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Boron Chemosterilants Against Screw-Worm Flies:[1,2,3] Structure-Activity Relationship

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ABSTRACT

Organoboron compounds that hydrolyzed readily to boric acid were effective chemosterilants of *Cochliomyia hominivorax* (Coquerel) and the activity was generally proportional to their boron content. However, this relationship was completely valid only with alkoxyboranes that hydrolyze almost instantaneously; aminoboranes and derivatives of boronic acids are more resistant to hydrolysis, and their effectiveness as chemosterilants could not be directly correlated with their content of boron. In all, 83 boron compounds were administered orally to both sexes of screw-worm flies, and their effects on mortality, oviposition, and hatch were determined.

Recent studies of the toxicology and pharmacology of organic derivatives of boron (Soloway 1964) indicate a broad spectrum of biological activity of the compounds in various organisms ranging from plants (Lee and Aronoff 1967) to animals (Zimmer et al. 1967). However, the effects of boron compounds on fertility have not been reported previously. In our continuing search for new insect chemosterilants (Bořkovec 1966), we detected indications of sterilizing activity in several esters of boric acid and in 2-phenyl-1,3,2-benzodiazaborole (Bořkovec and Settepani 1968). Subsequently, 4 series of structurally related boron compounds were tested as sterilants in the screw-worm fly, *Cochliomyia hominivorax* (Coquerel). These compounds (Table 1) were classified according to the groups attached to boron:

1. Alkoxy- and aryloxyboranes,
2. Aminoboranes,
3. Alkyl- and arylboronic acids and their derivatives,
4. Miscellaneous inorganic and organic derivatives of boron.

The nomenclature of boron compounds is complex; thus, for simplicity, only the generic names (followed by numbers referring to structures shown in Table 1) are used in this report.

MATERIALS AND METHODS.—All compounds listed in Table 1 have been described in the chemical literature. Those that were not available commercially or from industrial and academic sources were synthesized in our laboratory according to published procedures.

Candidate chemosterilants were screened orally against laboratory-reared adult screw-worm flies less than 24 hr old by allowing groups of 75 or 100 caged flies of both sexes to feed freely for 5 days on 1% of a compound in sugar syrup (saturated aqueous solution of sucrose) spread over the gauze roof of the cage. When this concentration was lethal or highly toxic, effects on fertility were assessed at the highest concentration that permitted adequate survival. Mortality, egg production, and hatchability of eggs were determined at 7 days posttreatment. Oviposition and hatch were estimated visually as normal (N), reduced (R), or none (O). These estimates of the extent of oviposition relate only to the size of egg masses and not to the number of females ovipositing. Tests in which oviposition or hatch was inhibited were replicated once. The data shown in Table 1 are not corrected for the results obtained with controls for each test series.

The data in Tables 2–4 were obtained similarly except that the medicated sugar syrup was prepared fresh daily (Tables 2 and 3), or the period of treatment was limited to 1 day (Table 4). Eggs were separated in a 1% aqueous solution of sodium hydroxide and allowed to settle in a graduated centrifuge tube; then the total number was estimated from the volume of the compacted mass (based on 1140 eggs/0.1 ml). About 100 eggs from each treatment were transferred to moist filter paper in each of 2 petri dishes, and the percentage hatch was calculated from the pre- and posthatch tallies. Percentage sterility was defined as $100(1\text{-}fh)$, where f and h were the corrected decimal fractions of the percentages of fecundity and egg hatch, respectively. These data were then corrected on the basis of the results obtained with controls by the formula:

[1] Diptera: Calliphoridae.
[2] Accepted for publication October 22, 1968.
[3] Mention of proprietary product or company does not necessarily imply endorsement by the USDA.
[4] Mission, Tex. 78572.

Table 1.—Effect of boron compounds on the fertility of screw-worm flies. Adult flies of both sexes were fed sugar syrup containing the indicated concentrations of candidate compounds for 5 days.

