Frank S. Santamour, Jr.¹

The importance of eastern white pine (Pinus strobus L.) to the forest economy of the Northeast is well known, That the white-pine weevil (Pissodes strobi Peck) is the major cause of the declining production and use of this species also is well known, And most people would agree that the development of weevil-resistant strains would be the most economical means of restoring white pine to its former high place among Northeastern timber species.

The initiation of an effective intra-specific breeding program for weevil resistance in eastern white pine depends on the finding of resistant trees. A question of paramount importance is whether there are, in fact, resistant specimens of P. strobus. Few studies have been directed toward the detection or selection of resistant trees, and so far no individual trees have been proved to be resistant.

The difficulties involved in the selection of resistant trees are many and varied. The insect may not be present in a particular area; or if it is present, its numbers may fluctuate. The likelihood that a tree will be attacked depends to some extent on growth rate, morphological characters, and environmental conditions such as shading. All of these factors, and more, are compounded with time, since even one successful attack during the first 15 to 20 years of growth may appreciably lower the value of the basal 16-foot log.

However, the principal reason for the scarcity of reports on weevil resistance has been because the trees that have been examined have lacked an absolute identity. That is, information about the particulars of their origin has been inadequate for classifying them by parentage or provenance in such a way that meaningful comparisons between groups could be made.

Provenance test offer a limited possibility for classification. Pauley, Spurr, and Whitmore² reported that trees of three Ontario provenances were weeviled less than those from Massachusetts, New York, or New Hampshire. But, they stated, "No evidence was found indicating genetic resistance to weeviling independent of tree size or exposure." Wright and Gabriel³ found less weeviling in an Ontario source than in three New York sources in an unreplicated test where "all plantings were of approximately equal height." The evidence from these two studies does not justify any firm conclusions regarding inherent differences in resistance among provenances.

Results have been even more inconclusive where less was known about the origins of the trees being observed. Wright and Gabriel³ differences in resistance among and within small local populations of eastern white pine: during 32 man-days spent in examining 614 stands, they found an "estimated 15 apparently resistant phenotypes," They concluded that "The possibility of developing a weevil-resistant strain by intra-ecotypic selection and breeding within eastern white pine seems remote."

¹ Geneticist, Northeastern Forest Experiment Station, U. S. Forest Service, stationed at Durham, New Hampshire, in cooperation with the University of New Hampshire.

² Pauley, Scott S., Spurr, Stephen H., and Whitmore, Frank W. Seed source trials of eastern white pine, Forest Sci. 1: 244-256, 1955.

³ Wright, Jonathan W., and Gabriel, William J, Possibilities of breeding weevil-resistant white pine strains. U. S. Forest Serv., Northeast, Forest Expt. Sta. Paper 115, 35 pp., 1959. The present study is concerned with differences in weeviling, and in some tree characters thought to influence weevil attack, as observed in one small plantation of eastern white pine of known parentage.

MATERIAL AND METHODS

The eastern white pine trees used in this study were derived from openpollination and artificial crosses of six ornamental trees in the Morris and Swarthmore Arboretums, near Philadelphia, Pa. The only criteria for selection as parents were availability and fecundity. The crosses were made in 1947 by Dr. Jonathan W. Wright. The seed was collected in the fall of 1948, stratified, and sown in the greenhouse in January 1949. The seedlings were potted in March 1949 and outplanted as 3-year-old stock in 1952. Thus, at the time of the most recent examination, in 1962, the trees were 1L1 years old from seed.

The plantation (GP-14-52) was established at the Hopkins Experimental Forest, Williamstown, Mass. The trees were planted in rototilled strips in an old field at a spacing of 6 feet between trees in two rows 6 feet apart. The rows run in an east-west direction and lie between young plantings of paper birch and green ash., which have not yet begun to shade the pines. The seedlings from each seedlot were divided into two approximately equal groups, and one group of each pair was assigned at random in each row. Originally there were 58 trees representing 8 seed lots in the planting. Eleven trees died prior to 1955, but since then no mortality has occurred.

In the summer of 196, after height growth had ceased, height, d.b.h. and total number of weevilings were recorded for each tree. In addition, determinations of bark thickness at the midpoint of the 1960 internode and at breast height were made on all trees of progenies Pi-259 and Pi-2600 Bark thickness was taken on the 1960 growth rather than on the 1961 growth because weevil attacks in 1962 had damaged the 1961 growth of some of the trees. So, to get complete and comparable data, the growth of the preceding year was sampled. Bark thickness was obtained by caliper measurements on bark disks cut from the tree with a cork borer.

RESULTS AND DISCUSSION

Data on weeviling in the various progenies are presented in table 1. Because of the small and variable number of trees in each seedlot group and the high mortality in some seedlots, no meaningful analysis of variance was possible. Therefore chi-square tests were used for purposes of comparison.

