WEEVIL ATTACKS ON CAGED SEEDLINGS OF THREE WHITE PINE SPECIES[1]

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A breeding program for improving the resistance of Pinus strobus L. and related white pines to the white pine weevil, Pissodes strobi Peck, would be greatly facilitated if selection against susceptible trees could be practiced while they are still small enough to be tested in large numbers in a uniform environment with a high level of infestation. Artificially confined insects have been used to screen for resistance to Cooley aphid (Beier-Peterson and Soegaard, 1958) and to the pine reproduction weevil (Callaham, 1960; and Smith, 1960). Experiments by Connola (1965), Heimburger (Gerhold et al., 1966), and Plank and Gerhold (1965) showed that it should be possible to develop a screening method of this type for white pine weevil resistance. Several other entomologists and tree breeders have made suggestions for developing such a method (Gerhold et al., 1966). A system was envisioned in which seedlings would be raised conventionally, transplanted into cages to be screened for resistance between ages 2 and 5, and then outplanted for further genetic evaluations. The trees to be screened could consist of provenance collections, individual-tree progenies of one or several species, or hybrids.

[1] The study was supported by Forest Service, U. S. Department of Agriculture, Washington, D. C., Grant #1; and by Northeast Regional Research Project NE27, U.S.D.A. Authorized on September 27, 1966, as Paper No. 3179 in the Journal Series of the Pennsylvania Agricultural Experiment Station.
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Here we describe the initial results from one of our experiments designed to provide information for developing a screening method for small trees. We wanted to learn how extensively adult weevils in cages would feed upon trees much smaller than those normally attacked, and whether it mattered if species were mixed or kept in separate cages. Hopefully, some data on larval development might also be obtained, although feeding and oviposition on three-year-old trees would be considered very unusual under ordinary circumstances.

METHODS

Three species likely to be included in a weevil resistance breeding program were chosen for the experiment, namely Pinus strobus L., P. monticola D. Don, and P. griffithii McClel. Several seedlots of the latter came from trees planted in Italy and the United States, so that there is a possibility that hybrids are included. We are indebted to J. B. Genys of the University of Maryland for supplying two-year-old seedlings that were lifted in 1965 from the Maryland Forest Tree Nursery. The seedlings were potted in asphalt felt-paper cylinders 6 inches by 12 inches deep, and transplanted intact in April, 1966, at a spacing of 1 foot in the desired experimental design. They then were covered with aluminum screen cages 4 feet by 6 feet by 3 feet high.

The experimental design consisted to two replicates containing six combinations of the three species in sets of two which were mixed in alternate rows:1/

Replicate 1

mmmmmm	gggggg	gggggg	ssssss	ssssss	ssssss
mmmmmm	mmmmmm	gggggg	gggggg	ssssss	mmmmmm
mmmmmm	gggggg	gggggg	ssssss	ssssss	ssssss
mmmmmm	mmmmmm	gggggg	gggggg	ssssss	mmmmmm

Replicate 2

gggggg	ssssss	ssssss	ssssss	mmmmmm	gggggg
gggggg	gggggg	ssssss	mmmmmm	mmmmmm	mmmmmm
gggggg	ssssss	ssssss	ssssss	mmmmmm	gggggg
gggggg	gggggg	ssssss	mmmmmm	mmmmmm	mmmmmm

1/ Each rectangle represents a cage and each tree is represented as follows:

s = P. strobus
m = P. monticola
g = P. griffithii

Nine combinations were available for analysis:
- strobus associated with itself
- strobus associated with monticola
- strobus associated with griffithii
- monticola associated with itself
- monticola associated with strobus
- monticola associated with griffithii
- griffithii associated with itself
- griffithii associated with strobus
- griffithii associated with monticola

Each species group of twelve trees was constituted from twelve different seed sources; the same sources of a species were used in every group, with the exception of monticola which was represented by only eight sources, half of which were duplicated. The sampling was widespread for the range of strobus, but more restricted for the other two species. The seed sources are listed in table 1, together with the average dimensions of the trees. Measurements of height, leader length, leader diameter, and stem diameter were made just before the start of the experiment. These dimensions differed significantly among species. Terminal elongation began during the weevil exposure period.