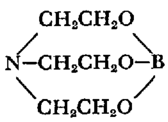
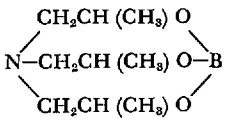
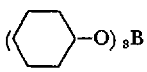
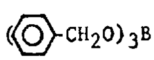
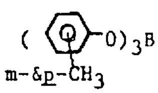
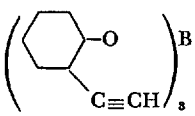
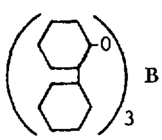
Compound			H_3BO_3/ com- pound	Concn (%)	Mor- tality (%)	Ovi- posi- tion[b]	Hatch[b]
No.	Structure	Source[a]					
	Alkoxy- and aryloxyboranes						
1	$(CH_3O)_3B$	A	0.60	1.0	69	R	O
2	$(C_2H_5O)_3B$	A	.43	1.0	71	R	O
3	$(C_3H_7O)_3B$	A	.40	1.0	53	N	O
4		B	.33	1.0	47	N	O
5	$[(CH_3)_2CHO]_3B$	A	.33	1.0	52	N	O
6	$(NH_2CH_2CH_2O)_3B$	B	.32	1.0	55	N	O
7		A	.31	1.0	57	N	R
8	$(C_4H_9O)_3B$	A	.27	1.0	43	N	R
9	$[(CH_3)_2CHCH_2O]_3B$	A	.27	1.0	53	R	O
10	$[CH_3CH_2CH(CH_3)O]_3B$	C	.27	1.0	53	N	R
11	$[(CH_3)_3CO]_3B$	C	.27	1.0	54	N	R
12	$(ClCH_2CH_2O)_3B$	B	.25	1.0	79	N	R
13	$(C_5H_{11}O)_3B$	A	.23	1.0	45	N	R
14	$[(C_2H_5)_2CHO]_3B$	C	.23	1.0	39	N	R
15	$[C_2H_5C(CH_3)_2O]_3B$	C	.23	1.0	51	N	N
16	$(C_6H_5O)_3B$	C	.21	1.0	28	N	R
17	$(C_6H_{13}O)_3B$	A	.20	1.0	40	N	R
18		A	.20	1.0	29	N	N
19		A	.19	1.0	56	N	R
20		B	.19	1.0	48	N	R
21	$(C_8H_{17}O)_3B$	A	.16	1.0	57	N	N
22		B	.16	1.0	69	N	R
23	$[(CH_3)_2C(NO_2)CH_2O]_3B$	B	.16	1.0	73	N	R
24	$(C_{10}H_{21}O)_3B$	A	.14	1.0	66	N	N
25		A	.13	1.0	39	N	N

Table 1.—(Continued)

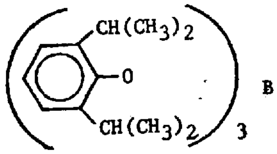
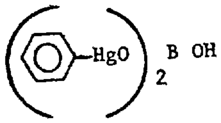
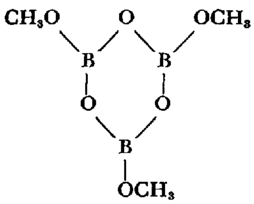
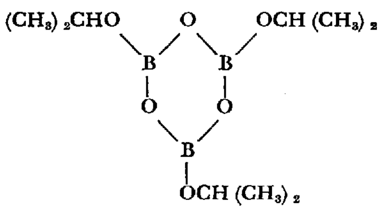
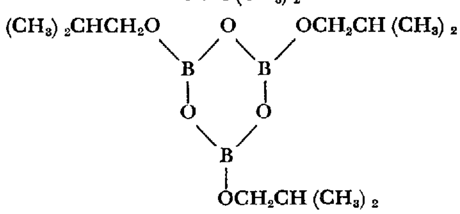
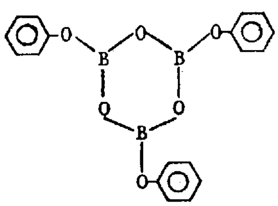
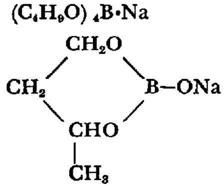
Compound			H_3BO_3/ compound	Concn (%)	Mortality (%)	Oviposition[b]	Hatch[b]
No.	Structure	Source[a]					
26		B	.11	1.0	51	N	N
27		A	.07	.1	59	N	N
28		B	1.07	.5	59	R	O
29		B	.72	1.0	80	R	O
30		B	.62	.5	53	N	R
31		B	.52	.5	60	R	O
32	$2[(CH_3)_2CHO]_3B\cdot3B_2O_3$	B	.84	.5	67	R	O
33	$2(CH_3O)_3B\cdot3B_2O_3$	B	1.19	.1	44	N	R
34	$(CH_3O)_3HB\cdot Na$	A	.48	1.0	65	R	O
35	$(CH_3O)_4B\cdot Na$	C	.39	1.0	70	N	O
36	$(C_2H_5O)_4B\cdot Na$	C	.29	1.0	81	N	O
37	$(C_4H_9O)_4B\cdot Na$	C	.19	1.0	49	N	R
38		B	.47	1.0	45	N	R

