored scales have intermediate resistance	JONES *et al.*, 1946
Lespedeza sp. Sericea lespedeza	High protein content	Low tannin content	Suggested; phenotypic correlations = −0.23, −0.53, and −0.51 respectively	COPE, 1962; DONNELLY and ANTHONY, 1969
Avena sativa L. Oat	Yield	Serological differentiation	Suggested; consistent ranking of seven varieties	KLEESE and FREY, 1965; SMITH and FREY, 1970
Triticum aestivum L. and *Zea mays* L. Wheat and corn	High protein content	High nitrate reductase activity[1]	Suggested; consistent ranking of varieties	BEEVERS and HAGEMAN, 1969
Nicotiana tabacum L. Tobacco	High yield	Low alkaloid content	Suggested; $r_a = -0.50$	MATZINGER and WERNSMAN, 1968
Nicotiana tabacum L. Tobacco	Resistance to the root-knot nematode, *Meloidogyne incognita* KOFOID and WHITE	Susceptibility to vascular necrosis caused by potato virus Y	Successful; testing for susceptibility to the virus is 5—9 times faster than testing for resistance to the nematode	HENDERSON and TROUTMAN, 1963; LAPRADE and HENDERSON, 1968
Nicotiana tabacum L. Tobacco	Low nornicotine content	Cherry red leaves	Successful; undesirable genotypes selected against	WERNSMAN and MATZINGER, 1970
Gossypium hirsutum L. Cotton	Insect resistance	Glabrous[1], nectariless[1], high gossypol content[1]	Successful; phenotypic correlations	LUKEFAHR and HOUGHTALING, 1969
Gallus gallus L. Domestic chicken	Disease resistance	Blood group	Suggested; strong association in five out of 10 genotypes. Pleiotropy or very close linkage	GILMOUR, 1969; GILMOUR and MORTON, 1970
Pinus sylvestris L.	Resistance to *Lophodermium pinastri* (SCHRAD.) CHEV.	Inhibited growth of the pathogen on needle-juice-agar[1]	Suggested; consistent ranking of over 20 clones	SHÜTT, 1966
Pinus elliottii ENGELM. var. *elliottii* Slash pine	Tall oil yield	Yield of ethanol-benzene extractives[1]	Suggested; $r_a = 0.58$	FRANKLIN, *et al.*, 1970
Pinus teada L. Loblolly pine	Resistance to *Cronartium fusiforme* HEDGC. and HUNT *ex* CUMM.	Content of β-phellandrene in branch cortex	Suggested; $r_a = 0.54$ and 0.78	ROCKWOOD, 1972

[1] The causal relationship between the desired and marker trait is known.

INDIRECT SELECTION FOR DISEASE RESISTANCE IN FOREST TREES

Three reports have been published on the use of indirect selection in forest trees, but only two are concerned with breeding for disease resistance. SHÜTT (1966) reported on the use of press juice from needles of *Pinus sylvestris* L. to identify clones resistant to *Lophodermium pinastri* (Schrad.) CHEV. ROCKWOOD (1972) found that content of β-phellandrene in branche cortex of *Pinus taeda* was genetically correlated with resistance to *Cronartium fusiforme* .Selection for high yield of tall oil in slash pine by using a simple measurement of benzene-ethanol extractives as a marker is a promising application of indirect selection (FRANKLIN *et al.* 1970).

In a comprehensive review on the biochemistry of resistance of *Pinus monticola* DOUGL. to *Cronartium ribicola* J. C. FISCH *ex* RABENH., HANOVER (1966) concluded that the objective of finding a marker for indirect selection of white pines could be attained, although not without difficulty. Despite HANOVER's cautious optimism, other efforts to discover biochemical markers for rust-resistance in white pine (HANOVER and HOFF, 1966; HOFF, 1968, 1970) have not been successful. Discouraged with these unsuccessful efforts, BINGHAM (1970) decided that further physiological studies to identify markers should be deferred by his research group until a more complete understanding had been obtained of the inheritance, physiology, and mechanisms of resistance to the pathogen.

In order to determine whether further efforts to discover markers for indirect selection of rust-resistant pines were justified, an investigation was made to determine the magnitude of gain in resistance to fusiform rust that could be achieved by indirect selection compared to the predicted gains achieved by presently applied direct selection. The comparison was made by using the following relationship (FALCONER, 1967, p. 320):

$$\frac{G_x}{D_x} = r_a \cdot \frac{i_y}{i_x} \cdot \frac{h_y}{h_x}$$

where G_x = gain in resistance to rust by indirect selection using the marker Y

D_x = gain in resistance to rust by direct selection

r_a = genetic correlation between resistance to rust and a marker Y

i = selection intensity achieved for the marker Y and resistance to rust X, respectively

h = square root of the heritability for the marker Y and resistance to rust X, respectively

Assuming that a potential marker Y has the same heritability as resistance to fusiform rust in loblolly pine, in this case 0.22 (BLAIR, 1970), the formula can be reduced to:

$$\frac{G_x}{D_x} = r_a \cdot \frac{i_y}{i_x}$$

This simplified formula shows that the relative efficiency of indirect vs. direct selection is dependent on the genetic correlation between the marker trait and rust-resistance and the selection intensities that can be achieved by the direct and indirect selection procedures, respectively.

Using genetic parameters obtained from the Loblolly Pine Heritability Study, BLAIR (1970) calculated the following estimates of predicted gain in rust-resistance (expressed in arbitrary units of resistance) that could be achieved by three procedures of direct selection: (i) mass selection = 0.71, (ii) family + within family selection = 0.88, and (iii) mass selection + progeny testing = 1.40. The gains achieved by the three methods relavtive to that achieved by mass selection only were thus 0.71/0.71 = 1.00, 0.88/0.71 = 1.24, and 1.40/0.71 = 1.97. Solving the previous equation for these relative gains and increasing values of i_y/i_x, three curves were obtained, one for each of the three estimates of gain (Figure 1).

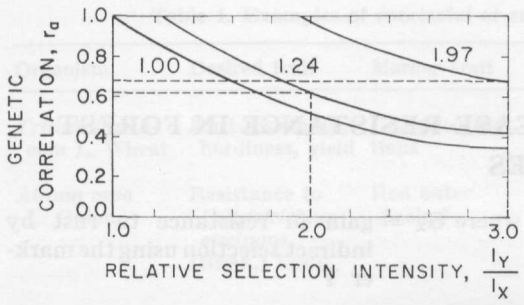

Figure 1. Relationship between genetic correlation and selection intensities for 1.00, 1.24, and 1.97 units of relative gain.

If the selection intensity for indirect mass selection (i_y) can be made twice as great as that of the selection intensity for direct selection ($i_y/i_x = 2$), then a genetic correlation of 0.62 is sufficient to achieve a predicted gain from simple indirect mass selection equal to that for expensive direct family + within family selection. Similarly, if $i_y/i_x = 3$, a genetic correlation of 0.70 is required to achieve a predicted gain equal to that for direct mass selection + progeny testing. Thus gains from simple indirect mass selection can be made equal or superior to the gains presently predicted (Blair, 1970) from direct selection methods provided that: (i) the heritability of the marker is equal to that of resistance to fusiform rust (0.22 according to Blair, 1970), (ii) the genetic correlation between the marker and resistance is moderate (0.62—0.70), and (iii) the selection intensity is two or three times greater than that achieved when direct selection is applied. Each of these conditions are examined in detail below.

If the marker has a heritability higher than that of resistance to fusiform rust, the genetic correlation or selection intensity can be smaller without decreasing the predicted gain. Since heritability values for a number of traits in loblolly pines often are even larger than that reported for resistance to fusiform rust (Goggans, 1962; van Buijtenen, 1962; Stonecypher *et al.*, 1964), it was assumed that chemical traits with a sufficiently large heritability could be found.

Since selection intensity is a function of the ratio of the number of plants selected as having a desirable trait to the total number of plants tested for that trait, any trait that can be measured more quickly or at less cost than by direct tests for resistance will permit a higher selection intensity to be applied with the same investment of time and effort. Thus the relatively high selection intensity required for indirect selection can be achieved by measuring a suitable marker by means of any of a large number of routine chemical or other analytical procedures. Considering the modest selection intensities achieved by large scale direct selection of clones delivering resistant offspring in most breeding programs in the southeastern United States, it appears that doubling and in some cases even tripling such intensities is possible by means of efficient indirect selection (Table 2). In addition the breeder can provide a sufficiently large number of selected plants to achieve further gains from subsequent cycles of selection for both resistance to rust and other important characteristics as well.

The need for a moderate to high genetic correlation required for successful indirect selection probably can be met if the marker trait is a component trait of resistance. A component trait is defined as a trait that directly or indirectly contributes to the disease-resistance of the pine host. Genetic correlations often are high for component traits

Table 2. Maximum proportions to be selected from a large base population in order to achieve a given selection intensity, i_x, and to double or triple it. Figures obtained from Namkoong and Snyder (1969).

i_x	$1 \times i_x$	$2 \times i_x$	$3 \times i_x$
	(Maximum proportion to be selected)		
0.50	—	0.380	0.166
0.75	—	0.166	0.031
1.00	0.380	0.058	0.004
1.25	0.260	0.016	0.0002
1.50	0.166	0.004	—
1.75	0.100	0.0006	—

in forest trees (FRANKLIN *et al.*, 1970) as well as in agronomic crops (MILLER *et al.*, 1958; AL-JIBOURI *et al.*, 1958; MOLL and ROBINSON, 1966; STUBER *et al.*, 1966; MILLER and RAWLINGS, 1967b). High genetic correlations also, however, can be found between traits that have no apparent or well-understood causal relationship to each other. Examples of such correlations are those found between high yield and low alkaloid content in tobacco and opaque endosperm and high percentage of basic amino acid content in proteins of corn (MATZINGER and WERNSMAN, 1968; MERTZ, 1968).

The mechanisms of resistance to fusiform rust in loblolly pine are not well understood at the present time. In the western white pines (*Pinus monticola* and *Pinus lambertiana* DOUGL.) several different mechanisms of resistance to *Cronartium ribicola* have been found and the genetic control of some of these mechanisms are partly known (MCDONALD and HOFF, 1970; KINLOCH *et al.*, 1970). In slash pine, three different mechanisms of resistance (host responses) to fusiform rust have been demonstrated recently (MILLER, 1971), but no information is available on the genetic control and relative frequency of these mechanisms in the pine population. Several mechanisms of resistance also may be discovered in loblolly pine. If some of these different mechanisms occur with a large frequency in the host population, markers may be used which are highly correlated with these mechanisms. Direct selection may be used to breed for resistance controlled by less frequent mechanisms. It is therefore of great importance both to identify various mechanisms and to determine their frequency in the population.

There are two additional major factors involved in discovering markers with sufficiently high genetic correlations with resistance to fusiform rust:

(i) Some recent reports (SNOW and KAIS, 1970; KAIS and SNOW, 1972) have suggested that geographic variation in the pathogenicity of *Cronartium fusiforme* occurs. Such variation may further reduce the probability of finding a marker sufficiently correlated with resistance to such races of the pathogen as may occur in the region where the improved trees will be grown.

(ii) To determine the potential value of a marker trait, accurate estimates of its heritability and genetic correlation with rust-resistance ultimately must be obtained. Genetic studies satisfactory for this purpose will be very costly to establish and require considerable time to complete.

Presently there is insufficient information to decide about the relative importance of factor (i). The unique and comprehensive Loblolly Pine Heritability Study established in southwestern Georgia in the United States (CECH *et al.*, 1962; STONECYPHER, 1966; KINLOCH and STONECYPHER, 1969; BLAIR, 1970) provides satisfactory genetic data with which to estimate the heritability and genetic correlation for possible marker traits. Considering the potential advantages of indirect selection previously discussed, it was concluded that continuing efforts to find a marker were justified. Thus, the purpose of the experimental part of this study was to find some chemical characteristics in loblolly pine with such a high phenotypic correlation with resistance to fusiform rust that further studies on such markers can be justified.

THE CHOICE OF PLANTS AND MARKER TRAITS TO BE INVESTIGATED

Criteria for Choice of Ideal Plant Material

In previous efforts to find markers for disease resistance in pines, several different types of plant material have been used. SHÜTT (1966) compared over 20 clones of *Pinus sylvestris* with known and varying resistance to *Lophodermium pinastri*, and KLINGSTRÖM (1969) compared four clones of *Pinus sylvestris* with known resistance to *Melampsora pinitorqua* (BRAUN) ROSTR. Both HANOVER and HOFF (1966) and HOFF (1968, 1970) used mature trees of *Pinus monticola* selected for phenotypic resistance in natural stands, while HARE (1970) compared seedlings of resistant western and susceptible eastern sources of loblolly pine as well as pollen, seeds, and seedlings from six species of pines with varying resistance to fusiform rust from the southern United States. ROCKWOOD (1972) used full- and half-sib families of loblolly pine. Except for ROCKWOOD's results, the more promising ones invariably have been obtained in studies of clonal material (SHÜTT, 1966; KLINGSTRÖM, 1969).

In the section of this paper dealing with the properties of a marker trait, it was pointed out that a marker trait should meet several qualifications (p. 10). In order to find a marker meeting such qualifications it is necessary to consider the problem of choice of plant material more in detail.

To maximize the probability of discovering potentially useful chemical markers for resistance to fusiform rust, the plant material investigated ideally should meet the following criteria:

1. The material should lend itself to analysis of components of additive variance and covariance in order to estimate the heritability of the marker and its genetic correlation with disease resistance. In order to obtain reliable estimates of these parameters, elaborate genetic studies ultimately will be required.

2. The trees, clones, or families should differ greatly in resistance. Large variation in resistance and other traits within the clones and families as well as lack of precision in the measurements of the marker trait, may reduce the possibilities of obtaining satisfactory estimates of phenotypic and genotypic correlations.

3. The clones or families should have a small genotype $\times$ environment (G $\times$ E) interaction in order to minimize errors in estimates of resistance associated with the environment where the plants have been grown and samples collected for the study.

4. The resistance of the plants should be reliably estimated, preferably in several environments, and especially during the first seven years of the seedling's life during which time fusiform rust is most damaging.

