

with heavier rates of nitrogen was higher than normally found.

*Yields.*—The yield data show differences among all rates of nitrogen and between treated and untreated plots. The yield increases were approximately linear with each increase in rate of nitrogen in both the treated and untreated plots regardless of the fact that the larval population increased in the untreated and remained approximately the same in the treated. Since the interaction, treatments  $\times$  rates of nitrogen, was not significant, the increase in yields from the seed treatment was the same regardless of the amount of nitrogen applied. These data also indicate that yield losses may not be accurately predicted from larval populations alone.

*DISCUSSION.*—Since there was no correlation between any two of the four measurements taken, it was not possible to isolate the factor or factors responsible for the in-

crease in number of larvae with increase of nitrogen. The data on number and total length of feeding scars would indicate that palatability was not a factor affecting adult activity. However, palatability should not be ruled out until data are obtained after a longer feeding period. Since the female rice water weevil oviposits in or around the root of the rice plant, it is possible that the plants in plots receiving higher rates of nitrogen had larger roots and thereby provided a more favorable oviposition site.

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## Toxicity of Pine Resin Vapors to Three Species of *Dendroctonus* Bark Beetles

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#### ABSTRACT

The vapor toxicity of saturated resin vapors of both host and non-host pines was determined for three species of *Dendroctonus*: *D. brevicomis* Le Conte, *D. monticolae* Hopkins, and *D. jeffreyi* Hopkins. Results with hard pines substantiate the hypothesis that bark beetles of this genus can tolerate saturated vapors of host resin but not of non-host resin, suggesting that resin is a determining factor in host specificity. Results with soft pines do not substantiate the hypothesis, suggesting that other properties of resin or non-resinous characteristics of these pines determine

host specificity. A delayed effect in many tests with hard pine host resin suggests that even host resins can be deleterious under certain conditions.

Variable results were obtained with hybrid pines. Resin vapors of non-host  $\times$  non-host hybrids were toxic. *D. brevicomis* and *D. jeffreyi* were usually significantly affected by non-host  $\times$  host hybrid resin vapors while *D. monticolae* was not.

The overall results suggest that resin in some capacity may be an important factor in host resistance.

Pine resin has been considered an important factor in the success or failure of the attacks of tree-killing bark beetles and, therefore, in the resistance of pines to these insects. Toxicity of resin is one way in which beetles might encounter resistance. Though resin may be toxic in various ways, the vapor state was the most readily studied. Recent research (Smith 1961b) indicates a close association between the vapor toxicity of pine resins to *Dendroctonus* and generally accepted host relationships. This association may be stated as a hypothesis: bark beetles of the genus *Dendroctonus* can tolerate saturated resin vapors of host pines but not those of nonhost pines. This paper reports on experiments which expand on earlier studies to determine the validity of the hypothesis.

These studies are part of a project investigating the resistance of pines to bark beetles. They seek a better understanding of the relationship between bark beetles and pines and a definition of how the trees resist insect attacks so that improvement can be made in the system for (a) rating the risk of bark beetle attack on ponderosa pines, (b) selecting superior phenotypes for breeding for resistance, and (c) assessing bark beetle resistance of pine species and hybrids at an early age.

In the United States speculation about the relationship

of pine resin to bark beetles dates back to Hopkins (1902) Since then there have been scattered efforts to determine the nature and the extent of this relationship, and these efforts have been briefly reviewed by the author (1961b, c). Since these articles Vité & Wood (1961) have reported a series of tests in which low oleoresin exudation pressure was associated with the incidence of success of *D. brevicomis* LeConte and *D. monticolae* Hopkins attacks on second-growth ponderosa pine.

*MATERIALS AND METHODS.*—This work covers three species of *Dendroctonus*—*D. jeffreyi* Hopkins, *D. brevicomis* LeConte, and *D. monticolae* Hopkins, respectively the Jeffrey pine, western pine, and mountain pine beetle. Pine species and pine hybrids were selected at the Institute of Forest Genetics in Placerville, California, to include hosts and nonhosts of each beetle (Table 1).