Table 1.—(Continued)

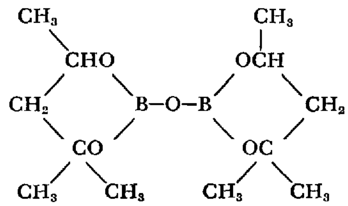
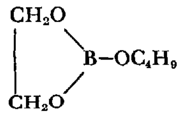
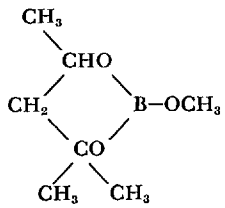
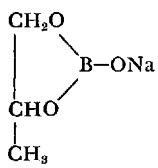
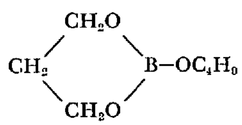
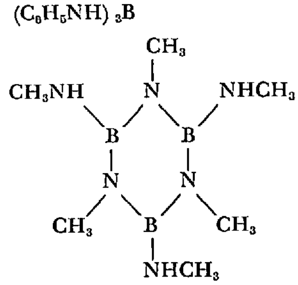
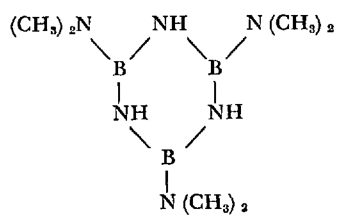
Compound			H_3BO_3/ com- pound	Concn (%)	Mor- tality (%)	Ovi- posi- tion[b]	Hatch[b]
No.	Structure	Source[a]					
39		D	.44	1.0	73	R	R
40		B	.43	1.0	85	O	
41		B	.39	1.0	83	N	R
42		B	.50	.5	55	N	R
43		B	.39	.5	59	N	R
44	$[(CH_3COO)_2B]_2O$	C	.59	1.0	83	R	O
		Aminoboranes					
45	$[(CH_3)_2N]_3B$	C	.44	1.0	68	N	O
46	$[(CH_3)_2CHNH]_3B$	C	.34	1.0	50	N	O
47	$[(C_2H_5)_2N]_3B$	C	.28	1.0	89	N	N
48	$(C_6H_5NH)_3B$	C	.22	1.0	74	N	R
49		C	.90	1.0	73	R	O
50		C	.90	1.0	53	R	R

Table 1.—(Continued)

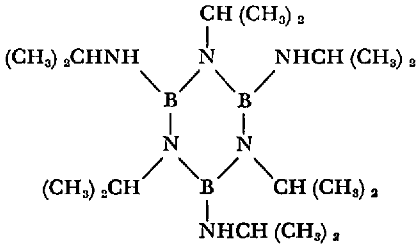
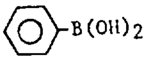
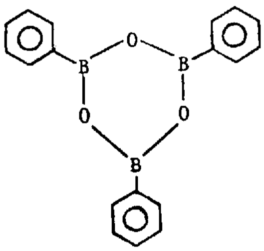
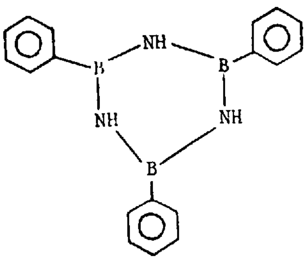
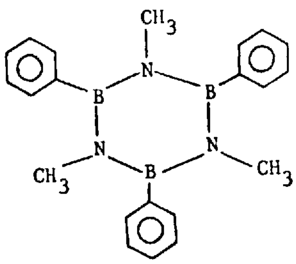
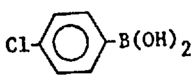
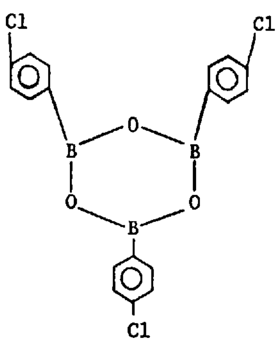
Compound			H_3BO_3/ compound	Concn (%)	Mortality (%)	Oviposition[b]	Hatch[b]
No.	Structure	Source[a]					
51		B	.48	1.0	33	N	R
	Alkyl- and arylboronic acids and their derivatives						
52		B		.1	75	N	N
53		C		.05	64	N	R
54		B		1.0	76	N	R
55		B		1.0	88	N	N
56		E		.1	76	N	N
57		E		.05	35	N	R