Table 1.--Seedling dimensions and seed sources of the three white pine species exposed to weevils at age 3.

Character	P. strobus Mean	P. strobus Standard error	P. monticola Mean	P. monticola Standard error	P. griffithii Mean	P. griffithii Standard error
	(millimeters)					
Height	245.0	8.1	104.8	3.8	164.2	3.9
Leader length	107.8	4.8	46.0	2.4	65.3	2.2
Leader diameter	3.3	0.07	2.8	0.06	5.2	0.12
Stem diameter	5.8	0.13	4.5	0.09	7.4	0.16

J. B. Genys Md F numbers and states or countries	P. strobus	P. monticola	P. griffithii
	1 Minn.	41 Ida.	103 Pakistan
	3 Queb.	44 Ida.	106 Italy *
	10 N. C.	116 Wash.	280 Pakistan
	51 Tenn.	153 B. C.	281 Pakistan
	54 Minn.	154 Ida.	282 Pakistan
	148 Me.	640 Ida.	296 India
	566 N. H.	654 Ida.	297 India
	570 S. C.	724 Ida.	586 Caton., Md.*
	618 Pa.		589 Oxf., Md.*
	653 Mich.		719 Pakistan
	655 N. Y.		721 Bhutan
	700 Ohio		771 India

* Seeds from trees planted as exotics - may include hybrids.

Weevils were picked by hand from P. strobus leaders in a single plantation in Huntingdon County, Pennsylvania, on April 26 and again on May 17, 1966. On May 20, 48 weevils were evenly distributed over the soil in each cage. Earlier trials had indicated that this method of release gave a more uniform distribution pattern than placing the weevils on aluminum screening platforms suspended in similar cages. They dispersed by walking and occasionally by flying. After 17 days the adult weevils were killed with a 5 percent parathion spray and the cages were removed. Within three days the feeding cavities in each tree were counted, and for about five weeks the trees were checked every few days for symptoms of larval development. These included dying and dead needles, brown discoloration of the green or grey bark, and a spongy feel to the lower stem when squeezed slightly. By June 20 symptoms were common in all species, especially griffithii. The larvae had completely girdled many stems and had fed down into the root systems, killing the trees. Trees with larval symptoms were dug out with a trowel, and their bark was cut or pulled off so that the larvae could be counted.

RESULTS

The adult weevils fed at average rates of 0.9 to 2.1 cavities per day in the various cages. These were similar to rates of 1.0 to 2.0 on strobus, 2.0 to 3.5 on monticola, and 2.1 on various strobus hybrids when weevils were caged on leaders of large trees in other experiments; and also to rates of 0.7 to 4.7 when cut leaders of the same species were exposed in small cages (Plank and Gerhold, 1965). The insects fed along the entire length of the stem, but very commonly near the top of the previous year's internode, and seldom on the new growth. The feeding on individual trees within plots was highly variable, ranging from 0 to 223 cavities. Standard deviations were roughly proportional to and of about the same magnitude as plot means, indicating that the data probably comprise a Poisson or negative binomial distribution. No real differences were detected among cage totals, but surprisingly one replicate had only two-thirds as many feeding cavities as the other.[3] One replicate had feeding on 90 percent of the trees, the other on 75 percent. A 3 x 3 factorial analysis of variance of plot totals showed that there was a significant species effect and that the amount of feeding on species was influenced by the species associated with them (table 2). Duncan's Multiple Range Test revealed that the feeding on griffithii > monticola, and in the presence of griffithii the feeding on its associate was depressed. At the 5 percent level the interaction term is not quite significant, and strobus does not quite differ from the other two species; otherwise, the interpretation would change in several ways.