5. The material should represent subpopulations of different age and geographic distribution since different patterns of inheritance and mechanisms of resistance may occur in various subpopulations.

The first three criteria will be considered in greater detail with respect to the choice of trees, clones, and families as well as the problem of estimating G $\times$ E interaction for resistance to fusiform rust.

Mature trees and their clones have the advantage of limited variation within each clone or tree. This allows for less intensive sampling, but on the other hand, estimates can rarely be obtained of the components of strictly additive genetic variance and covariance required for computation of heritability and genetic correlation. Mature trees may be growing in different environments (*e.g.* plus-trees in natural stands selected for seed orchards) which may call for intensive sampling of neighboring trees in order to make approximate corrections for the environmental effect on the trait studied. Both mature trees and their clones provide material

for identification of markers for indirect selection of parents that deliver resistant offspring. Provided that estimates of the resistance as well as the components of additive genetic variance and covariance are available, the parent-offspring genetic cross-correlation and the heritability can be computed (FALCONER, 1967, p. 317).

Full- and half-sib families may have a large within-family variation. This will require more intensive sampling of families than of clones. Families from genetic experiments or progeny tests may provide satisfactory estimates of components of variance and covariance, and the genetic correlation and heritability can be computed (FALCONER, 1967, p. 317).

Other types of plant material, such as resistant and susceptible species and various geographical provenances used by HARE (1970), do not provide possibilities for estimation of heritability and genetic correlation, and must therefore be considered much less suitable for studies concerned with finding markers useful for indirect selection.

Although several studies on resistance to fusiform rust in loblolly pine have indicated only small or no G × E interaction (WELLS and WAKELEY, 1966; KRAUS, 1967; KINLOCH and STONECYPHER, 1969; BLAIR, 1970; KINLOCH and ZOERB, 1971), families with intermediate resistance commonly show large G × E interaction (KINLOCH and STONECYPHER, 1969; BLAIR, 1970). Substantial interaction also was found in some progeny tests of slash pine (SCHMIDT and GODDARD, In press). Likewise, examination of the records for some 200 families of loblolly pine evaluated for rust-resistance in 32 progeny tests during several years, suggested that a large number of families performed differently with respect to their resistance in tests located in different environments. Therefore a method was developed by which comparisons could be made of the magnitude of G × E interaction of resistance to fusiform rust for individual families.

Choice of Potential Marker Traits

Useful markers for indirect selection of loblolly pines resistant to fusiform rust might be found among component traits of resistance but such traits are not known, although some have been suggested. The surface-area of susceptible pine tissue exposed to basidiospores has been suggested as a factor in resistance (BALTHIS and ANDERSON, 1944) but later this hypothesis was discredited on the basis of field observations of the association between the size of pines and the frequence of infection (GILMORE and LIVINGSTON, 1958; KINLOCH and STONECYPHER, 1969; DINUS and SCHMIDTLING, 1971). It also has been suggested that severe infection may be caused by early development of susceptible tissue which subsequently would be exposed to inoculum for a longer period of time (SIGGERS and LINDGREN, 1947; SIGGERS, 1955). Experimental support for this hypothesis has not been found (BLAIR, 1970; DINUS and SCHMIDTLING, 1971.).

Our present knowledge of the nutritional requirements of *Cronartium fusiforme* is too limited for development of hypotheses about resistance based on deficiency of available nutrients in the host. This limitation in our knowledge is due partly to the difficulties encountered in attempts to study obligate parasites in axenic culture (SCOTT and MACLEAN, 1969).

It generally has been assumed that inhibition of germination of basidiospores could be a factor in resistance to rusts. Doubt about the validity of this assumption has been expressed (HANOVER, 1966), but evidence sufficient to invalidate it has not yet been presented. Several attempts have been made to discover markers for rust-resistance in pines among host extractives that would inhibit spore germination (HANOVER and HOFF, 1966; HOFF, 1968, 1970; KLINGSTRÖM, 1969; HARE, 1970), but none has so far led to the discovery of a useful marker.

The wide variety of host extractives that have been studied for the purpose of finding a marker include: sugars, amino acids, organic acids, anthocyanins, chlorophylls, carotene, macronutrient elements, monoterpenes, and pectic and cellulolytic enzymes (HANOVER, 1966), polyphenols and simple phenols (HANOVER and HOFF, 1966), tannins and natural toxic compounds (HOFF, 1968, 1970), thermolabile enzymes, water-soluble needle diffusates, lipid extracts, chloroplast extractives, vacuolar pigments, and various frac-

tions obtained through organic solvent partitioning (HARE, 1970).

In studies of as yet unidentified growth-inhibiting substances, clones of *Pinus sylvestris* resistant to pine twisting rust caused by *Melampsora pinitorqua* were found to contain high concentrations of inhibitors compared to susceptible clones; both growth inhibitors and resin acids inhibited germination of basidiospores of the pathogen (KLINGSTRÖM, 1969), suggesting that these compounds may be component traits of rust-resistance and therefore merit further investigation.

Resin acids are major components of pine oleoresins; they occur mostly in resin ducts but also in epithelial and other parenchyma cells throughout the tree (MUTTON, 1962) and are reserve metabolites mobilized at times of intensive synthesis of sugars, starch, and fatty acids (BERNARD-DAGAN, 1961).

The effect of resin acids on development and metabolism of bacteria and fungi has not been studied extensively. Recent research indicates, however, that resin acids may act as inhibitors and stimulators or provide nutrients for some organisms. Resin acids of the abietic type can serve as a sole source of carbon for growth of some bacteria (RAYNAUD *et al.*, 1968). Levopimaric acid, a common resin acid, can be a sole source of carbon for *in vitro* growth of *Fomes pinicola* (SWARTZ) CKE. (SHRINER and MERRILL, 1970). Both oleoresin and abietic acid inhibited *in vitro* mycelial growth of *Fomes annosus* (FR.) CKE. (SHAIN, 1970). Studies on the effect of resin acids on *Cronartium fusiforme* is very limited; it has been found that an ethanol extract, containing phenols and resins from rust-susceptible slash pine, stimulated germination of basidiospores of fusiform rust (HARE, 1970).

The effects of fatty acids on wood-inhabiting and tree-rust fungi have been studied more extensively than have the resin acids. Studies on germination of basidiospores of *Lenzites saepiaria* (WULF.) FR. indicated that esters of short-chain fatty acids inhibited germination while esters of long-chain fatty acids, in this case palmitic, stearic, and oleic acids, stimulated germination (WALKINSHAW and SCHELD, 1965; SCHELD and PERRY, 1970). The latter authors suggested that one of the main roles of exogenous organic acids, including fatty acids, could be the generation of CO_2 for lipid and membrane synthesis in the fungus. Unsaturated fatty acids may stimulate formation of rhizomorphs of *Armillaria mellea* (VAHL.) QUÉL. *in vivo*. (MOODY and WEINHOLD, 1970). *In vitro* studies indicated that linoleic acid, a common unsaturated fatty acid, may be important in the resistance of *Alnus rubra* BONG. to infection by *Poria weirii* MURR.; it also inhibited growth of *Fomes annosus* (LI *et al.*, 1970).

The physiological mechanisms underlying the influence of fatty acids on fungal growth have been studied using *Boletus variegatus* SWARTZ *ex* FR. as a test organism (PEDERSEN, 1970; LODE and PEDERSEN, 1970). The authors concluded from *in vitro* experiments, that exogenously applied short-chain fatty acids, especially octanoic acid, rapidly interacted with lipophilic parts of the cell membranes leading to inhibition of respiration and leakage of low molecular weight compounds, such as pentoses, pentosphosphates, purine and pyrimidine bases, nucleosides, and mono- and dinucleotides. Further, loss of oligoribonucleotide peptides, structural elements of membranes, occurred after 1.5—2 hours exposure to octanoic acid. Loss of this compound led to irreversible stagnation of growth by the fungus. The leakage of compounds was smaller when the mycelium was exposed to a 12-carbon fatty acid. Fatty acids with more than 12 carbon atoms in the chain were not tested.

In studies on the effect of fatty acids on germination of spores of *Cronartium fusiforme* it has been found that long-chain fatty acids stimulated germination of aeciospores (WALKINSHAW and SCHELD, 1965) suggesting that germination occurred at the expense of exogenous fatty acids which possibly could act as emulsifiers for—or stimulants of—lipase activity (WALKINSHAW, 1965). Later it was found that oleic acid increased germination of stored aeciospores and prolonged germination of metabolically active aeciospores of the fungus (WALKINSHAW, 1968a), perhaps by stabilizing cellular membranes in the germinating spores. In further studies (WALKINSHAW, 1968b) it was observed that exogenous unsaturated fatty acids with 18 carbons in the chain (*i.e.*, linoleic and linolenic acids) stim-

ulated the consumption of oxygen by aeciospores. These results were related to the natural conditions of the pathogen:

«These acids occur in high concentration in the natural host (MAX, 1945) and in aeciospores of many rust fungi (TULLOCH and LEDINGHAM, 1962). They probably play an active role in aeciospore metabolism . . .»

While Walkinshaw's studies pertained to the effect of fatty acids on aeciospores, which do not infect the pine host, other studies were concerned with the effects of oleic acid and lipid-containing extracts on germination of basidiospores, which do infect pines (HARE, 1970). Hare concluded that:

«Oleic acid strongly promoted germ tube formation on agar but the tubes were short, bent, and branched or otherwise malformed.»

The lipid extract obtained by water partitioning of a crude chloroform-methanol extract from slash pine did not promote germ tube formation and growth as compared to the water fraction of the crude extract. The specific chemical composition of the extracts was not reported.

The amount of certain long-chain fatty acids in cells of *Chlorella fusca* SIHIRA and KRAUS increased with CO_2-fertilization (DICKSON *et al.*, 1969), but no reports have been found of similar changes in forest trees or any other plants due to fertilization with CO_2 or other nutrients. Fertilization with nitrogen, phosphorus, and potassium as well as site preparation of pine plantations have increased the susceptibility of seedlings of pines to *Cronartium fusiforme* (BALTHIS and ANDERSON, 1944; SIGGERS and LINDGREN, 1947, BOGGESS and STAHELIN, 1948; GILMORE and LIVINGSTON, 1958; KINLOCH and STONECYPHER, 1969; MILLER, 1972; DINUS and SCHMIDTLING, 1971.).

The various reports mentioned above suggest that quantitative and qualitative properties of fatty acids may affect development of *Cronartium fusiforme* and thus may have some influence on resistance. The information available on the effect of resin acids (HARE, 1970) was very limited compared to that of fatty acids. Experiments were therefore conducted on the effects of certain resin and other organic acids on germination of basidiospores. Growth-inhibiting compounds and fatty acids were considered promising as potential marker traits.

MATERIALS AND METHODS

Choice of Available Plant Material

An intensive search was made in several tree-improvement and tree-breeding programs in the southern United States for clones, full-sib, and half-sib families of loblolly pine that met the criteria for choice of ideal plant materials. The programs included the N. C. State University-Industry Cooperative Tree Improvement Program, the tree-improvement program of the Georgia Forestry Commission and the Southern Forest Experiment Station (U.S.D.A, Forest Service) as well as the Cooperative Loblolly Pine Heritability Study of N. C. State University and International Paper Company. More than 200 different genotypes established in some 50 progeny- or specialtests in six states were assessed for resistance to fusiform rust in choosing the plant materials used in this study. A method was developed to estimate the amount of G × E interaction and used as much as possible as an important aid in choosing the material.

Several methods for estimation of G × E interaction for individual families and varieties of agronomic crops have been developed by others (PLAISTED, 1960; FINLAY and WILKINSON, 1963; WRICKE, 1966; SCOTT, 1967; HANSON, 1970) and for height growth in *Pinus devaricata* AIT. (MORGENSTERN and TEICH, 1969). In the present study, the standard error of the average Standard Relative Resistance (SRR, see equation below) was taken as a measure of the family's G × E interaction over several environments (Stability Index, SI). SRR was obtained by a linear transformation of the percentage of infected seedlings in all tests where it had been tested (Figure 2). This transformation was necessary to avoid over-estimation of the variation in percentage of plants infected due to the varying amounts of infection in the different tests. SRR was calculated as the ratio between a family's deviation ($MP_j - x_{ij}$) from the midpoint (MP_j) of the range (R_j) of percentage of plants infected in the test and half of this range ($R_j/2$):

$$SRR_{ij} = \frac{2}{R_j}(MP_j - x_{ij})$$

where SRR_{ij} = Standard Relative Resistance of the i^{th} family in the j^{th} test

MP_j = midpoint of the range of plants infected among all families in the j^{th} test

x_{ij} = percentage of plants infected in the i^{th} family in the j^{th} test

R_j = range of percentage of plants infected among all families in the j^{th} test

A mean $SRR_i = +1.00$ for a family that has the lowest percentage, while $SRR_i = -1.00$ for a family with the highest percentage of plants infected in all tests where it has been evaluated. It was assumed that all families in a test had been exposed to equal amounts of inoculum. No attempt was made to determine the relative contribution of various environmental factors, such as (i)

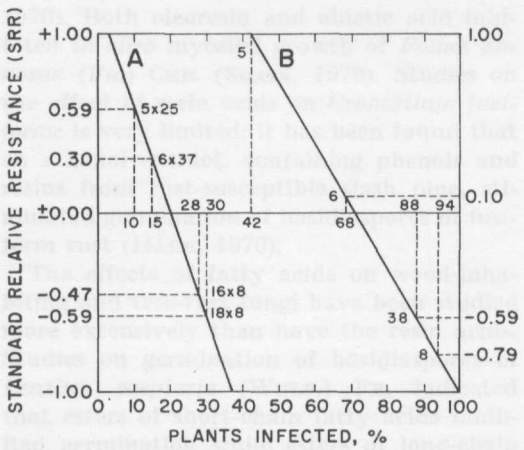

Figure 2. Transformation of percentage of plants infected of full-sib families (A) and half-sib families (B) to Standard Relative Resistance values. The transformation lines are for data in Test III, Set 3, and Test II, Set 4, Table 3, respectively.

variation in the pathogen population, (ii) geographical location and site index of the test, (iii) year of planting, *etc.*, to the magnitude of the observed G × E interaction. Families with very small SI-values were considered to meet the third criterion for material to be used in this study.

