

PATHOGENICITY OF SOME ENTOMOGENOUS  
FUNGI, THEIR COMPATIBILITY AND INTEGRATED ACTIVITY  
WITH CHEMICAL INSECTICIDES AGAINST *PISSODES STROBI* (PECK)

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Report CC-X-69  
Canadian Forestry Service  
Department of the Environment  
September, 1974

## RÉSUMÉ

Des études préliminaires sur la vulnérabilité de Pissodes strobi (Peck) à un mélange de champignons entomogènes et d'insecticides chimiques a montré que: 1. les larves, les pupes et, dans une moindre mesure, les adultes étaient très vulnérables aux champignons Beauveria bassiana et Metarrhizium anisopliae; 2. l'exposition des conidies pendant 4 semaines à une température de  $-40^{\circ}\text{C}$  n'a pas affecté leur viabilité; 3. les deux espèces croissaient bien entre  $14$  et  $26^{\circ}\text{C}$  pendant une incubation de 6 jours. M. anisopliae sporulait à  $26^{\circ}\text{C}$ ; 4. de faibles concentrations des insecticides chimiques Gardona<sup>R</sup>, Phoxim<sup>R</sup> et Dursban<sup>R</sup>, lorsque mélangées aux conidies, augmentaient apparemment la virulence des pathogènes en réduisant leur  $\text{LT}_{50}$  de 3 à 5 jours.

## ABSTRACT

Preliminary studies on susceptibility of *Pissodes strobi* (Peck) to mixtures of entomogenous fungi and chemical insecticides indicated that 1. larvae, pupae, and to a lesser extent, adults were highly susceptible to the fungi *Beauveria bassiana* and *Metarrhizium anisopliae*. 2. Exposure of the conidia for 4 weeks to  $-40^{\circ}\text{C}$  did not affect their viability. 3. Both species grew well between  $14$  and  $26^{\circ}\text{C}$  during 6 days incubation. *M. anisopliae* sporulated at  $26^{\circ}\text{C}$ . 4. Low concentrations of the chemical insecticides Gardona<sup>(R)</sup>, phoxim<sup>(R)</sup> and Dursban<sup>(R)</sup> when mixed with conidia apparently enhanced the virulence of the pathogens by reducing their  $\text{LT}_{50}$  by 3-5 days.

## INTRODUCTION

The white pine weevil *Pissodes strobi* (Peck) (Coleoptera; Curculionidae) is a very destructive pest of white pine (*Pinus strobus* L.) in eastern North America. "For more than 60 years, according to DeBoo (Sutherland and DeBoo, 1973) the weevil has wrought havoc on reforestation programs, and attempts to establish the eastern white pine have been abandoned in several regions due primarily to the combined impact of the weevil and white pine blister rust (*Cronartium ribicola*) (Fisher)." Furthermore Brace (1972) stated that in Canada this insect reduced tree heights in the 60 year old white pine stands by 10 feet, and cubic volumes by 3 to 20%. The average loss estimated was about 25% of the total value of lumber sawn from the weeviled logs. Thus control of this pest would mean the protection of young plantations and improved quality of old stands.

Several chemical insecticides at one time used to control this pest are no longer recommended because of their stability in the environment and residual hazards (Nigam 1973). The results of preliminary experiments with aerial spray applications of methoxychlor, the insecticide currently in use, did not yield good control of adult weevils. At best only 80% reduction has been achieved during operational sprays in Ontario at rates of 1-3 lbs. active methoxychlor/acre.

On the other hand several authors, Ellinboe *et al* (1957), Frye and Raun (1961) McLaughlin (1962), Walstad and Anderson (1971), Tedders *et al* (1973), and Taylor and Franklin (1973) have shown that many curclionids are susceptible to infection by *Beauveria bassiana* (Bals.)

Vuill. and *Metarrhizium anisopliae* (Metsch.) Sorok. spores. Taylor and Franklin (1973) determined the percentage of *B. bassiana* infection in the population of *Hylobius pales* and *Pachylobius pictivorus* adult weevils. They found 17.54% *H. pales* and 13.39% *P. pictivorus* were infected in the natural habitat by *B. bassiana*.