Table 2.--Weevil feeding in species mixtures.

Species	Associated species			Species average *
	P. strobus	P. monticola	P. griffithii	
	Cavities per seedling in 12-tree plots			
P. strobus	56	48	39	47
P. monticola	52	43	18	38
P. griffithii	53	74	44	57
Asso. species av.*	54	55	34	
Experiment av.				47

* Statistically significant at the 5 percent level.

No information was obtained about oviposition. Whether or not the number of eggs would be in the same proportion to the number of feeding cavities for each species is a matter of conjecture. The excavated insects were observed in several stages, most of them as larvae but also as pupae and adults. Their behavior seemed normal in all respects, except that their feeding progressed into the root system due to its proximity to the zone of oviposition. Because of the possibility of underground losses of larvae, it was deemed undesirable in this experiment to study pupal development and adult emergence.

In strobus 147 larvae (or pupae or adults) were counted, in monticola 91, and in griffithii 1,861. Of the 83 percent of trees that had feeding cavities, strobus had larvae in 26 percent, monticola in 47 percent, and griffithii in 80 percent. Nine of the latter had more larvae than feeding cavities. ,Table 3 gives the experimental results in percentages, however the statistical analysis was conducted using number of trees containing larvae per plot. Only the species effect is significant at the 5 percent level; however, the variance due to associated species is nearly significant and the F value for the interaction term is also fairly large. A comparison of the means shows that the number of trees with larvae in griffithii > monticola > strobus. Thus to a great extent the frequency of larvae was correlated with the amount of feeding, with the exception that strobus had fewer trees with larvae than might have been expected.

[3] We remembered that about half of the weevils in each cage came from the earlier collection date, and that in some of these insect containers there had been heavy mortality for reasons unknown; possibly the survivors from such containers fed at a lower rate, and these may have been placed in the replicate that had less feeding. Insect counts taken on May 24 and June 4 showed significant differences between dates but not between replicates.

Table 3.--Trees with larvae in species mixtures.

Species	Associated species			Species average *
	P. strobus	P. monticola	P. griffithii	
	Percent of seedlings containing larvae in 12-tree plots			
P. strobus	33	21	12	22
P. monticola	42	54	17	38
P. griffithii	75	62	71	69
Asso. species av.	50	46	33	
Experiment av.				42

* Statistically significant at the 5 percent level.

Multiple correlation and regression analyses of the seedling measurements were calculated on an IBM 7070-7074 digital computer at the Computation Center of The Pennsylvania State University. Simple correlations among tree characters and measures of insect attack are shown in table 4. Multiple regression with stepwise elimination of independent variables was used to determine the relative contribution of the various tree characteristics in explaining number of feeding cavities (table 5) and number of larvae (table 6). Stem diameter and leader diameter were the most important contributors to the regression sum of squares for number of feeding cavities and number of larvae, respectively,

DISCUSSION AND CONCLUSIONS

Our primary concern in the interpretation of these results is what can be learned about developing a method for testing resistance, rather than to evaluate the particular trees in this experiment. Therefore, we shall largely refrain from comparing individuals, provenances, or even species, and rather think of the species as being representative of entities that would be tested in a breeding program.

The most important practical question that cannot be answered now is how well these types of data can predict the number of times trees will be "weeviled" after they have been exposed in plantations. Thus it will be necessary to find out which measures of susceptibility have predictive value, under what conditions measurements should be made, and in what manner they should be expressed. For example, we have shown that mixing species can greatly alter measures of susceptibility. However, it is not entirely clear whether one white pine species will have the same effect on all others, or whether it may differ from species to species. Such interrelationships certainly need to be investigated further; until they are better understood, a minimal recommendation would be to test every species or hybrid separately and also in association with P. strobus, which is likely to be interplanted when a new variety is first released.