Table 1.—(Continued)

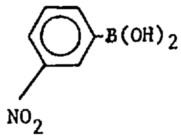
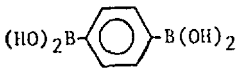
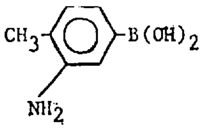
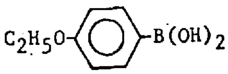
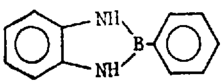
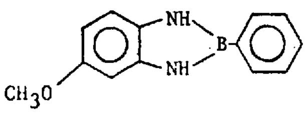
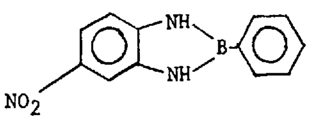
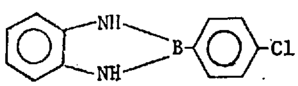
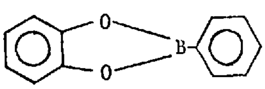
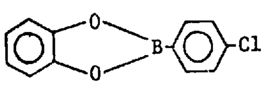
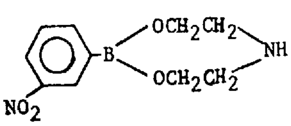
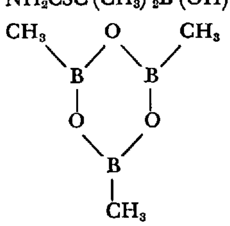
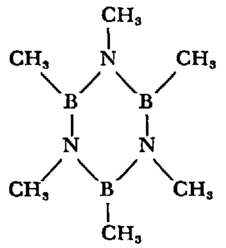
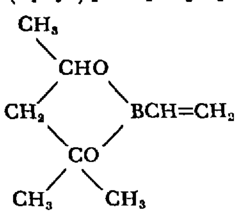
Compound			H_3BO_3/ compound	Concn (%)	Mortality (%)	Oviposition[b]	Hatch[b]
No.	Structure	Source[a]					
58		C		.05	86	R	R
59		F		1.0	88	R	O
60		F		1.0	84	N	R
61		F		1.0	73	N	R
62		A		1.0	23	N	R
63		C		1.0	30	N	N
64		C		1.0	25	N	N
65		C		1.0	48	N	N
66		C		.1	92	N	R
67		C		.1	65	N	O
68		F		.05	79	N	R
69	$NH_2C(=NH \cdot HBr)SC(CH_3)_2B(OH)_2$	G		1.0	63	R	O
70		A		.5	76	R	O

Table 1.—(Continued)

Compound			H_3BO_3/ com- pound	Concn (%)	Mor- tality (%)	Ovi- posi- tion[b]	Hatch[b]
No.	Structure	Source[a]					
71		B		1.0	51	N	N
72	$[(CH_3)_3CO]_2BCH_3$	G		.5	85	N	N
73	$(C_4H_9O)_2BC_4H_9$	G		1.0	62	N	R
74	$(C_4H_9O)_2BCH{=}CH_2$	G		.1	94	N	O
75	$(C_4H_9O)_2BCH_2CH_2SC_2H_5$	G		.1	68	N	R
76		B		.1	72	N	N
	Miscellaneous boron compounds						
77	H_3BO_3	A	1.00	1.0	95	O	
78	HBO_2	C	1.41	.5	79	R	O
79	B_2O_3	A	1.78	.05	65	N	N
80	$Na_2B_4O_7{\cdot}10H_2O$	A	0.65	1.0	87	O	
81	$(CH_3)_3N{:}BH_3$	A		1.0	75	R	R
82	$(C_2H_5)_3N{:}BH_3$	A		.5	42	N	N
83	$(CH_3)_3CNH_2{:}BH_3$	A		.1	55	N	N