Many fungi are known to be pathogenic to insects and for this reason they are known as "entomogenous" fungi. Some entomogenous fungi such as *Coelomomyces* and *Massospora* spc, (Phycomycetes) *Septobosidium* (Basidiomycetes) and *Hirsutella* (Fungi Imperfecti), are limited to attacking only members of a single family, genus or even only a few species of insects. However, fungi with wide host ranges are most commonly isolated during surveys. *B. bassiana* is considered to be the most widely used fungus for the practical control of insect pests and *M. anisopliae* is the second most widely used (Roberts and Yendal 1971).

In Canada, the Insect Disease Surveys in British Columbia carried out during 1947 to 1969 inclusive (Morris - 1963, and Morris and Olsen 1970) mentioned only one case of *Pissodes* sp. parasitized by polyhedral virus. In central Canada, Sault Ste. Marie during 1967 to 1972 inclusive (Burke) *P. strobi* was represented by 14 specimens, from which 5 were parasitized by *Microsporidia* and one by a *Microsporidian* combined with *Isaria* sp. The remaining 8 specimens were not parasitized. Surveys in the Province of Quebec carried out during the years of 1956-60 (Smirnoff, 1961) did not mention *P. strobi* at all.

The small numbers of *P strobi* specimens submitted for examination indicate the difficulties connected with finding dead insects in forest litter and grass.

The present preliminary investigation was planned as follows:

1. To find out if the entomogenous fungi are able to parasitize *P. strobi*.
2. To select the most virulent isolates of entomogenous fungi for further investigations.
3. To study the effect of various temperatures on survival and germination of conidia of entomogenous fungi.
4. To determine the compatibility of entomogenous fungi with chemical insecticides.
5. To assay the integrated activity of chemical insecticides and conidia of selected entomogenous fungi against *P. strobi* adults.

#### MATERIAL AND METHODS

The entomogenous fungi were obtained from Europe and Canada. *B. bassiana* (Bb-55) obtained from the Department of Insect Pathology, Institute of Entomology, Czechoslovak Ac. Sci. Prague, Czechoslovakia.

*B. bassiana* (Bb-29)

" " (Bb-33a)

*Metarrhizium anisopliae* (Ma 46)

*Paecilomyces farinosus* (Paec. f. 27)

were received from Biologische Bundesanstalt, Institute für biologische  
Schalingsbekämpfung, 61 Darmstadt, Heinrichstrasse 243.

*B. bassiana* - (HB-BR)

*M. anisopliae* - (8-P-1) from Research Station, Research Branch, Canada  
Agriculture, University Campus, Saskatoon, Sask. S7N 0X2.

*B. bassiana* DAOM - 71453

*M. anisopliae* DAOM 34470 from Plant Research Institute, Research Branch,  
Canada Agriculture, Ottawa.

*B. bassiana* - (Bb-Tim)

" " - (Bb Forest)

" " - (Bb Insect)

" " - (*Z-Paecilomyces farinosus*) were isolated by the senior author  
from grasshopper, forest soil, unidentified insects and from dead  
*P. strobi* adult.

The insecticides used in the experiments were selected on the  
basis of Nigam's (1973) data:

Dursban® Technical 28.8% - 0,0-diethyl 0-(3,5,6-trichlor-2-  
pyridyl) phosphorothioate. Dow Chemical Company, Midland, Michigan. 48640.

Gardona® WP 75% - 2,3,2-diloro-1-(2,4,5-trichlorophenyl) vinyl  
dimethyl phosphate, Shell Oil Company, 110 West 51st Street, New York,  
N.Y. 10020. U.S.A.

Methoxychlor 88% - 1,1,1-trichloro-2,2-bis(p-methoxyphenyl) ethane,  
DuPont de Nemours, E.I., and Company Inc., Wilmington, Delaware 19898, U.S.A.