The univoltine Jeffrey pine beetle is monophagous on Jeffrey pine. The multivoltine western pine beetle is oligophagous on ponderosa and Coulter pines. The univoltine mountain pine beetle is polyphagous on sugar, western white, ponderosa, Coulter, and lodgepole pines.

The three species of beetles were reared from naturally

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Table 1.—Species and hybrids of *Pinus* used as sources of resin.

SCIENTIFIC NAME	COMMON NAME
<i>Hard pines</i>	
<i>Pinus ponderosa</i> Laws	Ponderosa pine
<i>P. jeffreyi</i> Grev. and Balf.	Jeffrey pine
<i>P. coulteri</i> D. Don	Coulter pine
<i>P. sabiniana</i> Dougl.	Digger pine
<i>P. radiata</i> D. Don	Monterey pine
<i>P. jeffreyi</i> × <i>ponderosa</i>	Jeffrey × ponderosa pine
<i>P. jeffreyi</i> × ( <i>jeffreyi</i> × <i>coulteri</i> )	Jeffrey × (Jeffrey × Coulter) pine
<i>P. attenuata</i> × <i>radiata</i> , Stockwell and Righter	Knobcone × Monterey pine
<i>Soft pines</i>	
<i>P. lambertiana</i> Dougl.	Sugar pine
<i>P. monticola</i> Dougl.	Western white pine

infested material gathered from the forest of the central Sierra Nevada and held at the Institute. Ponderosa pine was the host brood material for both the western pine beetle and mountain pine beetle, and Jeffrey pine was the brood source for the Jeffrey pine beetle. The material was obtained when the brood had developed to near maturity, and put either directly into a screened outdoor insectary for rearing the adults or placed in a 35° F. coldroom until ready for use in the insectary. Thus, the beetles were allowed to mature and emerge under the prevailing summer weather at the Institute.

Satisfactory emergence was obtained with both the mountain pine beetle and western pine beetle. Emergence of the Jeffrey pine beetle was quite unsatisfactory, probably because of desiccation of the brood material. Emerged beetles were collected individually from the insectary walls in #000 gelatin capsules twice daily and kept at 35° F. until ready for use in tests. Collected beetles were held for less than 3 days, except for some tests with the Jeffrey pine beetle.

Fresh resin was obtained by a microtapping technique from trees growing at the Institute (Smith 1961a). Trees were tapped 6 to 48 hours before a test was established so that enough resin could be collected when the test began. All resin of a pine species or hybrid came from the same tree.

Mature beetles were exposed to an atmosphere saturated with resin vapors. The beetles were placed in individual cells in a 150-cc. screw-cap jar to which was then added a small vial containing freshly collected resin. The amount of resin in a vial, usually 200 to 300 mg., was enough to insure saturation by vapors of the atmosphere in the jar. Two or more samples of each resin in each test were weighed to determine the loss of weight during the time insects were in the chamber. Weighings were made to 0.1 mg. Weight loss was regarded as the weight of resin which had vaporized into the chamber. Each replicate consisted of 10 to 12 beetles, and 3 to 7 replicates were used for each test material in an experiment. Beetles were equitably distributed by size among all replicates of an experiment; replicates were randomly assigned to treatments. An untreated control group, included in each experiment, consisted of a set of replicates in capped chambers without any resinous test material.

All assembled tests were held at 73° ± 2° F. for a specified number of days. At the end of this treatment period the chambers were uncapped and the cells removed. Beetles were observed for mortality and those which did not move when agitated were considered dead. The basic figure used in analysis was the number of dead beetles in each replicate. After this first examination all beetles were held within their cells at 73° ± 2° F. in a non-resinous atmosphere and observed at 2-day intervals for subsequent mortality.

The mortality data for each set of observations were treated as an entity for analysis. That is, the examination after a treatment was considered one set of observations; the examination 2 days later was considered another set. The numbers of dead beetles in treatments and replicates were arranged in the block order which existed during the test and F-values calculated for each set of observations (Snedecor 1946, p. 218).

Duncan's (1955) multiple-range testing was applied to each set of observations having a treatment F-value greater than F at the 90% confidence level to determine treatments which differed significantly at the 95% confidence level. Mortality was converted to percentages and the results arranged in tabular form with notations of treatments causing a significantly greater mortality than the untreated control.