[a] A, commercial; B, U.S. Borax Research Corp.; C, synthesized; D, The Standard Oil Co., Ohio; E, Midwest Research Institute; F, A. H. Soloway, Massachusetts General Hospital; G, D. S. Matteson, Washington State University.
[b] N, normal; R, reduced; O, none.

$$\text{Corrected \% effect} = \frac{\text{(experimental \% effect)}}{\text{(control \% effect)}}.$$

The data of Tables 2 and 4 are unreplicated; those of Table 3 are averages of 2 replicates.

RESULTS AND DISCUSSION.—*Alkoxy- and Aryloxyboranes.*—The hydrolytic stability of the boron-oxygen bond is generally low, but steric and electronic factors strongly influence the rate of hydrolysis of alkoxy- and aryloxyboranes. In aqueous medium, boric acid is always the ultimate product of solvolysis but its evolution may be only gradual when the alkyl or aryl groups shield the boron atom from attack by the molecules of water. In the oral treatment of screw-worm flies, the candidate compound was always administered in aqueous sugar syrup.

When any of the simple alkoxyboranes (*e.g.*, compounds 1–3) were added to the syrup, the mixture almost instantly contained boric acid and the appropriate alcohol rather than the original borane. Boric acid is the only hydrolytical product that compounds 1–3 have in common so if it were the active principle of the treated food, then the sterilizing activity of any easily hydrolyzable alkoxyborane should be proportional to the amount of liberated boric acid and not to the amount of the candidate compounds. The amount of boric acid produced by the complete hydrolysis of a unit weight of each hydrolyzable candidate compound is shown in the 4th column of Table 1. Clearly, when the ratio of boric acid to the compound dropped below 0.32, the flies treated with 1% of the sterilant were seldom completely sterilized; when the figure was lower than 0.2, the compound tended to be ineffective.

The test method used to obtain the results shown

Table 2.—Effect of boric acid, H_3BO_3, on the fertility of screw-worm flies. (Adults of both sexes were fed sugar syrup containing the indicated concentration of the compound for 5 days.)

H_3BO_3 (%)	Mortality (%)	Avg no. eggs/♀	Hatch (%)	Sterility[a] (%)
0.1	36	228	63	34
.2	44	131	11	93
.3	62	51	10	98
.4	72	0		100
.5	62	0		100
Control	30	276	79	0

[a] Percent sterility = 100 (1-*fh*), where *f* and *h* are the corrected decimal fractions of percent fecundity and egg hatch, respectively.

Table 3.—Effects of feeding screw-worm flies with sugar syrup containing organoboron compounds or equivalent amounts of the respective hydrolytical products. (Adults of both sexes were fed sugar syrup containing the indicated concentration of the compound for 5 days.)

Compound	Concn (%)	Mortality (%)	Avg no. eggs/♀	Hatch (%)	Sterility[a] (%)
$(CH_3O)_3B$	0.5	59	46	15	96
$H_3BO_3 + CH_3OH$		73	13	4	99
Control		49	222	90	0
$[(CH_3)_2N]_3B$	1.0	34	205	66	41
$(CH_3)_2NH + H_3BO_3$		40	57	14	97
$[(C_2H_5)_2N]_3B$	1.0	28	177	50	67
(C_2H_5) NH + H_3BO_3		35	86	28	90
Control		31	274	85	0

[a] Percent sterility = 100 (1-*fh*), where *f* and *h* are the corrected decimal fractions of percent fecundity and egg hatch, respectively.

in Table 1 is only semiquantitative, but the exceptionally low activity of cyclic compounds 38, 39, and 41 merits an explanation. In aqueous solutions, the hydrolysis of hindered glycol borates 38, 39, and 41 reaches equilibrium (Steinberg 1964); thus, only a part of the theoretically available boric acid may be utilized by the organism. The relative hydrolytical stability of compounds 38, 39, and 41 may well account for their low activity.