Five factors seriously limited the choice of ideal material: (i) scarcity or complete lack of seed from many desirable crosses limited the number of families that could be raised from seed, (ii) lack of data on rust-resistance for offspring from mature trees or their clones limited the study of older material, (iii) bias from phenotypic selection of resistant parent trees limited the choice of highly susceptible trees, (iv) large G × E interaction for rust-resistance of families with intermediate resistance to rust (KINLOCH and STONECYPHER, 1969; BLAIR, 1970) limited the study to families with only extreme resistance or susceptibility, and (v) difficulties to find large numbers of genotypes within each subpopulation with reliable data on resistance and small G × E interaction; only a maximum of five clones or four families were chosen from each subpopulation. Because of these limitations, the material finally chosen did not meet all ideal criteria but was as good a compromise as possible.

Six sets of plant material were chosen containing both highly resistant and highly susceptible clones, full-sib, or half-sib families (Table 3):

Set 1 — The ortets [1] for these clones were located in the Coastal Plain region of North Carolina and South Carolina (Figure 3). Scions were collected when the ortets were 32—58 years old and grafted in 1959—1962 and in 1966 on root-stock from commercial seed. The ramets [2] were planted in a seed orchard located near Georgetown, South Carolina (Figure 3). Four clones were chosen from the 22 clones in this orchard based on the percentage of ramets infected with *Cronartium fusiforme*. The percentage of infection was determined for all ramets in 1968; for those grafted in 1966 it was determined again in 1970 (Table 3). The 35—37 ramets in each clone were evenly distributed over the whole orchard; infected ramets occurred

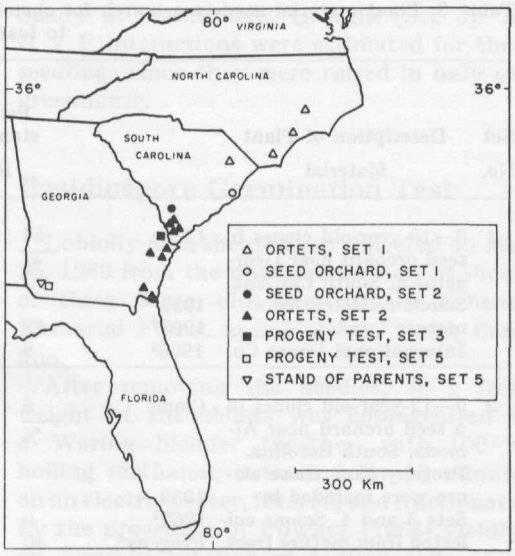

Figure 3. Geographical distribution of the plant material used in a search for chemical markers of loblolly pines resistant and susceptible to fusiform rust.

in all but two 0.4-ha sections of the 6-ha orchard. Thus, variation in the percentage of infected ramets per clone apparently was due predominantly to variation in resistance of the ramets rather than to uneven distribution of inoculum and (or) differences in environmental conditions leading to development of galls. Because these clones were planted in only one environment, no estimate could be made of G × E interaction.

Set 2 — The ortets for these clones were located in the Coastal Plain region of South Carolina and Georgia (Figure 3). Scions were collected when the ortets were 19—46 years old and grafted in 1959—1962 to root-stocks derived from commercial seed. The 32—54 ramets in each clone were planted randomly in a seed orchard near Almeda, South Carolina (Figure 3). Five clones were chosen from the 16 clones in this orchard based on the percentage of ramets infected with fusiform rust as determined in 1967. Data on the resistance of open pollinated progeny from the clones also were available (see Set 4, Table 3). Rust infection on pines in the surrounding area was severe — in a plantation established in 1958 within 170 m of the orchard, 98 % of the trees were infected. (KINLOCH and ZOERB, 1971). Because the

[1] Tree from which scions are collected for grafting.
[2] Grafted member of a clone.

Table 3. Plant material used in a search for chemical markers of loblolly pines resistant and susceptible to fusiform rust.

Set No.	Description of Plant Material		Percentage of plants infected and standard relative resistance (SRR)[1] Resistant		Susceptible		Range of % infected plants in all clones or families in the test
1	3—10-year-old clones in seed orchard near Georgetown, South Carolina.	Clone	59 %	58 %	102 %	71 %	
	Scions collected from mature trees.	1959—1962[2]	18	23	84	89	18—89
	International Paper Co.	1966[3]	9	11	18	55	9—55
2	9—12-year-old clones in a seed orchard near Almeda, South Carolina.	Clone	6 %	5 %	13 %	38 %	18 %
	Progeny from these clones were included in Sets 3 and 4. Scions collected from mature trees.	1959—1962[4]	0	19	40	43	65	0—100
	Union Camp Corporation	Progeny[5]	40	38	64	69	65	11—100

3	3-year-old trees from full-sib families grown in progeny test near Savannah, Georgia. Parents in seed Orchard, Set 2	Family	5 × 25 %	SRR	6 × 37 %	SRR	18 × 8 %	SRR	16 × 8 %	SRR	
		Test I	11	0.79	7	1.00	—	—	25	— 0.05	7—45
		Test II	12	1.00	20	0.74	56	— 0.44	66	— 0.77	12—73
		Test III	10	0.59	15	0.30	30	— 0.59	28	— 0.47	3—37
		Test IV	4	0.97	17	0.59	45	— 0.22	72	— 1.00	3—72
		Averages	9	0.84	15	0.66	44	— 0.42	48	— 0.55	
		SI[6]		± 0.09		± 0.14		± 0.11		± 0.23	

4	9-week-old seedlings from half-sib families grown in greenhouse. Parents in seed orchard, Set 2	Family	5 %	SRR	6 %	SRR	38 %	SRR	58 %	SRR	
		Test I	48	0.71	41	1.00	80	— 0.67	86	— 0.92	41—88
		Test II	42	1.00	68	0.10	88	— 0.59	94	— 0.79	42—100
		Test III	23	0.14	11	1.00	39	— 1.00	30	— 0.36	11—39
		Averages	38	0.62	40	0.70	69	— 0.75	70	— 0.69	
		SI		± 0.25		± 0.30		± 0.13		± 0.17	

5	7-year-old trees from full-sib families grown in the Loblolly Pine Heritability Study near Bainbridge, Georgia. International Paper Co.	Family	10-B %	SRR	15-D %	SRR	20-B %	SRR	51-C %	SRR	
		Test I	35	0.50	65	— 0.25	75	— 0.50	95	— 1.00	15—95
		Test II	25	0.70	65	— 0.25	75	— 0.50	95	— 1.00	15—95
		Test III	25	0.55	15	0.77	45	0.11	95	— 1.00	5—95
		Test IV	15	0.72	25	0.43	45	— 0.14	75	—1.00	5—75
		Averages	25	0.62	43	0.18	60	— 0.17	90	— 1.00	
		SI		± 0.06		± 0.25		± 0.15		± 0.00	

6	9-week-old seedlings from full-sib families, grown in greenhouse. Parents same as in Set 5		10-B %	51-B %
			25[7]	80

[1] SRR = + 1.00 for the most resistant family, SRR = —1.00 for the most susceptible family in the test
[2] Percentage of infected ramets, grafted in 1959—1962, as examined in 1968
[3] Percentage of infected ramets, grafted in 1966, as examined in 1970
[4] Percentage of infected ramets, grafted in 1959—1962, as examined in 1967
[5] Average percentage of infected plants in open pollinated progeny, Set 4
[6] Stability Index = standard error of average SRR
[7] Average percentage of infected plants in full-sib families, Set 5

clones were planted in only one environment, no estimates could be made of G × E interaction.

Set 3 — These full-sib families (Table 3) were obtained from the ramets in Set 2 by controlled pollinations. Four families were chosen from 30 available in four progeny tests established in 1965, 1966, and 1967 on two different sites near Savannah, Georgia (Figure 3). The progeny tests were established as randomized complete blocks with 10-tree row-plots. Tests I, II, and III contained six replications while test IV contained three replications (Table 3). The families were examined for rust infection in 1969 (Table 3). The most resistant family (5 × 25) had the least (SI = ± 0.09) while the most susceptible family 16 × 8 has the largest G × E interaction (SI = ± 0.23).

Set 4 — These half-sib families were obtained from the ramets in Set 2 by open pollinations. Four families were chosen from 12, whose resistance had been determined in three special tests completed in 1968 and 1969. Tests I and II were artificial inoculation tests while test III was a field test in a Rust Nursery (DRIVER *et al.*, 1966) near Bainbridge, Georgia (Table 3). The G × E interaction was large especially for the two resistant families. Seedlings of the selected families were raised for this study in a greenhouse at Raleigh, North Carolina.

Set 5 — The parent trees for these full-sib families were located in an area «typical of the Piedmont region» (STONECYPHER, 1966) of southwestern Georgia (Figure 3) and were evaluated for rust-resistance in the Loblolly Pine Heritability Study at Bainbridge, Georgia. Fifty-five full-sib families were planted on four sites in 1963. Sites I, II, III, and IV (Set 5, Table 3) were referred to by STONECYPHER (1966), KINLOCH and STONECYPHER (1969), and BLAIR (1970) as the «Peanut Field», «Rust Nursery», «Fallow Field», and »Residual Forest,» respectively. The families were evaluated for rust resistance in 1966 (Table 3). The two intermediate families, 15-B and 20-B, showed larger G × E interactions than the extreme families, 10-B and 51-C. Family 51-C showed no G × E interaction.

Set 6 — These full-sib families were raised in a greenhouse at Raleigh, North Carolina from the same seed lots used to raise the corresponding families in the Heritability Study at Bainbridge, Georgia (Set 5). No G × E interactions were estimated for these seedlings since they were raised in only one greenhouse.

Basidiospore Germination Test

Loblolly pine shoots were collected on May 25, 1969 from the uppermost whorl of shoots of three 3-year-old trees in the Schenck Memorial Forest near Raleigh, North Carolina.

After removing the needles, 50 g fresh weight of the shoots was homogenized in a Waring blender together with 100 ml boiling methanol, extracted for 10 minutes on an electric heater, filtered, and fractionated by the procedure of POWELL (1964) yielding an aqueous solution of sodium salts of organic acids. The acids were extracted with diethyl ether after acidifying the solution to pH 3 with 6 N HCl. The acidic extract was further fractionated by preparative thin layer chromatography (TLC) on silica gel G (E. Merck, Darmstadt, Germany). Using butanone:hexane (40:60, v:v) the chromatogram was developed by the ascending method twice to 6 cm and twice to 12 cm with air drying between developments (STAHL, 1967). Eight zones were located with short wave UV-light and Ehrlich's reagent. The silica gel containing these eight zones was scraped off, all eight were combined, extracted with ether and rechromatographed in the same solvent system twice to 6 cm and twice to 12 cm. In the last two developments, the solvent ratio was changed to 30:70, v:v. The eight zones were located as before, scraped off, extracted with ether individually, and the extracts were subjected to germination tests with basidiospores of *Cronartium fusiforme* using concentrations of the extract corresponding to 0.1 g, 1.0 g, and 10 g fresh weight of tissue per 10 µl solvent. Ten µl of each concentration was applied in three replications on the surface of 10 ml of one percent water agar in petri dishes. The control was agar with an ether extract of silica gel treated with butanone and hexane.

Leaves of northern red oak (*Quercus rubra* L.) bearing telia of *Cronartium fusiforme* were incubated above the agar at 20° C for eight hours during which time basidiospores were produced and cast upon the agar surface.

The leaves were removed and the dishes incubated for 48 hours. The response of 3 groups of 200 spores to the extractives was observed after 24 hours. The average percentage of spores showing the following three forms of response to the extract (or controls) was recorded: no germination, indirect germination, and direct germination. Indirect germination was defined as formation of secondary sporidia, while direct germination was defined as the formation of a germ tube with a minimum length equal to the diameter of the basidiospore.

The effects of the following known resin acids on germination of basidiospores were determined in a second germination test: Pimaric, isopimaric, levopimaric, dehydroabietic, and abietic acid. Each acid was dissolved in ether in four concentrations: 1, 10, 100, and 1000 ppm. Ten µl of each concentration was applied to agar surfaces in three replications per plate and the response of spores was measured as described previously. The control consisted of agar with ether only.

Constituents of the extracts tested on basidiospores were identified tentatively by co-chromatographing the unknowns with the resin acids used in the second spore-germination test and the following indole-compounds: 3-indole-acetic acid, 3-indole-butyric acid, 3-indole-propionic acid, and L-tryptophane. The tentative identifications were checked by gas-liquid chromatography as follows.

The extractions from the eight zones on the TLC-plate corresponding to 1 mg fresh weight of tissue were silylated in a 2-ml vial with 0.5 ml bis(trimethylsilyl)acetamide (Applied Science Laboratories, Inc., State College, Pa., U.S.A.) dissolved in pyridine. The solution was dried with a stream of nitrogen and injected into the gas chromatograph as a methylene chloride solution. The chromatographic conditions were adapted from procedures described by Davis *et al.* (1968) and Zinkel *et al.* (1968). The copper column (180 × 0.6 cm) was packed with 5 % SE-30 liquid phase coated on Chromosorb P 80/100 mesh (Applied Science Laboratories, Inc., State College, Pa., U.S.A.), the temperature of the oven was 233° C, that of the injection port and flame ionization detector was 241° C, and the helium carrier gas flow rate was 75 ml/minute. The reference compounds used in the TLC-identification also were silylated and injected separately to obtain reference data.

Analysis of Growth-inhibiting Substances

This test was undertaken to measure the amount of growth-inhibiting compounds present in resistant and susceptible loblolly pines. Whole branches were collected in October 1968 from the uppermost whorl of branches of two loblolly pines per family on the «Residual Forest» location (Test IV, of Set 5, Table 3). The branches were frozen with dry ice immediately after harvest and stored at —20° C.