Progeny number Pi-	Parentage	No. trees with weevilings					Total no.	Total no.	Number weevilings
		0	1	2	3	4	trees	weevilings	per tree
259	730 x 748	0	2	3	5	2	12	31	2.58
260	730 x 740 ,	3	4	2	1	2	12	19	1.58
730-48	730 x OP 1/	1	2	2	2	0	7	12	1.71
271	723 x 736	0	1	0	1	0	2	4	2.00
273	723 x 740	2	2	4	1	0	9	13	1.44
267	719 x 740 ,	0	2	0	0	0	2	2	1.00
719-48	719 x OP 1	0	0	1	0	0	1	2	2.00
288	740 x 748	0	0	1	1	0	2	5	2.50

Table 1.--Weeviling in eastern white pine progenies

1/ Open pollinated.

The first comparison of interest is that between progenies Pi-259 and Pi-2600 The female parent of both progenies was the same tree (G-730), crossed with two different males. The chi-square test showed highly significant differences between the two progenies in amount of weeviling (based on the distribution of trees weeviled various numbers of times), the progeny from the cross with G-740 (Pi-260) being superior in apparent resistance.

When all trees having G-740 as one parent were compared to trees of other parentage, the difference were again highly significant. Further, when the trees with and without G-740 in their parentage are grouped only as "weeviled" or "un-weeviled", the difference is highly significant.

What is the cause of these differences in amount of weeviling? Suppression and/or shading was not a factor. Only one tree in the entire planting could be considered as being shaded to some extent. Growth rate can likewise be discounted as a cause. Trees of Pi-260 were larger than those of Pi-259 in both height and diameter; and within P1-260 as well as other seedlots, the unweeviled trees were as large or larger than those that were weeviled. Differences in bark thickness between trees of P1-259 and P1-260 and between weeviled and unweeviled trees of P1-260 were extremely slight and decidedly nonsignificant.

Having discounted as probable causes the various environmental and morphological factors believed to contribute to variability in weevil susceptibility, the obvious inference is that unknown gene-controlled characteristics were responsible.

With the limited amount of material of known parentage now available, only a modest beginning can be made in elucidating the causes of supposed resistance.

Some preliminary chemical analyses of resin and wood have been made, and the possibility that resistance has a chemical basis appears promising at this time. However, much more work along this line is needed.

DISCUSSION

<u>MORROW</u> - It bothers me that the reduction in weeviling appears so little. It's a beginning, I'll admit, but it just doesn't look terribly promising to me at the moment.

<u>SANTAMOUR</u> - There is a very slight reduction when the average numbers of weevil ings in the progeny groups are compared. The difference is in the number of trees not weeviled in these groups. There is where the significance lies.

<u>MORROW</u> - In southern New York we'll get 3-5 weevils in ten years per tree. To grow good white pine, we're going to have to cut this down to less than one weevil. It can't be a reduction in weeviling; it's got to be a big reduction.

<u>GERHOLD</u> - I believe it's true, Frank, that no genetic selection was practiced in this case in regard to weeviling. Is that right?

- <u>SANTAMOUR</u>-That the parents were not selected for any particular weevil resistance.
- <u>GERHOLD</u> These were randomly selected trees with respect to weeviling and this is no indication of how much progress is possible.

<u>SANTAMOUR</u> At the present time, we are not interested in the average amount of weeviling in a progeny; we are interested in those members of certain progenies that are not weeviled. As I have said, with even one weeviling in the first 10-15 years a tree may be ruined. We will be working with unweeviled trees-testing and breeding.

FOSTER I'd like to ask Dr. Stairs what levels of radiation work with pine and oak that are mutagenic and those which will kill nuclei.

STAIRS The radiation dose for mutation breeding will depend on the species used. For oaks and most hardwoods acute irradiation of mature pollen up to levels of 3,000 roentgens will probably be permissible in terms of maintaining In so doing, one might select at least five treatment levels; nuclear viability. for example, 500r 1,000r, 2,000r, 3,000r and a control. With pines and other conifers the radiosensitivity will be increased by a factor of about 5. In this case, one might use lower levels of radiation such as 100r, 250r, 500r, 1,000r plus a control. Both Dr. Mergen and myself are continuing work in this area; we feel that data from many experiments will be necessary to quantify the mutational vield for a given dose rate. Basically, we expect a linear increase of mutation rate as radiation dose increases; we also know from the work of Dr. Arnold Sparrow and others at Brookhaven that radiosensitivity is directly related to nuclear volume. Thus having established that the ranges I suggest above are reasonable for experimentation, we now request that other workers join in further defining tolerance and mutation yield data.

SANTAMOUR You mentioned the possibility of overcoming incompatibility and non crossability by mixing pollen. You killed the compatible pollen in this mixture you also used a non-compatible pollen. What mechanism is operative, in your mind, that would cause the incompatible pollen to actually affect fertilization?

STAIRS In terms of defining incompatibility, we probably need to differentiate between known sterility alleles and the type of general cytoplasmic incompatibility that has been reported for most forest trees. It is dangerous to generalize at this time since we can only hypothesize about mechanisms. However, I would suggest the possibility of growth substance stimulation by the compatible but nuclear inviable pollen, thus allowing the growth of otherwise incompatible pollen. Certainly we need further study in this area prior to attempting a more specific definition of mechanisms.

<u>GENYS</u> How different would be the mutagenic effects, caused by different irradiating agents such as ultraviolet light, X, alpha, beta- and gamma rays?