In discussing the results, treatments which caused a significant increase in mortality are considered toxic; those causing a nonsignificant increase are considered nontoxic.

**RESULTS.**—*Dendroctonus monticolae*.—Tests with mountain pine beetle included resin of hosts (ponderosa, Coulter, sugar, and western white pines); nonhosts (Jeffrey, Monterey, and Digger pines); and hybrids of Jeffrey × ponderosa and of knobcone × Monterey pines (Table 2).

Jeffrey pine resin vapor was toxic in the 3-, 5-, and 7-day treatments. Resin vapors of the three host pines and the Jeffrey × ponderosa pine hybrid were nontoxic during these treatment periods. Host resins tended to produce a delayed effect, evidenced by a significant increase in mortality during the posttreatment periods. Perhaps even host resins in sufficient quantities for extended periods may adversely affect beetles. This posttreatment

Table 2.—Mortality of adult *Dendroctonus monticolae* held in a saturated atmosphere of different pine resin vapors for varying periods.

PINE SPECIES	EXPERIMENT <sup>1</sup> AND DAYS EXPOSURE						
	a 3	b 5	c 7	d 5	e 5	f 7	g 7
	Percent mortality <sup>2</sup>						
Control	3	12	27	5	23	23	32
Coulter	3	18	25				
Ponderosa	5	10	38				
Sugar	10	18	42				
Jeffrey × ponderosa #1	8	18	28				
Jeffrey	53*	98*	95*				
Western white				6			
Monterey				8	23	57*	
Knobcone × Monterey					20	63*	
Digger					33*	100*	
Jeffrey × ponderosa #2							43
Jeffrey × ponderosa #3							35

<sup>1</sup> Six, 10-beetle replicates for a, b, c, d, g; three for e, f.

<sup>2</sup> \*Indicates mortality significantly greater than control group at the 95% confidence level.

effect became more severe with increased periods of treatment.

Monterey pine resin vapor was nontoxic in a 5-day treatment, but had a delayed effect by causing a significant increase in mortality during the posttreatment period. In a 7-day treatment, resin vapors of Monterey and Digger pines and the knobcone×Monterey hybrid were toxic. The effect of Digger pine resembled that of Jeffrey pine. These two pines have similar volatile constituents (Mirov 1961). Western white pine was nontoxic in a 5-day treatment or in the posttreatment periods.

No significant differences were obtained with three different Jeffrey×ponderosa hybrid trees. Two of those (#1 and #2) have the same ponderosa male parent but different Jeffrey female parents. Thus, though the nonhost Jeffrey pine resin vapor is toxic to this beetle, its effect is apparently "diluted" to a sublethal level in the Jeffrey×ponderosa hybrid. In tests with this hybrid resin a partial paralysis of the beetle was noted. This effect was not reflected in mortality and, therefore, could not be measured.

The uniform quality of the beetle population is expressed by the mortality of the controls. In three tests, covering a 1-month period, mortality was 23, 27, and 32% in the 7-day treatment.

Results substantiate the hypothesis that *Dendroctonus* bark beetle can tolerate saturated resin vapors of host trees, but cannot tolerate those of nonhosts.

*Dendroctonus jeffreyi*.—The same resins used with the mountain pine beetle were used with the Jeffrey pine beetle. Except for size, these two species of beetles are practically indistinguishable morphologically. Jeffrey

Table 3.—Mortality of adult *Dendroctonus jeffreyi* held in a saturated atmosphere of different pine resin vapors for 5 days.

PINE SPECIES	EXPERIMENT <sup>1</sup>			
	a	b	c	d
Control	35	Percent mortality <sup>2</sup> 25 23		37
Jeffrey	32			
Jeffrey×ponderosa #1	40		30	
Sugar	50	53*		
Coulter	62*			
Ponderosa	67*			
Digger				22
Western white				32
Monterey				60*
Knobcone×Monterey				72*
Jeffrey×ponderosa #2			40	

<sup>1</sup> Six, 10-beetle replicates for a, b, d; three for c.