After removing all needles, the branches were extracted by homogenizing 50 g fresh weight of tissue in a Waring blender together with 100 ml 96 % aqueous ethanol and by subsequent shaking in the same solvent at room temperature for 3 hours, followed by fractionation procedures described in detail by Franson (1953) and Klingström (1969). Amounts of each final extract corresponding to 1 g fresh weight of tissue was further fractionated by ascending chromatography at 20° C on 3-cm paper strips (Whatman No. 1) in 70 % aqueous ethanol to a height of 15 cm. The paper strip was cut into five 3-cm-long portions which in turn were cut into small bits to facilitate extraction of any growth-regulating substances present.

Coleoptiles were obtained by soaking oat (*Avena sativa* L). seeds in distilled water for 2 hours and then allowing them to germinate on water-saturated vermiculite in total darkness at a temperature of 24 ± 1° C for 110—111 hours. Only coleoptiles with a total length of 20—23 mm and with the first true leaf extending less than 3 mm below the tip of the coleoptile were used. A 5-mm-long segment was cut off 3 mm below the tip of each coleoptile. After washing the segments in distilled water for one hour, 10 selected at random were put into a 3-ml screw-cap vial containing 1 ml citrate buffer (Klingström, 1969) and all the bits from one 3-cm portion of the chromatography paper described in the preceding paragraph. For each set of five vials used per chromato-

gram, one additional (control) vial was filled with 10 coleoptile-segments, buffer, and bits of chromatography paper treated with ethanol but not with extract of branch tissue.

The vials were placed in a horizontal plane in a rack. After rotating the vials around their long axis in total darkness at a temperature of 24 ± 1° C for 24 hours, the length of each coleoptile was measured to the nearest 0.1 mm.

The amount of growth-inhibiting substances present in the extract between Rf-values 0.6—1.0 on the chromatograms of each branch was expressed as the percentage reduction of elongation (X):

$$X = 100 \frac{2 (L_c - 1) - (L_{0\cdot6} + L_{0\cdot8})}{(L_c - 1)}$$

where:

L_c = average length of the coleoptile-segments in the control vial

L = average length of the coleoptile-segments exposed to extracts located between the Rf-values of 0.6—0.8 and 0.8—1.0, respectively

l = 2.262 × standard error of L_c

Analysis of Resin- and Fatty acids

Collection and Preparation of Plant Material

For the analysis of resin- and fatty acids young, succulent shoots were collected from trees in the field experiments at the time of natural rust infection or from seedlings at a susceptible age in the greenhouse. The time at which the shoots were collected was determined by observing the maturation of telial columns of *Cronartium fusiforme* on oak leaves adjacent to the pines at each field location, while seedlings were collected at 9 weeks of age when they can be infected readily (MILLER, 1970).

All shoots except those in Set 2 were collected from the same well-defined position of each tree — the whorl of lateral shoots surrounding the leader formed during the current season. From each tree two to three shoots were collected.

Some of the trees from which shoots were collected had been infected with fusiform rust. Although this may have affected the nature and (or) amount of resin- and (or) fatty acids, infected trees were not avoided since the objective was to randomly sample each clone or family.

The seedlings were grown in a greenhouse free of *Cronartium fusiforme*. Seeds were germinated under continuous light in flats containing steam-sterilized sand. After two weeks, seedlings were transplanted to flats containing a l:l, v:v, mixture of sterilized fine sand and peat moss. The families were transplanted in randomized order in rows of seven seedlings across the flats. The flats were kept in a greenhouse for seven weeks and systematically moved on the bench and turned 180° every second day in order to expose the seedlings uniformly to a mist of water applied during 0.5 minutes out of every 5 minutes. At 9 weeks of age, seedlings from each family were collected to provide 0.5—2.0 g fresh-weight of tissue for analysis.

Shoots or seedlings were collected as follows from each Set (Table 3):

Set 1 — Two shoots were collected on May 8, 1969 from each of three to five randomly selected ramets of each clone that was grafted in 1966.

Set 2 — Two terminal shoots were collected on May 7, 1971 from each of three to four randomly selected ramets of each clone that was grafted in 1959—1962. The shoots were taken from each of two branches on the northwest side of the crown at 40—60 % of the total height of the ramets. This position for collecting the shoots was an exception compared to the position chosen in plants of all other Sets where shoots were collected because the trees were too tall to reach the terminal with the equipment available.

Set 3 — Three shoots were collected on May 13, 1970 from each of seven randomly selected trees in each family that was planted in progeny test III in 1967 (Test III of Set 3, Table 3).

Set 4 — Six to ten randomly selected seedlings of each family were collected in the greenhouse.

Set 5 — Three shoots were collected on May 4, 1970 from each of three randomly

selected trees planted in 1963 at the »Residual Forest» location (Test IV of Set 5, Table 3).

Set 6 — Five randomly selected seedlings of each family were collected in the greenhouse.

All the plant material was frozen with dry ice immediately after collection and kept frozen at —20° C until prepared for extraction.

Choice of Tissue for Extraction

One or two grams fresh-weight of primary cortex of shoots and the whole stem above the cotyledonary needles of seedlings were chosen for extraction since natural infection by *Cronartium fusiforme* is believed to take place through these particular tissues (Powers, 1968; Miller, 1970). The cortex was removed at the base of each shoot resulting from the first flush of growth in the year of collection since (i) tip moths (*Rhyaconia frustrana* Comst.) frequently caused resin exudation on the cortex at the tops of shoots and (ii) the second flush emerging from the top of some shoots at the time of collecting was considered a possible cause of variation in physiological condition of the tissue to be extracted. In Set 2, however, the cortex was removed just below the terminal bud since no tip-moth damage occurred and no second flushes had developed in the middle part of the crown where the shoots were collected.

Extraction of Tissue and TLC-fractionation of Extracts

The plant tissue was prepared for quantitative and qualitative gas-chromatographic analysis by a modification of the extraction and fractionation procedures described by Pensar (1967, 1969). The primary cortex was cut into 70-μ sections and the stems of seedlings into 8-μ sections on a cryostat-microtome. The sections were put into 7-ml tared screw-cap vials, lyophilized, and then weighed in the vials.

The tissue was extracted by shaking with 4 ml anhydrous diethyl ether at 20° C except the extracts of Set 3, which were extracted at + 5° C, for 24 hours. The vial was then fitted with a tubing adaptor to a Pasteur pipette filled with 1.5—1.8 g anhydrous Na_2SO_4. By blowing a stream of air through a hypodermic needle into the vial, the extract was forced out of the vial, through the pipette in which it was simultaneously filtered and dried, and on into a 5-ml serum ampoule. The residual sections of tissue were washed twice with 1 ml ether which also was passed through the drying filter into the ampoule. The ampoule was then closed, the extract dried in a stream of nitrogen, and stored at —20° C until the crude extract was methylated at room temperature by adding 0.5 ml methanol as a catalyst (Schlenk and Gellerman, 1960) and 4 ml of freshly distilled diazomethane. The methylation reaction was completed in a few minutes and the extracts again dried in a stream of nitrogen.

Methyl esters of resin- and fatty acids were separated from other components in the extract by TLC-fractionation using petroleum ether : diethyl ether (95:5, v:v) and two developments for each plate. The zone including the methyl esters was located with shortwave UV-light at an Rf-value of 0.65. The zone was removed with suction to a Millipore (pore size = 5 μ) filter disc 2 cm in diameter and the compounds extracted from the silica gel with two 1-ml portions of diethyl ether into a 2-ml serum ampoule. This material was then dried under a stream of nitrogen and stored at —20° C.

Gas-chromatographic Analysis of Extracts

The extracts of Sets 1 and 3—6 were analyzed by gas-liquid chromatography for amounts of certain resin- and fatty acids while the extracts of Set 2 were analyzed for fatty acids only. The chromatographic conditions used were modified from procedures of Nestler and Zinkel (1967).

The extracts from Set 1 were analyzed on an F & M Research Chromatograph, Model 800, equipped with a flame ionization detector. The chromatographic conditions used as well as the detector response curve obtained for a representative extract of a shoot are given in Figure 4.

The identity of each compound was determined by comparing its retention time with those reported by Nestler and Zinkel

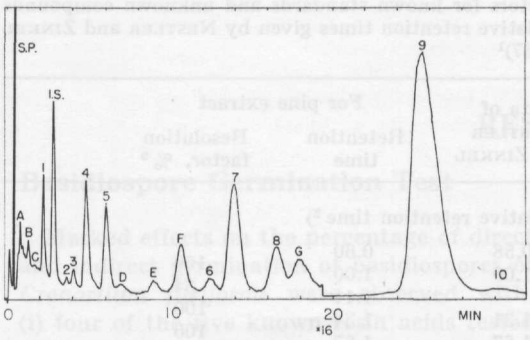

Figure 4. Gas chromatographic fractionation of methyl esters of resin- and fatty acids in a shoot from mature loblolly pine (Clone 57, Set 1). The acids were identified as follows:

Fatty acids;		Resin acids;
1 = 16:0	4 = 18:2	6 = pimaric
2 = 18:0	5 = 18:3	7 = sandaracopimaric
3 = 18:1		8 = palustric + levopimaric
A-E = minor fatty acids (?)		9 = abietic
		10 = neoabietic
		F = $\triangle^8$-isopimaric (?)
		G = isopimaric

S.P. = solvent peak, I.S. = internal standard; heptadecanoic. Conditions of chromatography: column 180 × 0.6 cm copper, liquid phase 20 % DEGS on ABS 80—90 mesh (Anakrom, Analabs Inc., Hamden, Conn., USA); temperature of oven 203° C, of inj. port 263° C, of flame ionization detector 254° C; helium carrier flow rate 63 ml/minute. Flame range set at 1, attenuation changed from × 8 to × 16.

(1967) and those obtained by injecting methyl esters of known resin- and fatty acids (Table 4). The study was limited to the 10 major compounds in the extract except that detector responses were obtained for some minor components of the extracts, but these were neither identified nor their amounts determined. The chromatographic conditions used did not allow resolution of methyl esters of palustric and levopimaric acids.

The amount of each of the 10 compounds in the extract was calculated by multiplying its peak area by a specific factor obtained for the detector response to a known amount of the corresponding reference compound. It was assumed that the relationship between peak area and the amount of the compound was linear. This assumption was confirmed on the Model 700 instrument described in the next paragraph. An approximation of the peak area was obtained as the product of retention time and peak height (KAISER, 1963).

The extracts from Sets 2—6 were analyzed on an F & M Laboratory Chromatograph, Model 700, also equipped with a flame ionization detector. Qualitative analyses on this instrument were made as described above. For the quantitative analyses a linear responce curve was obtained for each compound (KAISER, 1963; SHEPPARD *et al.*, 1968). This was done by preparing a carbon disulfide solution of a mixture of all 10 reference compounds in five concentrations covering approximately the range of amounts expected in the extracts. The proportions of compounds in the known mixture also corresponded approximately to expected proportions in the extracts. Each concentration of the solution was injected three times and the peak area obtained for each compound was plotted against its weight in the solution (Figure 5). The amount of each compound in the extracts was determined graphically from these response curves and expressed as µg compound/mg of freeze-dried tissue. The results are reported for resin acids as the weight of the acid only and for fatty acids as that of the uncorrected weight of the methyl esters.

All extracts were injected as carbon disulfide solutions containing a constant amount of the methyl ester of heptadecanoic acid as an internal standard, which was used to correct for both variation in amount of the esters injected and day-to-day variation in sensitivity of the gas chromatograph. The extracts were analyzed once each in a random time sequence or as a randomized complete block design with time sequence as a blocking criterion.

The amounts of five resin acids (primaric, sandaracopimaric, palustric + levopimaric, abietic, and neoabietic) and five fatty acids (16:0, 18:0, 18:1, 18:2, and 18:3 [1]) were measured in each extract. The sum of the amounts of all five resin or fatty acids was calculated and considered an estimate of total resin and total fatty acids in the extracts.

A study was made to establish the precision of the experimental procedure applied.

[1] The notation indicates the number of carbon atoms and double bonds in the molecule, respectively.

Table 4. Relative retention times and resolution factors for known standards and unknown compounds in an extract of mature loblolly pine compared to relative retention times given by NESTLER and ZINKEL (1967)[1]

Ester of acids	For standards	Data of NESTLER and ZINKEL	For pine extract	
			Retention time	Resolution factor, %[3]
Fatty acids	(Relative retention time[2])			
16:0[4]	0.57	0.58	0.60	100
18:0	1.00	1.00	1.00	84
18:1	1.11	—	1.11	100
18:2	1.32	1.31	1.34	100
18:3	1.66	1.67	1.67	
Resin acids				
Δ^8-isopimaric ..	0.87	0.87	0.86	100
Pimaric	1.00	1.00	1.00	95
Sandaracopimaric	1.18	1.13	1.13	100
Palustric	1.33	1.35	1.30	0
Levopimaric	1.33	1.33	1.30	69
Isopimaric	1.48	1.45	1.45	100
Abietic	2.23	2.10	2.02	96
Neoabietic	2.48	2.47	2.42	

[1] For chromatographic conditions, see footnote to Figure 4
[2] For esters of fatty acids, stearate = 1.00; for esters of resin acids, pimarate = 1.00
[3] Calculated according to KAISER (1960), complete resolution = 100 %.
[4] The notation indicates the number of carbon atoms and double bonds in the molecule, respectively.

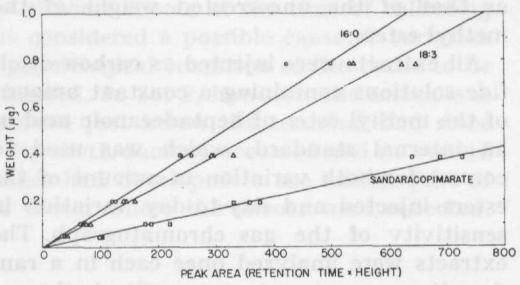

Figure 5. Standard response curves for two fatty acids (16:0 and 18:3) and one resin acid (sandaracopimaric).