Direct evidence supporting the hypothesis that alkoxy- and aryloxyboranes were active because they hydrolyzed to boric acid was obtained in 2 series of experiments. In the 1st series (Table 2), when sugar syrup containing 0.1–0.5% of pure boric acid was administered to the flies and the experimental conditions were the same as in the previous trials, concentrations of boric acid lower than 0.3% produced only partial sterility, but higher concentrations produced complete sterility. In the 2nd series (Table 3), the activity of a solution of alkoxyborane 1 was compared with that of a solution containing equivalent amounts of the hydrolytical products of compound 1, *i.e.*, boric acid and methanol. The results (Table 3) showed that the effectiveness of both solutions as sterilants was the same.

Aminoboranes.—Table 1 shows the sterilizing activity of 7 aminoboranes (compounds 45–51). The hydrolytic stability of the boron-nitrogen bond in alkylaminoboranes (compounds 45–47) appears to be somewhat higher than that of the boron-oxygen bond in alkoxyboranes (Steinberg and Brotherton 1966), and the sterilizing activity of compounds 45–48 and the proportion of boric acid agreed well. However, a more detailed comparison of dialkylaminoboranes 45 and 47 with their respective hydrolytical products (Table 3) indicated an incomplete hydrolysis. The mixtures of boric acid and the amine were more active than the solutions of the aminoboranes 45 and 47.

Table 4.—Effect of boric acid, H_3BO_3, on the fertility of screw-worm flies. (Adults of both sexes were fed sugar syrup containing the indicated concentration of the compound for 1 day.)

H_3BO_3 (%)	Mortality (%)	Avg no. eggs/♀	Hatch (%)	Sterility[a] (%)
0.1	38	279	74	0
.2	38	239	69	4
.3	36	177	33	63
.4	45	125	27	79
.5	48	91	39	78
.6	41	148	34	69
.7	52	120	28	79
.8	48	91	15	91
.9	39	23	11	98
1.0	43	6	5	100
Control	30	222	72	0

[a] Percent sterility = 100 (1-*fh*), where *f* and *h* are the corrected decimal fractions of percent fecundity and egg hatch, respectively.

Little is known about the hydrolytical stability of cyclic aminoboranes (borazines). The activity of borazines 49–51 (Table 1) suggested that the hindered borazines 50 and 51 hydrolyzed less rapidly than the relatively unhindered borazine 49. In general, the structure-activity relationship in aminoboranes is not so clear and direct as in alkoxyboranes. Nevertheless, the available evidence suggests that boric acid is again the active principle in aqueous solutions of aminoborane chemosterilants.

Alkyl- and Arylboronic Acids and Their Derivatives.—The physiological effects of boronic acids were reviewed by Soloway (1964). Although boronic acids can be oxidized and hydrolyzed to boric acid it is not certain whether such cleavage occurs in animals. For example, benzeneboronic acid (compound 52) was found unchanged in the brains of dogs that had received intravenous injections of this compound. In our experiments, the sterility effects of compounds 52–76 (Table 1) did not suggest that the active boronic acids (compounds 59, 67, 69, 70, 74) would yield boric acid shortly after they were administered. Also, except for compound 59, the boron content of boronic acids is low, and some compounds (67, 70, 74) were effective at concentrations lower than 1%. Of course, the boronic acid may be only a carrier of the boric acid moiety to the site of action, but the present experimental evidence is not sufficient to support such a hypothesis.

Miscellaneous Inorganic and Organic Derivatives of Boron.—The various chemical forms of boric acid shown in Table 1 (orthoboric acid 77, metaboric acid 78, and boric anhydride 79) are identical in aqueous solutions, and their effectiveness is proportional to their content of boron. The sterilizing effects of various concentrations of boric acid are shown in Tables 2 and 4. The results of the 5-day feeding tests shown in Table 2 have been discussed. Table 4 shows the effects of 0.1–1% of boric acid in a 1-day treatment. Obviously, concentrations of boric acid required for complete sterilization must be higher for the 1-day treatment than for the 5-day treatment, but the oviposition and hatch shown in Table 4 serve to emphasize that neither oviposition nor hatch alone would be a reliable measure of the sterilizing effects of boric acid.