<sup>2</sup> \*Indicates mortality significantly greater than control group at the 95% confidence level.

pine is the sole host of the Jeffrey pine beetle. The poor emergence of beetles from the brood material prevented completely parallel tests.

Jeffrey pine resin vapor was nontoxic (Table 3). Both Coulter and ponderosa pine resin vapors were toxic in the treatment period. This level of toxicity was maintained in posttreatment periods. Resin vapors of sugar pine and the Jeffrey×ponderosa hybrid were nontoxic during the 5-day exposure period, but a delayed effect became evident during the 2-day posttreatment period. In repeat tests, sugar pine resin vapor was toxic, and the hybrid was nontoxic. In this same test, no difference could be found between two hybrid individuals having different female Jeffrey pine parents. However, both hybrids showed a significant delayed effect.

Resin vapors of Monterey pine and the knobcone×Monterey hybrid were toxic in the treatment period (Table 3), and their toxicity was maintained during the posttreatment periods.

Digger pine resin vapors were not toxic during the treatment period and no delayed effect was evident. In this respect the insect responded similarly to both Jeffrey and Digger pine resins which have similar volatile constituents. Why Jeffrey pine beetle is not found on Digger pine is problematical. Digger pine occurs at a much lower elevation than the beetle. Perhaps the beetle cannot survive in the hot, dry climate of low elevations where Digger pine grows. Difficulties in rearing the beetle at low elevations lend support to this explanation. Also there may be effects of resin on the beetles which cannot be measured by the technique used in these studies.

Thus, except for Digger pine, results with the hard pine resins and Jeffrey pine beetle support the stated hypothesis. Results with both sugar pine and western white pine indicate the uncertainty of applying the hypothesis to soft pines. In general, the beetle could not tolerate hybrid pine resin vapors as well as it tolerated host resin vapors.

*Dendroctonus brevicomis*.—Tests somewhat parallel to those described above were made with western pine beetles, using ponderosa and Coulter pines as host resins. Jeffrey pine resin vapor caused 100% mortality in all treatment periods (Table 4). Ponderosa and sugar pine

PINE SPECIES	RESIN VAPOR SATUR.	<i>Dendroctonus</i>		
		<i>monticola</i>	<i>jeffreyi</i>	<i>brevicomis</i>
HARD PINES	mg.			
ponderosa	2.6	□○	●●	□○
Coulter	2.1	□○	●●	□○
Jeffrey	19.7	●●	□○	●●
Monterey	5.4	●●	●●	●●
Digger	20.4	●●	□○	●●
SOFT PINES				
sugar	2.3	□○	●○*	●○
western white	4.2	□○	●○*	●○
HYBRID PINES <sup>o</sup>				
Jeffrey x ponderosa	8.3	●○	●○*	●○*
Jeffrey x (Jeffrey x Coulter)	14.2	■	■	●●
knobcone x Monterey	6.1	●●	●●	●●

- Ecological host      ○ Resin vapor non-toxic
- Ecological non-host      ● Resin vapor toxic

<sup>o</sup> Hybrids are considered non-hosts if one parent is a non-host

\* Significant toxicity was achieved in some tests but not in others

FIG. 1.—Insect-host relationships between pines and three *Dendroctonus* species, based on host records and toxicity of saturated resin vapors.

Table 4.—Mortality of adult *Dendroctonus brevicomis* held in a saturated atmosphere of different pine resin vapors for varying periods of exposure.

RESIN	EXPERIMENT <sup>1</sup> AND DAYS EXPOSURE										
	a 3	b 5	c 7	d 5	e 5	f 5	g 5	h 5	i 5	j 5	
					<i>Percent mortality</i> <sup>2</sup>						
Control	4	26	51	11	42	18	17	11	36	21	
Ponderosa	7	29	65	31	56						
Sugar	4	24	63	13	60		12	14			
Coulter	11*	39	47	17	54						
Jeffrey × ponderosa #1	15*	31	76*	17	79*	65*					
Jeffrey × ponderosa #2					67*	52*				36*	
Jeffrey × ponderosa #3				25							
Jeffrey	100*	100*	100*								
Western white									33		
Monterey									54*		
Knobcone × Monterey									57*		
Jeffrey × (Jeffrey × Coulter)										100*	
Digger									100*		

<sup>1</sup> Six 12-beetle replicates for a, b, c, d, e; three for h, i, j; five for f; and seven 10-beetle replicates for g.