The standard deviation for one determination was ± 22 % for the amount of total resin acids, ± 14 % for the amount of total fatty acids, and of the same order of magnitude for each single resin- and fatty acid, respectively. These estimates of standard deviation included the variation contributed by extraction of tissue, TLC-fractionation, and gas chromatographic analysis. Additional studies were made to obtain information about the variation in amounts of compounds within whorls of shoots, within the cortical tissue of shoots and within the stem of 9-week-old seedlings. Considerable variation was found between different shoots in each whorl as well as between different portions of the tissue in a shoot or seedling (coefficients of variation ranged from 25—65 %). Therefore, the content of compounds for each individual member of a clone or family was estimated as the average content in all shoots collected from the individual. Estimates for a seedling were based on one determination only.

Resistant and susceptible clones and families within each Set were compared by their means. The data were subjected to analysis of variance and F-tests. Phenotypic correlation coefficients between amount of compounds and rust-resistance were calculated.

RESULTS

Basidiospore Germination Test

Marked effects on the percentage of direct and indirect germination of basidiospores of *Cronartium fusiforme* were observed with: (i) four of the five known resin acids tested (Figure 6); and (ii) eight zones detected on thin layer chromatograms of methanol extracts from shoots of loblolly pine (Table 5). One zone, which stimulated direct germination, contained a mixture of resin acids. Control spores mainly reacted by indirect germination.

When basidiospores were exposed to 1000 ppm of various known resin acids, pimaric acid showed no effect on the spores; but both levopimaric and neoabietic acids stimulated direct germination while isopimaric acid weakly inhibited and dehydroabietic acid strongly inhibited both direct and indirect germination (Figure 6). Similar but smaller effects were observed with 100 ppm of the acids except that neoabietic acid had a strong and inhibitory effect at this concentration. At 10 and 1 ppm the acids had little or no effect on germination.

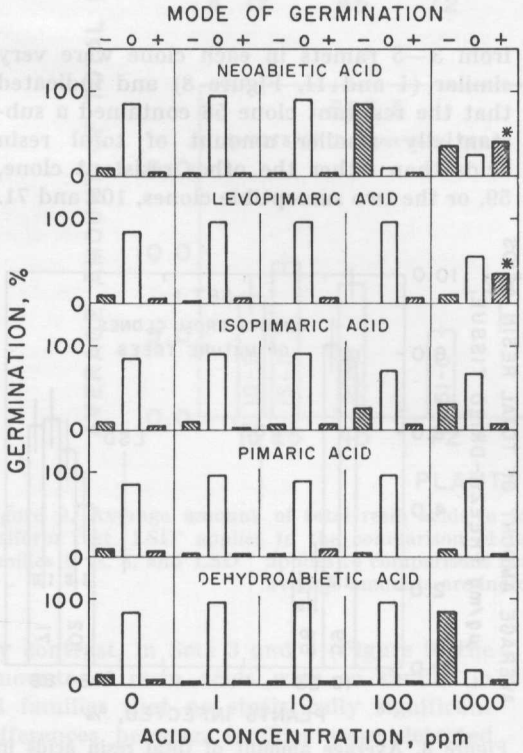

Figure 6. Effect of five resin acids on germination of basidiospores of *Cronartium fusiforme*. Modes of germination: — = no germination, 0 = indirect germination, + = direct germination. 0 ppm consisted of ether only on agar. * Indicates that germ tube formation occurred only when spores located on droplets of the acids.

Both stimulation and inhibition of direct germination also was observed with TLC-fractionated constituents of methanol extracts from pine shoots; zones with the Rf-values 0.76 and 0.90 stimulated direct germination while zones with the Rf-values 0.50, 0.57, 0.82, 0.87, 0.92, and 0.96 inhibited direct germination of basidiospores. The effects on direct germination were progressively smaller with decreasing concentrations of each of the constituents (Table 5). The extract from the zone Rf-value 0.76 contained a mixture of resin acids including pimaric and levopimaric acids as indicated by thin layer and gas chromatography. None of the compounds in the other zones were identified.

Analysis of Growth-inhibiting Substances

A curvilinear trend of increasing inhibition of coleoptile-elongation with increasing resistance to *Cronartium fusiforme* was observed with ethanol extracts of branches from the four full-sib families of loblolly pines in Set 5 (Figure 7). A quadratic curve was fitted to the observations.

Resin-acid Analysis

Neither the amount of total resin acids (Figures 8 and 9) nor the amount of five individual resin acids present in shoots of trees or stems of young seedlings at the

Table 5. Influence of extracts from shoots of loblolly pine on germination of basidiospores of *Cronartium fusiforme*. Spores were exposed for 24 hours on water agar to 3 concentrations of extractives from 8 zones of a TLC-fractionated methanol extract.

Rf-value of zone	(Concentration of extractives corresponding to the following g fresh-weight of tissue) 0.1			1.0			10.0		
	(mode of spore germination)[1] —	0	+	—	0	+	—	0	+
	(Average % germination of 3 groups of 200 spores)								
Control[2]	16	84	1	16	83	1	16	83	1
0.50	7	91	2	4	93	3	68	14	18
0.57	2	98	0	57	26	17	93	0	7
0.76	37	53	10	53	0	47	33	0	67
0.82	45	53	2	88	9	3	95	0	5
0.87	47	52	1	72	25	3	88	0	12
0.90	6	92	2	11	77	12	34	1	65
0.92	1	96	3	42	53	5	93	0	7
0.96	35	63	2	79	18	3	99	0	1

[1] — = no germination, 0 = indirect germination (formation of secondary sporidia), + = direct germination (formation of a germ tube with a minimum length equal to the diameter of the spore).
[2] Control consisted of agar with ether extract of silica gel treated with butanone and hexane.

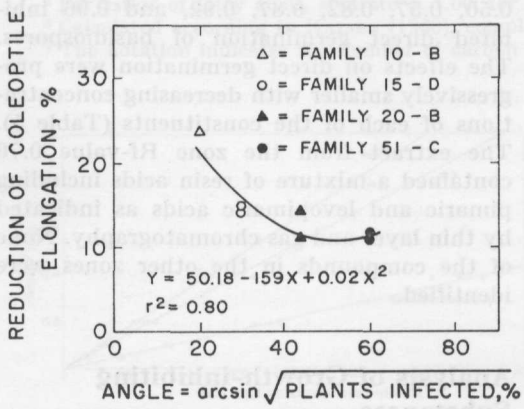

Figure 7. Relationship between amount of growth inhibiting compounds in branches of 7-year-old trees of loblolly pine (Set 5) and their resistance to fusiform rust.

time of natural infection (Tables 6—8) were associated with the resistance to fusiform rust in 4 clones and 14 families of loblolly pine.

Reproducible differences in amounts of total resin acids were detected among clones (Figure 8) and families (Figure 9) but these differences were not associated with the resistance of the plants to fusiform rust. In Set 1, for example, the results of two independent experiments with different shoots from 3—5 ramets in each clone were very similar (I and II, Figure 8) and indicated that the resistant clone 58 contained a substantially smaller amount of total resin acids than either the other resistant clone, 59, or the two susceptible clones, 102 and 71.

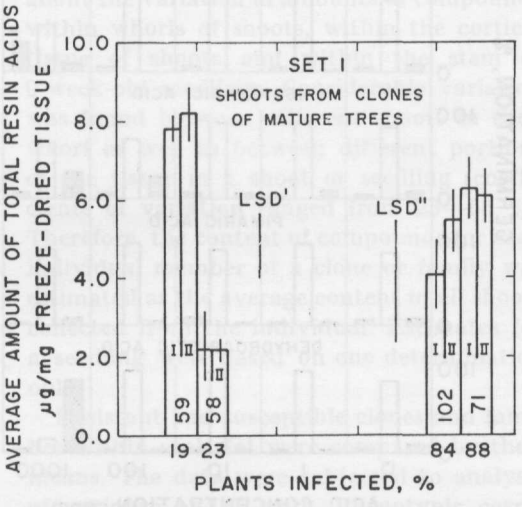

Figure 8. Average amount of total resin acids in clones of loblolly pine resistant and susceptible to fusiform rust. LSD′ applies to the comparison of clones 59 vs. 58 and LSD″ applies to the comparison of clones 58 vs. 71. I and II indicates results from two independently conducted experiments. Standard errors for the average amounts are indicated by a vertical line.

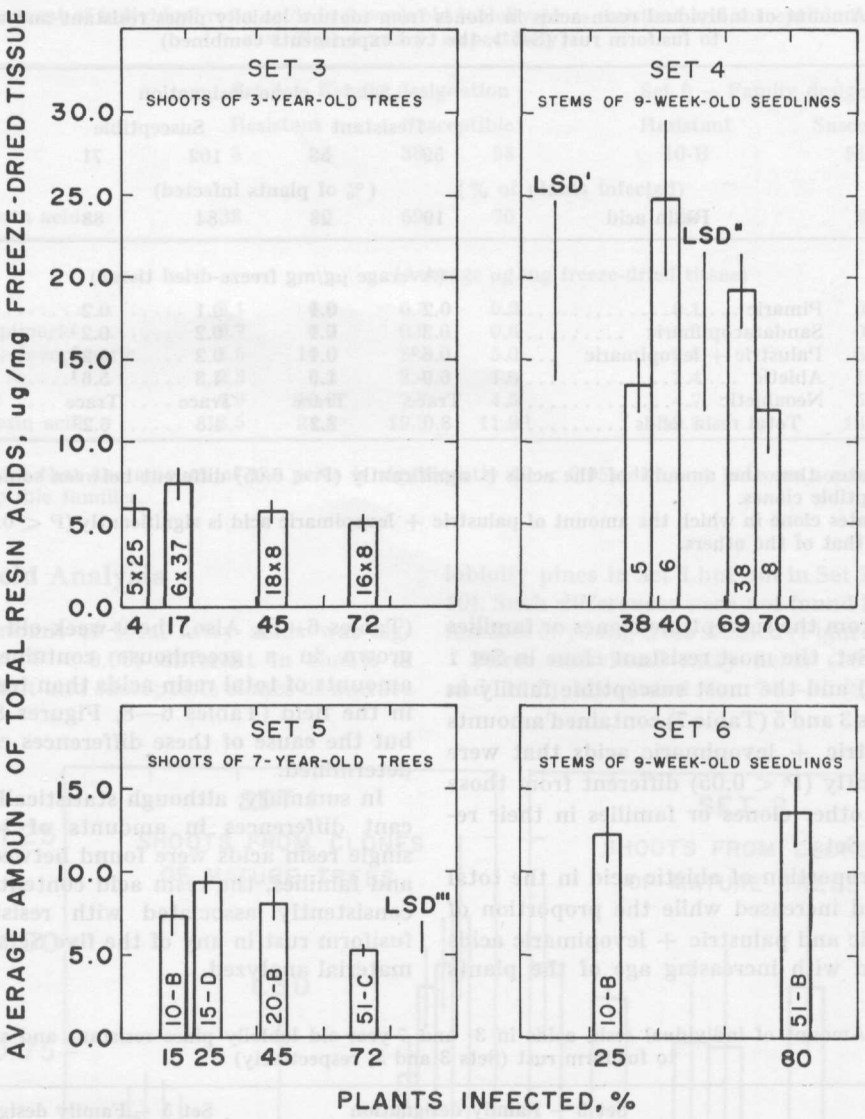

Figure 9. Average amount of total resin acids in families of loblolly pine resistant and susceptible to fusiform rust. LSD′ applies to the comparison of families 5 vs. 6, LSD″ applies to the comparison of families 6 vs. 8, and LSD‴ applies to comparisons between all families in the set. Standard errors for the average amounts are indicated by a vertical line.

By contrast, in Sets 3 and 6 (Figure 9) the amounts of resin acids were so similar in all families that no statistically significant differences between families were detected despite the substantial range of variation in resistance to fusiform rust. In Sets 4 and 5 (Figure 9) significant differences between some families could be detected but these also were not associated with differences in resistance.

In Sets 3—5 (Figure 9) the distribution of resin acids among families of increasing resistance was skewed to the left with intermediately resistant families containing larger amounts of total resin acids than the extremely resistant and susceptible families; but this trend was statistically significant only in Set 5 ($P < 0.05$).

Although none of the five individual resin acids could be used to distinguish the re-

Table 6. Amount of individual resin acids in clones from mature loblolly pines resistant and susceptible to fusiform rust (Set 1, the two experiments combined)

	Set 1 – clone designation			
	Resistant		Susceptible	
	59	58	102	71
	(% of plants infected)			
Resin acid	19	23	84	88
	(Average μg/mg freeze-dried tissue)			
Pimaric	0.2	0.1	0.1	0.2
Sandaracopimaric	0.3	0.1	0.2	0.2[1]
Palustric + levopimaric	0.6[2]	0.1	0.2	0.2[1]
Abietic	6.9	1.9	4.3	5.6[1]
Neoabietic	Trace	Trace	Trace	Trace
Total resin acids	8.0	2.2	4.8	6.2[1]

[1] Indicates that the amount of the acids is significantly ($P < 0.05$) different between some resistant and susceptible clones.

[2] Indicates clone in which the amount of palustric + levopimaric acid is significantly ($P < 0.05$) different from that of the others.

sistant from the susceptible clones or families in each Set, the most resistant clone in Set 1 (Table 6) and the most susceptible family in both Sets 3 and 5 (Table 7) contained amounts of palustric + levopimaric acids that were significantly ($P < 0.05$) different from those of most other clones or families in their respective Sets.

The proportion of abietic acid in the total resin acid increased while the proportion of neoabietic and palustric + levopimaric acids decreased with increasing age of the plants (Tables 6—8). Also, the 9-week-old seedlings grown in a greenhouse contained larger amounts of total resin acids than trees grown in the field (Tables 6—8, Figures 8 and 9), but the cause of these differences cannot be determined.

In summary, although statistically significant differences in amounts of total and single resin acids were found between clones and families, the resin acid content was not consistently associated with resistance to fusiform rust in any of the five Sets of plant material analyzed.