Sodium tetraborate (compound 80) also appeared to belong to the same category, i.e., its activity was proportional to the content of boron, though 1 hydrated tetraborate ion is quite stable. The alkylamine complexes of borane (compounds 81–83) appeared to be ineffective as chemosterilants of screw-worm flies.

The sterilizing activity of boron compounds should be studied further. The effects on the sexes and the results obtained with different modes of application would be of particular interest. Since boric acid was the actual sterilizing agent when many of the effective organoboron compounds were administered, boric acid itself would be the most convenient compound for such studies.

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Responses of the Pales Weevil to Natural and Synthetic Host Attractants[1]

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ABSTRACT

Hylobius pales (Herbst) responds to an attractant present in the cut pine stem. From observation of the weevil's behavior toward the natural attractant, a laboratory bioassay was developed in which paired chemicals were placed in a choice chamber to determine their attractiveness to the weevils. Using extracts of stems of loblolly pine, *Pinus taeda* L., we found that the principal attractant occurs in the phloem and concluded that it may be a monoterpenoid.

Bioassays of purified compounds, including some monoterpenoids, showed several which were attractive, particularly eugenol, anethole, and d-α-pinene. The response to the last mentioned, which makes up a large proportion of pine oleoresin, may explain the attractiveness of the cut pine stem to the pales weevil.

With the increased cutting and replanting of pines in the South, concern has developed over the accompanying increase in seedling losses cause by the pales weevil, *Hylobius pales* (Herbst) (Grady et al. 1967). The pales weevil problem is reduced if planting does not follow too soon after cutting. However, for economic reasons, delayed replanting has become unfeasible, and forest managers presently observe a policy of prompt replanting after cutting (Segur 1967). Under such a practice, the planted seedlings may suffer heavy attack from the weevil population which develops in the roots and stumps left by cutting. As the cutting and replanting sequence continues, weevils may become so numerous that control efforts with insecticides are only partially successful in preventing seedling losses. Consequently, alternatives are needed to supplement insecticides for seedling protection.

One promising alternative employs either an insecticide or antifertility agent with an attractive bait or other attractant source (Beroza 1966). In Europe, for example, a related weevil, *Hylobius abietis* L., is caught and killed in pitfall traps before it destroys the pine seedlings (Novak 1965). These traps contain fresh pine bark for attraction and an insecticide which kills the trapped weevils. The adult pales weevil is likewise attracted by odor to cut pine for food as well as for breeding material. This attraction, observed in America at least as early as 1862 by Harris, has been utilized to collect weevils by Pierson (1921), who set out pine chips, and by Ciesla and Franklin (1965), who used pine stem sections. The 2 last-mentioned cases suggest that the pales weevil can be manipulated in the field, perhaps in the same manner as the Europeans use in trapping and killing *H. abietis*. Our objective was to study the chemical basis for pales weevil attraction as a 1st step in deciding if it could be used to manipulate populations of the weevil.

WEEVIL RESPONSES TO CUT PINE.—Pales weevil attraction to cut pine is a vital process, since it is food-seeking behavior. The basic nature of the process was considered sufficiently important to warrant specific study. The results would be used to design a laboratory bioassay which utilized normal weevil behavior.

Simulating natural conditions and using the method of Ciesla and Franklin (1965), we confirmed that freshly cut sections of loblolly pine, *Pinus taeda* L., contain an attractant (unpublished). The method is based upon the behavior noted earlier by Pierson (1921), whereby the weevils hide beneath the pine sections and can then be collected or counted by an observer.

Traveling by the weevils to the attractant sections occurred at dusk or in the dark. By marking and releasing weevils in plots, we could determine the distances over which weevils responded to the attractant. When releases were made 20 ft from the plot center, an average of 32% of the weevils was recovered at the stem section in the center. A small percentage of weevils was recovered after release 100 ft from the plot center.

This information together with other observations indicate that after adult weevils migrate into a fresh cutting by flight, they remain localized, wandering and foraging on the surface of the ground. From the foregoing we concluded that : (1) the cut stem or trunk of pine is attractive to the pales weevil, (2) within 20–50 ft or more the weevils reach the attrac-

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