<sup>2</sup> \* Indicates mortality significantly greater than control group at the 95% confidence level.

resin vapors were nontoxic in the 3-, 5-, or 7-day treatments. Coulter pine resin vapor was toxic in the 3-day treatment but nontoxic in the 5- or 7-day treatment. All three resin vapors produced a significant delayed effect which became evident during the posttreatment periods, particularly with the longer time of treatment.

Variable results were achieved with Jeffrey × ponderosa #1. Some evidence of toxicity occurred in four different tests during the 3-, 5-, and 7-day treatments. No toxicity was found during two different 5-day periods. Posttreatment effects somewhat paralleled those produced during the treatment periods. In one test the resin vapors of two different Jeffrey × ponderosa hybrids were toxic in a 5-day treatment; and they also differed significantly from each other at the end of the treatment period. This variation suggests a difference between the two different Jeffrey parents.

The resin vapor of the Jeffrey × (Jeffrey × Coulter) hybrid—a backcross of a natural hybrid (Jeffrey × Coulter) to Jeffrey—caused 100% mortality in a 5-day treatment. Therefore, this hybrid, as might be expected, has more of the properties of Jeffrey than of Coulter pine.

Monterey and Digger pines and the knobcone × Monterey hybrid caused a significant increase in mortality in a 5-day treatment. Here again Digger pine resin vapor produced an effect similar to Jeffrey pine. Western white pine, like sugar pine, caused no significant effect.

Therefore, the hypothesis holds for this beetle using hard pines, but does not hold when soft pines are used. Since this effect to some extent was true for the Jeffrey pine beetle, it would seem that the hypothesis does not hold for soft pines.

CONCLUSIONS.—An attempt has been made to illustrate associations of the three species of *Dendroctonus* and the pine tested both on the basis of vapor toxicity studies and on the generally recognized ecological relationship (Fig. 1). This chart shows that for the hard pines the hypothesis that bark beetles can tolerate saturated resin vapors of host pines but not those of nonhost pines is supported in

14 out of 15 cases. This finding suggests that resin quality affects the resistance of hard pine to bark beetles.

The hypothesis is not supported by data for the soft pines. This fact suggests that resins of soft pines are quite different from those of hard pines or that, if resin quality is a factor in host resistance of soft pines, a different testing procedure is required to detect and demonstrate it.

The frequent occurrence of a delayed effect becoming evident during posttreatment period suggests that under certain, as yet unexplained, conditions even host resins may cause a harmful effect.

Two nonhost pines transmitted a toxic quality to the hybrid of the two. The variable results achieved with host × nonhost pine hybrids suggest that in the hybrid the toxic property of the nonhost pine may or may not be "diluted" to a nontoxic level by the host pine.

The manner in which resin actually functions in enabling a tree to resist beetle attack cannot be adequately explained by the results of vapor toxicity studies completed to date. Both death and inhibiting paralysis of the beetle are strongly suggested. Other possibilities, not investigated in this study, are that resin induces abnormal behavior after the beetle reaches the tree or repels the beetle before or after it reaches the tree.

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## Further Evaluation of Animal Systemic Insecticides, 1962<sup>1</sup>