Table 7. Amount of individual resin acids in 3- and 7-year-old loblolly pines resistant and susceptible to fusiform rust (Sets 3 and 5, respectively)

	Set 3 – Family designation				Set 5 – Family designation			
	Resistant		Susceptible		Resistant		Susceptible	
	5 × 25	6 × 37	18 × 8	16 × 8	10-B	15-D	20-B	51-C
	(% of plants infected)							
Resin acids	4	17	45	72	15	25	45	75
	(Average μg/mg freeze-dried tissue)							
Pimaric	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sandaracopimaric	0.1	0.2	0.1	0.1	0.2	0.2	0.3	0.2
Palustric + levopimaric	1.1	1.2	1.2	0.5[1,2]	1.2	1.7	1.5	0.7[1,2]
Abietic	2.6	3.6	2.1	2.3	3.5	4.3	3.6	2.7
Neoabietic	2.2	2.4	2.4	2.1	2.3	3.1	2.6	1.6
Total resin acids	6.1	7.5	5.9	5.1	7.3	9.4	8.1	5.3[1]

[1] Indicates that the amount of the acids is significantly ($P < 0.05$) different between some resistant and susceptible families.

[2] Indicates a clone in which the amount of palustric + levopimaric acid is different from that of most other families within respective Sets.

Table 8. Amount of individual resin acids in 9-week-old loblolly pines resistant and susceptible to fusiform rust (Sets 4 and 6, respectively)

	Set 4 – Family designation				Set 6 – Family designation	
	Resistant		Susceptible		Resistant	Susceptible
	5	6	38	58	10-B	51-B
			(% of plants infected)			
Resin acids	38	40	69	70	25	80
			(Average µg/mg freeze-dried tissue)			
Pimaric	0.1	0.3	0.3	0.2	0.1	0.1
Sandaracopimaric	0.7	0.7	0.6	0.6	0.3	0.3
Palustric + levopimaric	5.5	10.6	8.2	5.0	5.8	6.4
Abietic	2.3	3.2	2.4	1.6	1.4	1.6
Neoabietic	4.9	10.0	7.7	4.5	4.7	5.2
Total resin acids	13.5	24.8	19.2	11.9[1]	12.3	13.6

[1] Indicates that the amount of the acids is significantly ($P < 0.05$) different between some resistant and susceptible families.

Fatty-acid Analysis

The amount of total fatty acids was significantly ($P < 0.05$) different in shoots of the resistant and susceptible clones of mature loblolly pines in Set 1 but not in Set 2 (Figure 10). Such differences were not found in the 14 families of young trees studied (Figure 11).

Three individual fatty acids (16·0, 18:2, and 18:3) accounted for 74—95 % of the

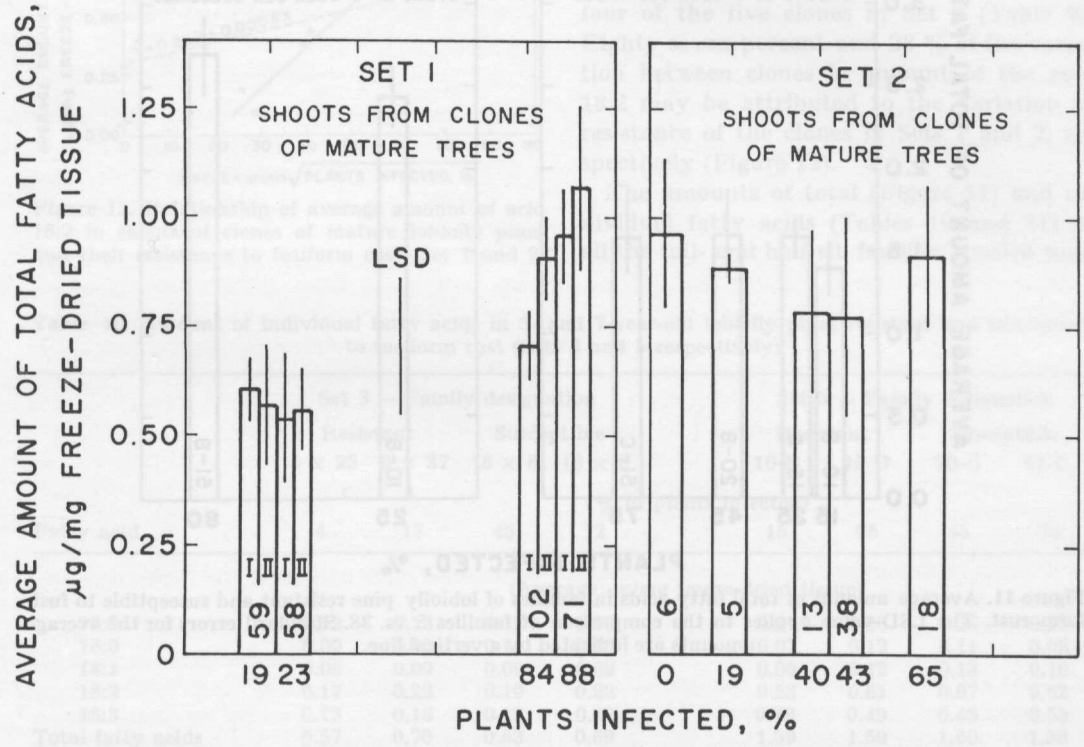

Figure 10. Average amount of total fatty acids in clones of loblolly pines resistant and susceptible to fusiform rust. The LSD-value applies to the comparison of the resistant vs. the susceptible clones. I and II indicates results from two independent experiments. Standard errors for the average amounts are indicated by a vertical line.

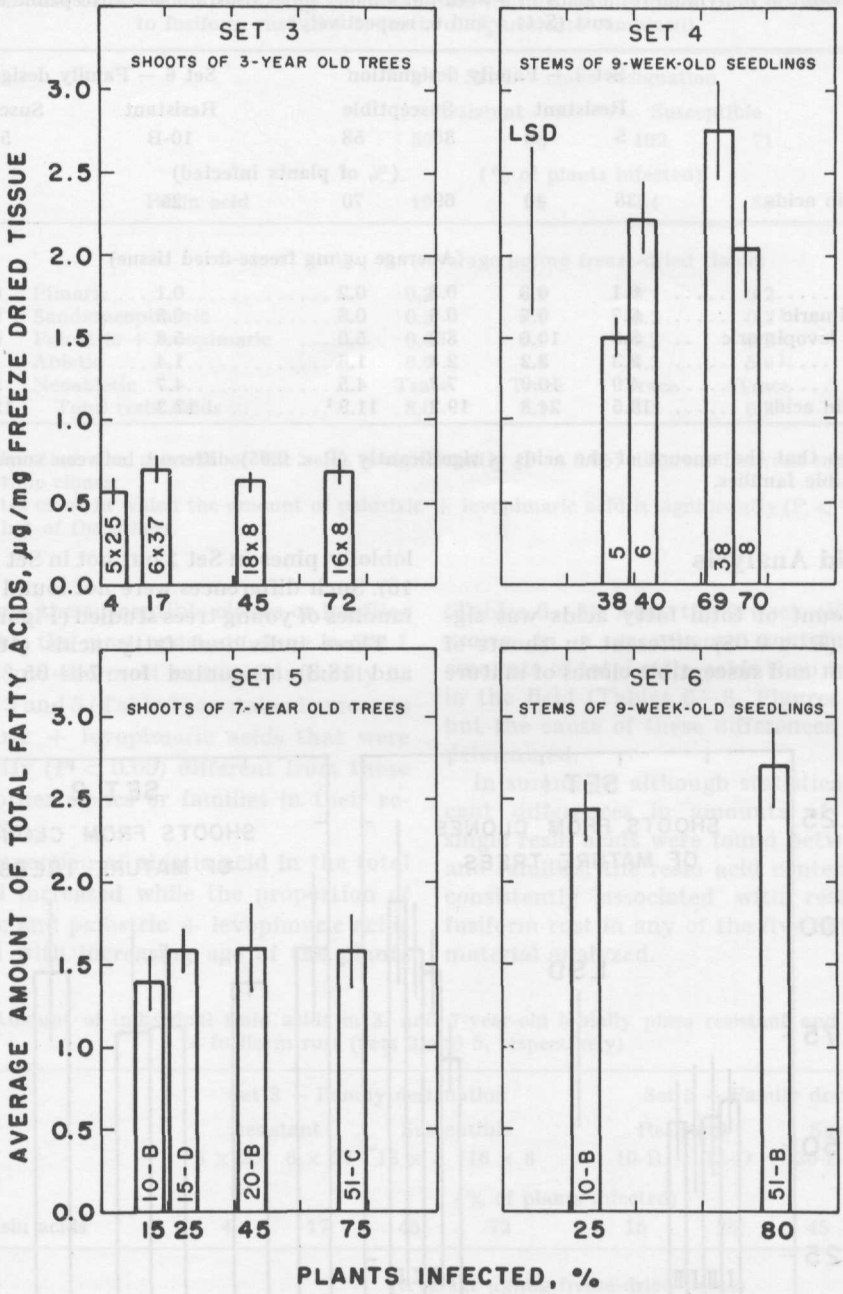

Figure 11. Average amount of total fatty acids in families of loblolly pine resistant and susceptible to fusiform rust. The LSD-value applies to the comparison of families 5 vs. 38. Standard errors for the average amounts are indicated by a vertical line.

Table 9. Amount of individual fatty acids in clones of mature loblolly pines resistant and susceptible to fusiform rust (Sets 1 and 2, in Set 1 the two experiments combined)

	Set 1 — Clone designation				Set 2 — Clone designation				
	Resistant		Susceptible		Resistant		Susceptible		
	59	58	102	71	6	5	13	38	18
	(% of plants infected)								
Fatty acid	19	23	84	88	0	19	40	43	65
	(Average μg/mg freeze-dried tissue)								
16:0	0.08	0.08	0.13	0.15 [1]	0.17	0.17	0.12	0.12	0.15
18:0	0.01	0.01	0.01	0.02	0.11	0.05	0.07	0.04	0.08
18:1	0.03	0.03	0.03	0.05	0.12	0.10	0.09	0.12	0.12
18:2	0.24	0.22	0.34	0.41 [1]	0.26	0.33	0.28	0.29	0.33
18:3	0.22	0.22	0.31	0.38 [1]	0.24	0.24	0.22	0.20	0.27
Total fatty acids	0.58	0.56	0.82	1.01 [1]	0.90	0.89	0.78	0.77	0.95

[1] Indicates that the amount of the acids is significantly ($P < 0.05$) different between some resistant and susceptible clones.

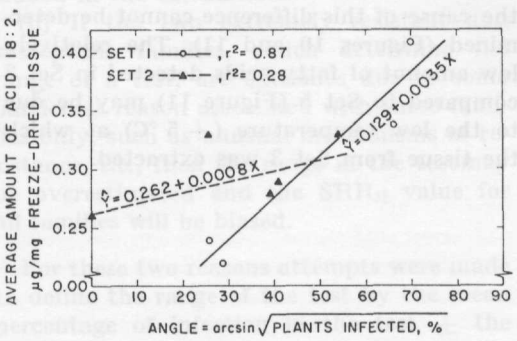

Figure 12. Relationship of average amount of acid 18:2 in shoots of clones of mature loblolly pines and their resistance to fusiform rust, Set 1 and 2.

amount of total fatty acids. All of them occurred in significantly ($P < 0.05$) larger quantitites in the susceptible compared to the resistant clones in Set 1 (Table 9). Acid 18:2 showed a similar but weak trend in four of the five clones in Set 2 (Table 9). Eighty-seven percent and 28 % of the variation between clones in amount of the acid 18:2 may be attributed to the variation in resistance of the clones in Sets 1 and 2, respectively (Figure 12).

The amounts of total (Figure 11) and individual fatty acids (Tables 10 and 11) in all the full- and half-sib families studied were

Table 10. Amount of individual fatty acids in 3- and 7-year-old loblolly pines resistant and susceptible to fusiform rust (Sets 3 and 5 respectively)

	Set 3 — Family designation				Set 5 — Family designation			
	Resistant		Susceptible		Resistant		Susceptible	
	5 × 25	6 × 37	18 × 8	16 × 8	10-B	15-D	20-B	51-C
	(% of plants infected)							
Fatty acid	4	17	45	72	15	25	45	75
	(Average μg/mg freeze-dried tissue)							
16:0	0.12	0.13	0.12	0.13	0.21	0.25	0.26	0.25
18:0	0.09	0.10	0.11	0.13	0.07	0.12	0.11	0.08 [1]
18:1	0.06	0.09	0.09	0.09	0.09	0.12	0.13	0.10
18:2	0.17	0.22	0.19	0.22	0.53	0.61	0.67	0.62
18:3	0.13	0.16	0.12	0.12	0.49	0.49	0.43	0.53
Total fatty acids	0.57	0.70	0.63	0.69	1.39	1.59	1.60	1.58

[1] Indicates that the amount of the acid is significantly ($P < 0.05$) different between some resistant and susceptible families.

Table 11. Amount of individual fatty acids in 9-week-old loblolly pines resistant and susceptible to fusiform rust (Sets 4 and 6).

	Set 4 – Family designation				Set 6 – Family designation	
	Resistant		Susceptible		Resistant	Susceptible
	5	6	38	58	10-B	51-B
	(% of plants infected)					
Fatty acid	38	40	69	70	25	80
	(Average μg/mg freeze-dried tissue)					
16:0	0.18	0.38	0.48	0.28[1]	0.56	0.58
18:0	0.08	0.18	0.48	0.26	0.72	0.62
18:1	0.28	0.38	0.46	0.34	0.40	0.44
18:2	0.68	0.92	1.02	0.84	0.56	0.78[1]
18:3	0.28	0.36	0.32	0.32	0.22	0.28
Total fatty acids	1.50	2.22	2.76	2.04[1]	2.46	2.70

[1] Indicates that the amount of the acids is significantly ($P < 0.05$) different between some resistant and susceptible families.

so similar that statistically significant ($P < 0.05$) differences could be detected only between a few families within some of the Sets.

The 9-week-old seedlings grown in the greenhouse had larger amounts of total fatty acids than the trees grown in the field but the cause of this difference cannot be determined (Figures 10 and 11). The relatively low amount of fatty acids detected in Set 3 compared to Set 5 (Figure 11) may be due to the low temperature (+ 5 °C) at which the tissue from Set 3 was extracted.