Inferences about relative resistance based on the evaluation of small trees should be made at this time only with caution, if at all. Resistance may change with age; for example, P.strobus might be expected to yield relatively more larvae as the leader diameter increases on older trees, Also, the number of feeding cavities might be only partly or not at all related to frequency of future weeviling. When weevils were caged on the leaders of 25 to 40 foot high trees near Maryland, N. Y., they made twice as many cavities in P. monticola as in P. strobus, contrasting with the results from exposing small trees. Furthermore, the number of cavities per tree was not at all closely correlated with the number of previous weevilings.

Table 4.--Simple correlations among tree characters and measures of insect attack.

	Species	Number of cavities	Number of larvae	Height (H)	Leader length (LL)	Leader diameter (LD)	Stem diameter (SD)	$(LD)^2 \times LL$	$(LD)^2 \times H$	$(SD)^2 \times LL$
Number of cavities	0.089									
Number of larvae	0.366*	0.346*								
Height (H)	-0.395*	0.202*	0.097							
Leader length (LL)	-0.384*	0.294*	0.028	0.866*						
Leader diameter (LD)	0.591*	0.400*	0.523*	0.257*	0.206*					
Stem diameter (SD)	0.408*	0.416*	0.511*	0.466*	0.408*	0.823*				
$(LD)^2 \times LL$	0.021	0.432*	0.279*	0.728*	0.836*	0.660*	0.711*			
$(LD)^2 \times H$	0.108	0.381*	0.404*	0.779*	0.679*	0.780*	0.803*	0.905*		
$(SD)^2 \times LL$	-0.140*	0.382*	0.215*	0.832*	0.920*	0.480*	0.698*	0.943*	0.846*	
$(SD)^2 \times H$	-0.087	0.321*	0.317*	0.899*	0.785*	0.558*	0.780*	0.850*	0.926*	0.917*

* Statistically significant at the 5 percent level.

There are three favorable indications that it may be feasible to screen small trees in cages for white pine weevil resistance. (1) The behavior of the insects during host finding and feeding was not atypical, so far as we could discern. (2) The weevils were able to discriminate among three species, in terms of both feeding and larval infestation. (3) Useful levels of selection appear to be possible. If the susceptibility of seedlings is directly related to that of older trees, individual tree selection in one year could remove 90 percent of the population on the basis of feeding, or 50 percent on the basis of larval infestation. A concurrent experiment (Soles and Gerhold, 1966) has shown that these figures can be manipulated by changing the insect population. Feeding in the fall is very similar to that in the spring, except that feeding is more dispersed over the tree, suggesting that trees could be exposed twice a year if the relationship between fall and spring feeding can be established experimentally. Under nursery conditions, trees could be exposed to feeding five times and twice to larval infestation over a two-year period.

Table 5.--Multiple regression of number of feeding cavities with seven seedling parameters listed in descending order of contribution to regression.[1]

Source of variation	df	≤ squares	Mean square	F ratio
Due to stem diameter	1	403.1	403.1	49.7*
Due to leader length, given stem diameter	1	63.4	63.4	7.8*
Due to $(SD)^2 \times H$, given stem diameter and leader length	1	73.1	73.1	9.0*
Due to leader diameter, given stem diameter, leader length, and $(SD)^2 \times H$	1	39.5	39.5	4.9*
Due to species, given stem diameter, leader length, $(SD)^2 \times H$ and leader diameter	1	61.0	61.0	7.5*
Due to $(LD)^2 \times LL$, given stem diameter, leader length, $(SD)^2 \times H$, leader diameter, and species	1	6.6	6.6	< 1 ns
Due to height, given stem diameter, leader length $(SD)^2 \times H$, leader diameter, species and $(LD)^2 \times LL$	1	0.4	0.4	< 1 ns
Total due to regression	7	647.1	92.4	11.4*
About regression	208	1686.8	8.1	
Total	215	2333.9		

Fraction of variance explained by the seven parameters 0.28
Multiple correlation coefficient 0.5266

1/ Number of larvae was omitted from the regression analysis for logical reasons. The variables (leader diameter)^2 × height and (stem diameter)^2 × leader length were eliminated by the computer program descending escalator option (backwards stepwise solution).