STAIRS Both x-rays and gamma rays are very similar with only slight differences in wave lengths. Virtually any source of ionizing radiation may be used in mutation induction work. Ionizing radiation does not render the material irradiated radioactive so that transmission of exposure is not possible. <u>MAHONEY</u> - I have something I'd like to question; as I understood, you're proposing the selection of trees with less vigorous leaders, slower growing leaders. Is this for the purpose of developing more resistant strains or reducing the incidence of weeviling in the existing stands.

<u>STROH</u> - I have tried not to propose any selection techniques. Based on what we know now, including the relationships I have developed and those reported by other workers regarding leader diameter and leader bark thickness, selection would be likely to favor thinner, less vigorous leaders. In other words--slower growing trees.

<u>MAHONEY</u> - From a silvicultural point of view, you could run into a problem even though you may have less instance of weeviling in any one year, if it takes you 3-8 years to get a 16-foot log.

<u>STROH</u> - That's true. I was attempting to point out a problem that we are in right now, What we need is a tree with the desirable characteristics from the standpoint of weevil resistance as well as the desirable characteristics from the standpoint of quality and quantity wood production.

<u>MAHONEY</u> - Entomologists have proposed much the same thing through the idea of keeping the partial shade over the trees to produce a slower growing leader. I think that if it's going to take at least eight years to get a 16-foot log instead of six years, we run the risk of weeviling for several additional years and may get more weeviling in the 16' although the incidence of weeviling one year may be lower.

<u>STROH</u> - That's right. The chemical resistance we have heard about today from Dr. Santamour may be a possible solution to this problem.

<u>CONNOLA</u> - You seem to have a pretty good correlation between height growth and your cortical resin data. When you take out the effect of height growth, how does it compare?

<u>STROH</u> - I attempted this using a co-variance technique and there was still a significant relationship. I also noted an increase in the differences between provenances with respect to the correlated characters after height growth was eliminated.

CONNOLA - How many bark samples were involved in this study?

<u>SANTAMOUR</u> - The comparisons on bark thickness were only made between those two progenies that had the largest number of trees, that's 24 trees.
One sample from the 1960 internode and one at d.b.h. on each of 24 trees.

<u>CONNOLA</u> - Do you plan to continue the same studies with bark samples along with your chemical analysis?

SANTAMOUR - No. I don't feel it's a very fruitful avenue of endeavor except perhaps in regard to the depth of the cortical resin canals as Stroh has pointed out here. But actual bark thickness, no. I think that bark thickness is too closely related to growth. And when you get down to a bark thickness that will inhibit weevil activity, you're down to a tree that you could jump over in ten years, and that's not what we want. The tree would be just too weak, too slow-growing; that's what they have found up in New Haven on bark thickness also. <u>STROH</u> - I would like to point out that I did observe some variation in bark thick ness that could not be explained on the basis of leader diameter or length. In some cases the more rapidly growing trees had relatively thinner bark than the more slowly growing trees.

<u>SANTAMOUR</u> - Yes, I would assume that there would be a relative variation., but the weevil works on absolute; and what I've run into consistently over the years that if there is an absolute bark there, the absolute weevil's going to chew on it.

<u>CONNOLA</u> - Well, I would think that under present conditions that you might be satisfied with a half a loaf rather than a whole loaf and go along with the slower growing trees until we find another answer; I think the whole problem itself is complex. I've been working with white pine weevil now for about ten years, and I'm convinced more and more that genetics is only one part of this complex. We have completed a five-year study in New York on the distribution of weeviling in the State, and as Bob Morrow mentioned, we have had a lot of weevil ing for several years. The correlation we found there is probably a physiologi cal thing. We found more weeviling over hard-pan, and the closer the hardpan to the surface the more weeviling, and this picture was the same in sandy and light soil as compared to the heavy soil.

SANTANOUR - Comments on that, Bob?

<u>STROH</u> - How was your growth rate over hardpank

CONNOLA - We found no correlations in growth rater

<u>SANTAMOUR</u> - The fact that you found the same in sandy and heavy soils would indicate that probably no chemical constituents of the soil were part of

it

CONNOLA - I would think so.

WEST - Any other questions?

<u>GERHOLD</u> - I might throw in one comment here that I think would be of interest to the group. A number of years ago Wright and Gabriel looked at a number of five-needle species in regard to weevil resistance and pointed out that one good possibility is western white pine, Bob Stroh and I had occasion to revisit one of the plantations that these two authors reported on, and obtained some dditional data. Their earlier results held up for this additional period in that western white pine was still weeviled about 1/5 as much as eastern white pine. The growth rate of the surviving western white pines I would say was fairly comparable, but there was a disease problem, This is an interesting pos sibility in the search for resistance

MAHONEY - Any other species such as Himilayan white pine?

<u>GERHOLD</u> - In that same paper, there were other observations on quite a number o species. Wright just recently published some data on Himilayan white pine which contradicts the earlier indications of resistance; that is, the more recent data shows that Himilayan white pine in Michigan was more heavily weeviled than eastern white pine. It was not the same material observed previously, possibly a different seed source.