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### ABSTRACT

Results are reported for 13 systemically active insecticides used in screening tests and in initial practical tests against cattle grubs (*Hypoderma* spp.). Results of additional tests against cattle grubs with eight compounds about which preliminary data were reported in 1960 or 1962 also are presented. Treatments highly effective against cattle grubs were: American Cyanamid CL-38023, *O,O*-dimethyl *O-p*-(dimethylsulfamoyl)phenyl phosphorothioate, 10 mg./kg./day for 10 days in feed or 20 mg./kg. intramuscular injection; Bayer 29493, *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate, 2.5 mg./kg./day for 10 days in feed or 5 mg./kg. intramuscular injection; Bayer 37341, *O,O*-diethyl *O*-4-(methylthio)-3,5-xylyl phosphorothioate, 10 and 50 mg./kg. drench, 5 mg./kg./day for 10 days in feed, or pour-on of 250 ml. of 2% in water; Bayer 37342, *O,O*-dimethyl *O*-4-(methylthio)-3,5-xylyl phosphorothioate, 10 mg./kg./day for 6 days in feed, 15 mg./kg. intramuscular injection, or pour-on of 125 ml. of 2% in oil or water; butonate, 5 or 10 mg./kg./day for 10 days in feed; Rhodia RP-9895, 2.5 mg./kg./day for 10 days in feed; Stauffer R-1504, *O,O*-dimethyl *S*-phthalimidomethyl phosphorodithioate, 2.5 mg./kg./day for 10 days in feed, 0.5% spray, or pour-on of 250 ml. of 2% in oil; Stauffer R-3352, *S*-diphenylmethyl *O,O*-diethyl phosphorodithioate, 25 mg./kg. by capsule; and Stauffer R-3828, *S*-(*p*-chlorophenyl)phenylmethyl *O,O*-diethyl phosphorodithioate, 100 mg./kg. by capsule.

A continuing research program to screen compounds for activity as animal systemic insecticides is carried on at the Kerrville, Texas, laboratory. Compounds active in screening tests are administered to small groups of cattle to determine systemic effectiveness against common and northern cattle grubs, *Hypoderma lineatum* (de Villers) and *H. bovis* (L.), respectively. Tests with 18 systemically active insecticides were reported in 1960 (Drummond 1960). Further tests with 8 of these compounds and initial results with 25 other insecticides were reported in 1962 (Drummond 1962). This paper presents the results of further tests against cattle grubs with 8 of the compounds reported in 1960 and/or 1962, as well as results in screening tests and in preliminary tests against cattle grubs with 13 additional systemically active compounds.

The 21 compounds tested are as follows; the first 8 are those for which initial tests were reported in 1960 and/or 1962:

American Cyanamid CL-38023, *O,O*-dimethyl *O-p*-(dimethylsulfamoyl)phenyl phosphorothioate

Table 1.—Systemic effectiveness of 13 compounds against screw-worms, stable flies, and ticks, when administered orally and subcutaneously to guinea pigs. Highest dosage given, 100 mg./kg.<sup>1</sup>

COMPOUND	METHOD OF ADMINISTRATION	LOWEST DOSAGE (MG./KG.)			
		Lethal to Guinea Pigs	Causing 100% Kill of Screw-Worms	Stable Flies	Ticks
Bayer 25316	oral	N	N	N	N
	s. cut.	100	100	50	N
Bayer 26405	oral	N	25	100	N
	s. cut.	100	N	N	N
Bayer 39193	oral	N	25	N	N
	s. cut.	100	50	N	N
Bayer 42600	oral	100	10	50	N
	s. cut.	100	25	25	N
Grace CPE	oral	N	100	N	N
	s. cut.	50	100	N	N
Hercules 7522-H	oral	50	50	50	N
	s. cut.	100	100	N	100
Rhodia RP-9895	oral	N	50	100	25
	s. cut.	N	N	N	50
Shell SD-1843	oral	N	N	N <sup>2</sup>	N
	s. cut.	100	N	N <sup>2</sup>	N
Stauffer N-3049	oral	50	25	50	N
	s. cut.	N	N	N	N
Stauffer R-3352	oral	N	100	100	N
	s. cut.	N	N	N	N
Stauffer R-3422	oral	N	50	50	N
	s. cut.	100	100	100	N
Stauffer R-3828	oral	N	N	N <sup>2</sup>	N
	s. cut.	N	N	N	N
Stauffer R-3829	oral	N	100	100	N
	s. cut.	N	N	N	N

<sup>1</sup> N indicates no dosage given was lethal to guinea pigs, or no dosage given caused 100% kill of arthropods.

<sup>2</sup> High, but not 100% kill of stable flies at 100 mg./kg.