DISCUSSION

The Stability Index

The method used in this study for estimating the G × E interaction for individual families was found to have two major weaknesses:

(i) If only a small portion of the families are common to all tests, the range may be severely biased by particular families in each test. Therefore the method is well-suited only for tests where a majority of families are common to all tests, or for tests where only those families which are common are included in the calculations.

(ii) If the two extremely resistant and susceptible families, which determine the range of a test, are extremes due to some particular reason associated with these families only, such as unusual mechanisms of resistance, *etc.*, then the range in the test may be overestimated and the SRR_{ij} value for all families will be biased.

For these two reasons attempts were made to define the range of the test by the mean percentage of infection in the test ± the 99.9 % confidence limits as well as by the mean ± multiples of the standard deviation of the mean. Both of these alternative methods were unsatisfactory due to the non-normal and skewed distribution of the values of percentage of plants infected. It therefore appears that the range may best be defined by the extreme families only; recently, this definition also was used for the estimation of G × E interaction for yields of soybean (*Glycine max* L.) (HANSON, 1970).

Comparison of Analytical Procedures

Three procedures were applied in this study, but only the procedure for analysis of resin- and fatty acids is rapid enough for routine analysis of large numbers of plants such as would be the case in an intensive breeding program testing clones, full-sib, and half-sib families for a marker for disease-resistance. For example, determination of resin- and fatty acid content in 40 extracts required 50 man-hours of laboratory work whereas the content of unknown biologically active organic acids could be determined in only 10 extracts by the basiodiospore- or coleoptile-test procedures within the same amount of time.

Basidiospore Germination Test

The results from the basidiospore germination tests indicate that some resin acids at a concentration of 1000 ppm, stimulate while other resin acids inhibit direct germination of basidiospores of *Cronartium fusiforme* (Figure 6). These observations are in agreement with those made by KLINGSTRÖM (1969), who found that three sources of crude «abietic acid», containing various resin acids, in concentrations of 1000 ppm and 100 ppm inhibited direct germination of basidiospores of *Melampsora pinitorqua*. One of the crude »abietic acids» was reported to contain mainly dehydroabietic acid (42.4 %) and large amounts of isopimaric acid (13.4 %). These acids also inhibited direct germination of spores of fusiform rust (Figure 6). Consequently, the basidiospores of the two rusts may react similarly to large concentrations of certain resin acids.

The discovery that different resin acids can both inhibit and stimulate direct germination of basidiospores of fusiform rust provided encouragement that resin acids may be component traits of resistance to fusiform rust and thus serve as possible marker traits. Similar encouragement was found in the observation that an extract from shoots of loblolly pine at the time of natural infection containing a mixture of resin acids, stimulated direct germination of spores (Table 5) and in reports in the literature on the biological effects of resin acids on germinating spores of *Melampsora pinitorqua* (KLINGSTRÖM, 1969) and *Cronartium fusiforme* (HARE, 1970).

During the progress of this investigation, however, new information about the development of symptoms of fusiform rust (KAIS and SNOW, 1972; SNOW, personal communication) indicates that both resistant and suceptible loblolly pines become infected by the pathogen. Similar observations also have been made with slash pine (KAIS and SNOW, 1972; SNOW, personal communication) and with *Pinus monticola* infected with basidiospores of *Cronartium ribicola* (HANOVER, 1966). The results of the resin acid analysis in the present study indicate that none of the resin acids with strong inhibitory effect on spore germination (isopimaric and dehydroabietic acids, Figure 6) occurred in more than trace amounts in the tissues analyzed. Therefore, suitable tissue of both resistant and susceptible plants contains resin acids which stimulate direct germination of basidiospores *in vitro*. These results may offer partial explanation for the observations that symptoms of infection develop in both resistant and susceptible loblolly pines. It therefore appears that the assumption according to which host extractives inhibiting spore germination are component traits of resistance, probably is not valid. Constituents of host extracts, which are known to effect spore germination *in vitro* only, such as those tested in this study (Table 5) or recently analyzed by HOFF (1970) therefore may not be component traits of resistance since the mechanism(s) of resistance apparently are effective after spore germination and infection.

Analysis of Growth-inhibiting Substances

A trend of increasing amounts of growth-inhibiting substances in branches of loblolly pines with increasing resistance to fusiform rust was found among four families tested (Figure 7). This trend is similar to that described by KLINGSTRÖM (1969) for clones of Scots pine resistant to pine twisting rust. The curve fitted to the observations available in the present study has to be viewed with caution, however, due to the few families analyzed.

Search for markers by applying the coleoptile-test procedures was discontinued since the coleoptile test was extremely time-consuming. In view of the association found between resistance and amount of growth-inhibiting compounds, however, the coleoptile-test procedures merit further consideration both for the purpose of indentifying markers and for studies on the physiology of rust resistance.

Resin-acid Analysis

No association was found between the content of total and five single resin acids as measured in this study and the resistance of four clones and 14 families analyzed.

The large content of the individual resin acids palustric + levopimaric acids in the more resistant clones and 3- and 7-years old families (Tables 6 and 7), however, may provide a basis for studies to gain understanding of the association between these acids and resistance to fusiform rust in loblolly pine. In the present study, no conclusions can be made about the relative amount of the two acids within the amount reported for their combination, but it is probable that a large part of the amount can be attributed to palustric acid since levopimaric acid tends to be decomposed in the gas chromatographic procedures applied (NESTLER and ZINKEL, 1967). Analytical procedures should be applied which provide complete resolution of the acids since they may have different biological activity, but the procedure suggested by CHANG and PELLETIER (1966) does not allow for simultaneous analysis of fatty acids (NESTLER and ZINKEL, 1967).

No published reports have been found on the resin acids present in extracts from primary cortex of loblolly pine. Isopimaric acid occurred only in trace amounts in some of the plant material analyzed in the present study. Trace amounts of isopimaric acid have been found in oleoresin of loblolly pine (JOYE and LAWRENCE, 1967), while considerable amounts of this acid have been found in oleoresin from cortex of slash pine (LAWRENCE, 1967). Neoabietic acid occurred only in trace amounts in most of the clones of mature trees while the acid was a major component in the younger material in the present study. Only moderate amounts of the acid were found in oleoresin of loblolly

pine (JOYE and LAWRENCE, 1967), while neoabietic acid occurred in large amounts in oleoresin of cortex from mature slash pine (LAWRENCE, 1967).

The distribution of the amount of total resin acids in families with different resitance to fusiform rust (Figure 9) is not readily explained by present understanding of the physiology of rust resistance.

Fatty-acid Analysis

The results of the qualitative analysis of fatty acids in this study are in close agreement with those obtained from analyses of fatty acids in the inner bark from the lower trunk of loblolly pines (RICHMOND *et al.*, 1970; RICHMOND, personal communication). In both studies the main acids were 16:0, 18:0, 18:1, 18:2, and 18:3. In the present study the acids 16:0, 18:2, and 18:3 accounted for the larger portion of the total fatty acids, while RICHMOND *et al.* (1970) found that the acids 18:1 and 18:2 occurred in large amounts. These quantitative differences may be due to differences in the tissues analyzed.

The amount of total and certain individual fatty acids may offer some promise as markers for clones of mature trees but not young families (age 9 weeks to 7 years) of loblolly pine resistant and susceptible to fusiform rust. The resistant clones could be identified by their low content of total fatty acids and the individual acids 16:0, 18:2, and 18:3 (Set 1, Table 9 and Figure 12). Four out of five other clones also showed a similar but weak trend of decreasing amounts of the acid 18:2 with increasing resistance (Set 2, Table 9 and Figure 12).

The small amount of fatty acids in the resistant clones in Set 1 may be due to an accident of sampling or be associated with a number of factors, one of which is previous natural infection with fusiform rust of the ramets analyzed. The observed differences between the clones was not attributed to previous infection, however, since (i) none of the ramets from which shoots were collected were infected in 1968, and (ii) in the spring of 1970, one year after collecting the material, only 6 of the 18 ramets analyzed were infected. One of the analyzed ramets was infected in each of the clones 59, 58, and 102, while three were infected in clone 71. Other possible causes for the observed small fatty-acid content were considered. They included: (i) variation in the geographical distribution (Figure 3), site index, and age of the ortets from which scions were collected for grafting the ramets, (ii) variation in the root stock to which the scions were grafted, and (iii) site differences in the orchard where the ramets were grown. The variation in fatty-acid content was not believed to be attributable to any of these causes.

The large amount of unsaturated, long-chain fatty acids (18:2 and 18:3) in the susceptible clones in Set 1 may provide a suitable carbon source for the pathogen according to the hypotheses suggested by WALKINSHAW (1968b) and SCHELD and PERRY (1970), discussed on page 14 of this study. Results from *in vitro* experiments with spores of the pathogens may not be applicable, however, to the *in vivo* interaction between host and pathogen. Unfortunately, in this study, no information was obtained about the content of short-chain fatty acids in the tissues analyzed.

The much smaller variation in the total and individual fatty-acid content between the clones in Set 2 , despite the large variation in resistance, may be due to the fact that the tissue analyzed was collected from the middle part of the crown of the ramets where shading or other effects from neighboring ramets as well as within-crown variation may change the pattern of variation in fatty-acid content. In other crops the variation in fatty-acid content and composition in seeds collected from various parts of plants such as *Glycine max* L. (COLLINS and CARTER, 1956), *Carthamus tinctorius* L. (WILLIAMS, 1962), and *Brassica napus* L. as well as *B. campestris* L. (BECHYNE and KONDRA, 1970) have been reported.

In spite of the uncertainty about the reliability of the association between small fatty-acid content and resistance observed in Set 1, it was important, in the opinion of the author, to gain more understanding of some of the problems encountered in this study. Therefore, assuming that the association found in Set 1 is real, attention was given to the fact that such an association was not detected in any of the young full-

and half-sib families analyzed. The low fatty-acid content may be due to either (i) a true cause-and-effect relationship or (ii) a non-causal relationship.

A true cause-and-effect relationship, based on the hypotheses of fatty acids providing a carbon source for the pathogen, involves the assumption that large amounts of fatty acids enhance the development of the pathogen in clones of mature trees but not in younger plants. Possible differences in fatty-acid metabolism between young and mature trees may render the fatty acids an important carbon source for the pathogen in mature trees, while some other sources of carbon are important for the pathogen in young trees. This hypothesis may be partly studied by applying the recently developed techniques of axenic culture of obligate parasites (Scott and Maclean, 1969; Harvey and Grasham, 1970; Quick and Cross, 1971) to culture experiments with *Cronartium fusiforme*.

On the other hand, the fatty acid content in young and mature trees may be directly or indirectly related to the modes of genetic control of rust-resistance in loblolly pine. Blair (1970) found that the inheritance of resistance changed from dominance to additive gene effects when young seedlings were in a condition of transplanting shock (the first season after planting) and had recovered from it (the second season after planting), respectively. Blair expressed the opinion that the most likely explanation for the two modes of inheritance was the differences in physiological conditions of the seedlings. Similar changes in the genetic control of rust-resistance may possibly occur between young and mature trees.

A non-causal relationship would possibly be due to linkage of genes, pleiotropic gene effects, or a combination of both. Such genes would control the two traits, fatty-acid content and resistance; expression of the gene effects would be a function of age and not detected in the younger plants. The expression of several gene effects controlling resistance to stripe rust, *Puccinia striiformis* West. of wheat, *Triticum aestivum* L. are functions of age (Allan *et al.*, 1966).

Whatever the reason for the observed association between small amounts of fatty acids and resistance to fusiform rust in loblolly pine, the usefulness of this association as a marker ultimately is dependent on whether resistant clones identified by the marker will deliver resistant offspring. Since resistant clones of loblolly pine tend to deliver resistant offspring (Kinloch and Zoerb, 1971) a marker which can readily identify such clones may be useful.

Other Possible Marker Traits

Discovery of other marker traits, not necessarily causally related to rust-resistance, but displaying satisfactory genetic correlation with resistance due to linkage or pleiotropy may be a lucky event rather than an expected result (Smith, 1967) since it is difficult to obtain any clues regarding such markers. It remains to be seen if the major polymorphic loci suggested to control fusiform rust in loblolly pine (Kinloch, 1972) also pleiotropically control some potential marker since pleiotropy is a common property of major genes (Falconer, 1967, p. 312).

Much hope has been expressed about the use of serological identification of genetic relationships among plants and animals (Nelson and Birkeland, 1929; Kleese and Frey, 1964; Beevers and Hageman, 1969; Gilmour, 1969; Smith and Frey, 1970; Volin and Welsh, 1970; Hare, 1970). Recently, however, Gilmour and Morton (1970) expressed some reservations about serological approaches to indirect selection in poultry breeding, while Smith and Frey (1970) required better qualitative serological techniques for predicting genetic relationships in wheat. On the other hand, differences in some protein and isozyme patterns were found associated with differences of six species of pines native to the southern United States and which show varying resistance to fusiform rust (Hare, 1970). Recently Rockwood found promising genetic correlations between β-phellandrene and resistance to fusiform rust in *Pinus taeda* (1972) and *Pinus elliottii* (personal communication, Oct. 1973).

Future efforts to discover useful markers for indirect selection of resistant loblolly pines have to be justified on the basis of a comparison of the estimated costs of indirect vs. direct selection. During the course of this investigation some progress has been

made in the techniques of direct selection. These include: (i) artificial inoculation techniques (MILLER, 1970; POWERS *et al.*, 1971) suitable for large scale testing (MATHEWS and ROWAN, 1972) of breeding material, (ii) the correlation between field and artificial inoculation tests (DINUS, 1972, GODDARD and SCHMIDT, 1971), (iii) phenotypic selection of resistant oftets from heavily infected stands (DINUS, 1972; WELLS and SWITZER, 1971), and (iv) field testing and selection of clones for seed orchards (KINLOCH and ZOERB, 1971). Such progress may result in increased efficiency of direct selection procedures and therefore eliminate the need to find possible methods of indirect selection.