* Statistically significant at the 5 percent level.

Table 6.--Multiple regression of number of larvae with eight seedling parameters listed in descending order of contribution to regression.[1]

Source of variation	df	≤ squares	Mean square	F ratio
Due to leader diameter	1	194.4	194.4	84.4*
Due to number of cavities, given leader diameter	1	15.7	15.7	7.1*
Due to $(LD)^2 \times$ LL, given leader diameter, and number of cavities	1	11.8	11.8	5.4*
Due to $(SD)^2 \times$ H, given leader diameter, number of cavities, $(LD)^2 \times$ LL	1	21.4	21.4	9.7*
Due to height, given leader diameter, number of cavities, $(LD)^2 \times$ LL, and $(SD)^2 \times$ H	1	17.3	17.3	7.9*
Due to stem diameter, given leader diameter, number of cavities, $(LD)^2 \times$ LL, $(SD)^2 \times$ H, and height	1	0.9	0.9	<1 ns
Due to species, given leader diameter, number of cavities, $(LD)^2 \times$ LL, $(SD)^2 \times$ H, height and stem diameter	1	0.8	0.8	<1 ns
Due to leader length, given leader diameter, number of cavities, $(LD)^2 \times$ LL, $(SD)^2 \times$ H, height, stem diameter, and species	1	0.4	0.4	<1 ns
Total due to regression	8	262.7	32.8	14.9*
About regression	207	447.1	2.2	
Total	215	709.8		

Fraction of variance explained by the eight parameters 0.37
Multiple correlation coefficient 0.6084

1/ The variables (leader diameter)$^2 \times$ height and (stem diameter)$^2 \times$ leader length were eliminated by the computer program descending escalator option (backwards stepwise solution).

* Statistically significant at the 5 percent level.

LITERATURE CITED

Beier-Peterson, B., and B. Soegaard. 1958. Studies on resistance to attacks of Chermes cooleyi (Gill) on Pseudotsuga taxifolia (Poir.) Britt. Det forstl. Forsgsv. Denmark 25: 37-45.

Callaham, R. Z. 1960. Observations on pine susceptibility to weevils. Pac. Southwest Forest & Range Exp. Sta,, Tech. Paper No. 51, 12 pp.

Connola, D. P. 1965. Summary report on study of resistance in eastern white pine to white-pine weevil attack in New York. N. Y. State Museum & Science Service, Albany, N. Y. Mimeo. report, 2 pp.

Gerhold, H. D., E. J. Schreiner, R. E, McDermott, and J. A. Winieski, eds. 1966. Breeding pest-resistant trees. Pergamon Press, Oxford. Discussions by Bedard, Callaham, Campbell, Connola, Gerhold, Goddard, Heimburger, Kulman, Plank, and Smith, pages 428-430, 487.

Plank, G. H., and H. D. Gerhold. 1965. Evaluating host resistance to the white-pine weevil using feeding preference tests. Ann. Ent, Soc. Amer. 58 (4): 527-532.

Smith, R H. 1960 Resistance of pines to the pine reproduction weevil, _Cylindrocoptorus eatoni._ J. Econ. Ent. 53 (6): 1044-1048.

Soles, R. L. 1967. Laboratory investigations of the vapor and contact repellency of terpenes to the adult white pine weevil, _Pissodes strobi_ Peck, Ph.D. Thesis, Pennsylvania State University.

_____, and H. Do Gerhold. (1966 ms). White-pine seedlings in cages attacked by white-pine weevil, _Pissodes strobi_ Peck. (Coleoptera: Curculionidae), at five population levels. ms submitted to the Annals of the Entomological Society of America.