CONCLUSIONS AND RECOMMENDATIONS

The most important conclusions and results from the present study are briefly summarized as follows:

1. Predicted gain from simple indirect mass selection in resistance to fusiform rust in loblolly pine can be equal or superior to the gain presently predicted (BLAIR, 1970) from conventional direct selection methods, which all require the expensive raising and testing of progeny, provided that: (i) the heritability of the marker is at least equal to that of resistance to fusiform rust (0.2 according to BLAIR, 1970), (ii) the genetic correlation between the marker trait and resistance is moderate ($r_a = 0.6 — 0.7$), and (iii) an indirect selection intensity can be achieved which is two or three times greater than that presently achieved by direct selection.

2. The possibilities of discovering and determining the potential value of markers for indirect selection will be reduced if: (i) several physiological races of the pathogen occur in significant portions of the host and pathogen populations respectively and (ii) the genetic studies necessary to obtain accurate estimates of heritability and genetic correlations are not available or are considered too costly to establish compared to the present costs of direct selection.

3. A new stability index was developed to measure the amount of genotype × environment interaction of relative rust-resistance for individual genotypes tested in several environments.

4. The resin acids neoabietic and levopimaric acids stimulated direct germination while isopimaric and dehydroabietic acids inhibited both direct and indirect germination of basidiospores of *Cronartium fusiforme*. Pimaric acid showed no effect on the spores.

5. This is believed to be the first published report on qualitative and quantitative analysis of certain resin- and fatty acids extracted from primary cortex of loblolly pine. Abietic and neoabietic acids usually accounted for the largest portion of resin acids while the acids 16:0, 18:2, and 18:3 accounted for the largest portion of fatty acids.

6. No association was found between the content of total and five individual resin acids as measured in this study and the resistance of four clones and 14 families analyzed.

7. A trend of increasing amounts of growth-inhibiting substances in branches of loblolly pines with increasing resistance to fusiform rust was found among four full-sib families analyzed.

8. The amount of total and certain individual long-chain fatty acids in young shoots from the uppermost whorl of branches may offer some promise as a potential marker for resistant clones of mature trees but not young families (age 9 weeks to 7 years) of loblolly pine.

Assuming that indirect selection is a suitable alternative to present direct selection it is recommended that the following measures be taken to facilitate search for markers:

1. Since markers in old and young trees may be different, two types of markers should be distinguished: (i) markers to identify parents delivering resistant offspring and (ii) markers to identify resistant offspring only.

2. In genetic studies of traits such as growth characteristics, wood quality, and resistance to other diseases and insects, continuous efforts should be made, whenever possible, to estimate the genetic correlation between such traits and resistance to fusiform rust.

3. Traits both causally and non-causally associated with resistance should be studied since, regardless of the underlying cause, high genetic correlations can be found which are due to linkage, pleiotropy, or a combination of both. Efforts should be made to identify

component traits of each mechanism of resistance.

4. Efforts should be made to find markers correlated with the most frequently occurring mechanism(s) of resistance.

5. Since use of clones for preliminary identification of possible marker traits has been the most successfull approach in previous as well as in the present study, continuous efforts should be made to determine the resistance of clones, their individual ramets, and their offspring. Seeds and clones of both resistant and susceptible plants should be preserved in seed- and clone banks to provide material for future studies.

6. To determine statistically significant and encouragingly high phenotypic correlations for preliminary marker traits, it appears necessary to analyze at least 15—20 clones or families. Accurate estimates of genetic correlations may require genetic studies as comprehensive as the Loblolly Pine Heritability Study (*cf.* pp. 9—11) although more limited studies may be satisfactory.

7. An equal number of both infected and non-infected members of resistant and susceptible clones and families should be analyzed when available to provide estimates which are (i) unbiased by possible effects of previous infection on the marker trait analyzed and (ii) representative of the variation within a clone or family.

8. The reliability of the possible association found in the present study between low fatty-acid content and resistance of four clones of mature loblolly pines should be determined by analysis of a larger number of genotypes. The magnitude of a possible genetic cross-correlation (*cf.* p. 13) between the fatty acid content in shoots from clones or parent trees and resistance of their offspring should be estimated. Tissue for analysis should be collected from the uppermost whorl of shoots in each tree; analysis of shoots collected from the middle part of the crown do not suggest any strong association between fatty-acid content and resistance.

Plant material required for studies of possible marker traits is now available in several seed orchards (*e.g.* Union Camp Corporation, orchards in Almeda, South Carolina and Egypt, Georgia) and in the valuable and outstanding Loblolly Pine Heritability Study in Bainbridge, Georgia. These plant materials provide a unique opportunity for comprehensive analyses of the important genetic relationship between parent trees and their offspring as well as physiological properties of the plants and resistance to the most devastating and increasingly important forest tree disease in the southeastern United States.

SUMMARY

This investigation was designed to: (i) examine the theory of indirect selection and its application in breeding programs, (ii) review earlier efforts to use indirect selection in breeding forest trees for disease resistance in general and for resistance to *Cronartium fusiforme* in loblolly pine in particular, (iii) develop a method for evaluation of environmental influences on the stability of relative rust resistance of genotypes tested in several environments, and (iv) conduct a preliminary search for chemical markers of resistance to fusiform rust in loblolly pine in the hope that possibly discovered phenotypic correlations with resistance may suggest genotypic correlations strong enough to justify further attempts to use indirect selection in breeding for rust resistance.

To achieve, by simple indirect mass selection, gains in resistance equal to or superior to those presently predicted for time-consuming and expensive direct-selection methods, a marker must meet three major criteria: (i) its heritability should approach or exceed that of resistance itself, (ii) it should have at least a moderate genetic correlation with resistance, and (iii) it should permit large numbers of plants to be tested rapidly and at low cost. Such a marker possibly could be found among chemical traits that are component traits of resistance.

A new stability index was developed to measure the amount of genotype × environment interaction of relative rust resistance for individual genotypes tested in several environments. This index was used to choose a number of highly rust-resistant and highly rust-susceptible clones and full- and half-sib families from among approximately 1000 genotypes of loblolly pine available in tree-improvement and genetic studies in the southern United States. These genotypes were used in a preliminary search for chemical markers of rust-resistance.

Basidiospore germination tests showed that extracts of pine shoots and various known resin acids stimulated direct germination of basidiospores of *Cronartium fusiforme*. Gas chromatographic analysis of resin acids in primary cortex of young shoots at the time of natural infection, however, did not reveal any association between rust-resistance and the amount of total or several individual resin acids in the four clones or the 14 families analyzed.

Coleoptile tests showed that the amount of growth-inhibiting substances present in branches from four full-sib families of loblolly pine was correlated with their resistance to rust — the greater the amount of growth-inhibiting substances present, the greater the rust-resistance of the family. Despite the encouraging phenotypic correlation observed, this analysis for a marker was not pursued since the method is very time-consuming.

Gas chromatographic analysis of extracts from the primary cortex of young shoots showed a strong association between rust-resistance and small amounts of certain long-chain fatty acids in four clones of loblolly pine in one seed orchard and a weak association in five clones in another seed orchard. No association was found, however, between rust-resistance and the content of total or individual fatty acids in any of 14 families analyzed.

In future studies of indirect selection it is recommended that: (i) the reliability of the phenotypic correlations observed in this study be tested by analyzing a larger number of genotypes, (ii) the possibility be investigated that there may be a causal relationship between the content of long-chain fatty acids and rust-resistance of clones, and (iii) the genetic cross-correlation be determined between the content of long-chain fatty acids in parental clones and the rust-resistance of their offspring.

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SELOSTE

Epäsuora valinta *Cronartium fusiforme*-kestävyyden lisäämiseksi loblolly-männyllä *(Pinus taeda L.)*

Monet männyn jalostusohjelmat Etelä-Yhdysvalloissa ovat ottaneet *Cronartium fusiforme*-kestävyysjalostuksen erääksi päätavoitteekseen. Kestävä jalostusmateriaali valitaan pääasiassa kenttäkokeiden perusteella. Koska kokeiden tulokset usein eivät ole yhdenmukaisia, valintatyö vaikeutuu. Kenttäkokeiden hyväksikäyttämiseen ovat liittyneet seuraavat vaikeudet: 1) Taudinaiheuttajan kannan suuruus vaihtelee vuosittain, 2) taudinaiheuttajan virulenssi vaihtelee vuosittain, 3) pienilmastolliset ja maaperälliset olot jotka vaikuttavat saastunnan runsauteen vaihtelevat ja 4) tyydyttävien kenttäkokeiden toteuttaminen vaatii suuria kustannuksia ja paljon aikaa.

Tehokkaampien testausmenetelmien löytämiseksi on voimaperäisesti tutkittu mahdollisuuksia kehittää ja käyttää jalostusmateriaalin laajamittaiseen testaamiseen soveltuvia keinollisia saastuttamismenetelmiä. Toistaiseksi kehitetyillä menetelmillä ei kuitenkaan voida testata muuta kuin isäntäkasvipopulaation hyvin pientä osaa. Nykyisten ns. suorien valintamenetelmien puutteellisuuksien takia on huomio kiinnittynyt mahdollisuuksiin käyttää epäsuoraa valintaa — so. vaikuttamista tavoiteominaisuuteen kohdistamalla valinta toiseen, tavoiteominaisuuden kanssa korreloivaan osoitinominaisuuteen (marker trait).

Nyt esillä olevan tutkimuksen tavoitteet olivat: 1) Tarkastella epäsuoran valinnan teoriaa ja sen soveltamismahdollisuuksia jalostusohjelmissa, 2) luoda katsaus aikaisempiin pyrkimyksiin käyttää epäsuoraa valintaa metsäpuiden taudinkestävyysjalostukseen yleensä ja erikoisesti *Cronartium fusiforme* -kestävyyden lisäämiseen *Pinus taedalla*, 3) kehittää menetelmä eri genotyyppien suhteellisen kestävyyden muuttumattomuuden (stability) mittaamiseksi eri ympäristöissä ja 4) suorittaa alustava tutkimus *Cronartium fusiforme* -kestävyyden kemiallisten osoitinominaisuuksien löytämiseksi *Pinus taedassa*.

Epäsuoralla valinnalla saavutettava hyöty taudinkestävyydessä oli sama tai suurempi kuin se ennustettu hyöty joka olisi saavutettu käyttämällä hidasta ja kallista suoraa massavalintaa. Tämä edellyttää kuitenkin että käytetty osoitinominaisuus täyttää seuraavat vaatimukset: 1) Sen heritabiliteetin on lähestyttävä tai ylitettävä tavoiteominaisuuden heritabiliteettia, 2) sen on oltava ainakin kohtalaisessa geneettisessä riippuvuussuhteessa taudinkestävyyteen ja 3) sen on oltava muilta ominaisuuksiltaan sellainen, että voidaan testata suuri määrä taimia pienin kustannuksin. Näitä vaatimuksia täyttävän osoitinominaisuuden oletettiin löytyvän sellaisten kemiallisten ominaisuuksien joukosta, jotka ovat taudinkestävyyden osaominaisuuksia (component trait).

Uusi muuttumattomuusindeksi (stability index) kehitettiin yksityisten genotyyppien suhteellisen taudinkestävyyden ja ympäristön välisen interaktion mittaamiseksi. Tämän indeksin avulla valittiin joukko erittäin ruosteenkestäviä ja erittäin ruosteenalttiita *Pinus taeda*- klooneja, puolisisar- ja täyssisarperheitä noin 1 000 genotyypistä, jotka olivat käytettävissä metsänjalostusohjelmissa ja geneettisissä tutkimuksissa Etelä-Yhdysvalloissa. Valittuja genotyyppejä käytettiin kemiallisten osoitinominaisuuksien alustavassa tutkimuksessa.

Idätyskokeet *Cronartium fusiforme* -sienen kantaitiöillä osoittivat, että männyn versoista saatavat uutteet ja erilaiset tunnetut hartsihapot elvyttivät iturihman muodostumista. Luontaisena saastumisajankohtana nuorten versojen primäärisessä kuoressa esiintyvien hartsihappojen kaasukromatografiset analyysit eivät kuitenkaan paljastaneet riippuvuussuhteita ruosteenkestävyyden ja useiden hartsihappojen erillis- ja yhteismäärien välillä.

Kestävien mäntyjen oksissa esiintyi suurempi konsentraatio kasvua estäviä aineita verrattuna kestämättömien mäntyjen oksiin.

Voimakas korrelaatio esiintyi neljän kloonin ruosteenkestävyyden ja nuorten versojen primäärisessä kuoressa luontaisena saastumisajankohtana esiintyvien pitkäketjuisten rasvahappojen määrien välillä. Lievempää korrelaatiota todettiin viiden muun kloonin ruosteenkestävyyden ja rasvahapon 18:2 määrän välillä. Tutkittaessa taas 14 *Pinus taedan* puolisisar- ja täyssisarperhettä, ei todettu korrelaatiota kestävyyden ja rasvahappojen erillis- eikä yhteismäärien välillä.

Vastaisissa epäsuoraa valintaa koskevissa tutki-

muksissa ehdotetaan että: 1) Tässä tutkimuksessa havaittujen fenotyyppisten korrelaatioiden luotettavuutta tarkistetaan tutkimalla suuria määriä genotyyppejä, 2) tutkitaan mahdollisten syy-yhteyksien esiintymistä ruosteenkestävyyden ja pitkäketjuisten rasvahappojen määrän välillä sekä 3) mitataan geneettinen ristikorrelaatio (genetic cross-correlation) vanhempaiskloonien rasvahappojen määrien ja jälkeläistöjen ruosteenkestävyyden välillä.

WEISSENBERG, KIM von O.D.C. 165.62, 443.3, 892.6

1973. Indirect Selection for Resistance to Fusiform Rust in Loblolly Pine. – ACTA FORESTALIA FENNICA 134. 46 pp. Helsinki

Indirect selection for resistance is superior to direct selection under certain conditions. The phenotypic correlation was studied between resistance to fusiform rust in loblolly pines, chosen by a newly-developed stability index, and the amount of resin acids, growth-inhibiting substances, and fatty acids extracted from pine tissue. No association was found between rust resistance and the amount of total or individual resin acids in clones or families. The amount of growth-inhibiting substances present in branches from full-sib families was greater in rust-resistant compared to rust-susceptible families. A strong association was found between resistance and the amount of long-chain fatty acids in four clones. A weak association was found between resistance and the amount of the fatty acid 18:2 in five other clones.

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