Phoxim (Baythion®) 73% - 2,3 phenylglyoxylonitrile oxime 0,0  
-diethyl phosphorothioate.

Farbenfabriken, Bayer A.G., Chemargo Corp., Kansas City, Missouri.

The insects for experimental purposes, adults of *P. strobi*, were reared from the infested white pine shoots collected in July, 1973. Larvae and pupae were obtained by dissecting the infested shoots and carefully removing them by means of soft forceps and camels brush. They were stored, for a few minutes, in moist sterile paper towels before using them in experiments.

The fungus inocula were prepared as dry conidia suspended in sterile tap water containing one drop of Tween 60 in 500 ml. Inoculum contained approximately 50,000 ( $\pm$  5,000) conidia/ml.

The insects were inoculated by dipping them into the inoculum and placing them individually into tubes. Each tube contained 3 ml of distilled water. The water was separated from rest of the space by a synthetic foam plug and the tube was plugged with non-absorbent cotton batting (Fig. II-2). Tubes thus prepared were then autoclaved at 15 psi for 30 minutes.

Inoculated adults were kept in 15 oz. jars containing moist sterile sand and short, freshly cut, white pine branches. The cut end was embedded into sand. The top of the jar was covered with cheese cloth which was secured with elastic rubber bands.

The tubes with inoculated and untreated larvae or pupae were incubated at room temperature (19-21° C) whereas jars with inoculated and control adults were kept in water bath that contained a shallow layer of water and was tightly covered with glass cover and incubated

also at room temperature (19-21° C).

The experimental insects were examined daily and, to ascertain if the dead insects (adults) were killed by the fungus inoculum, they were placed into tubes prepared the same way as were those used for fungus virulence tests (Fig. II-2).

In the case of the larvae and pupae they were surface sterilized first (one minute in Javex and rinsed several times in sterilized tap water) and then placed onto plates that were previously poured with PDA (Difco) medium (Fig. I, 1 and 2).

The compatibility of entomogenous fungi and chemical insecticides was determined by the streak method. Each dilution of insecticides, calculated on the basis of 100% purity, contained approximately 50,000 ( $\pm$  5,000) conidia/ml. The mixtures were incubated for one hour at room temperature (19-21° C) and then PDA plates were streaked in duplicate, with 0.01 ml calibrated loopfuls of the mixtures or with untreated suspension of conidia, containing approximately the same number of conidia/ml. Streaked plates were incubated at 28° C until the visible growth appeared and the toxicity of insecticides, if any, was scored. Incubation was continued to see if the ability of fungi to sporulate was affected by the insecticides.

## RESULTS AND DISCUSSION

### Effect of medium composition on the pathogenicity of fungi.

In spite of the fact that virulence of all isolates was improved by repeated passage through White pine weevil larvae, the data presented in Table I show a considerable variation in the pathogenicity of the same isolate due to the composition of the medium on which it was cultivated. This may explain the apparent difference in pathogenicity sometimes obtained by different workers using the same isolate. Furthermore, from the data presented it is apparent that plate count agar (Difco) is superior to soybean mash (Timonin 1937) and PDA (Difco). However, greater sporulation occurred on Sabouraud's agar than on any other medium during the same incubation period. Plate count agar was used for production of conidia for further investigations.

### Virulence of fungi to larvae and pupae.

The data presented in Table 2 and 3 show that an average 100% Kill of the larvae and pupae tested was obtained with *Beauveria* and *Metarrhizium* in 48 and 72 hours respectively, whereas among control larvae only one died out of twenty, during 11 days of incubation. Three adults emerged from the 20 control pupae during the same time of incubation.

To verify that the fungus inoculum was responsible for parasitism, the dead larvae or pupae were incubated at 28° C on PDA (Difco) medium, until the fungus developed good sporulation (Fig. I-1 and 2). The cultures could then be identified even with the naked eye or with a

magnifying glass.

Effect of temperature on survival and germination of fungus conidia

In survival and germination experiments dry conidia were suspended in sterile tap water containing 1 drop of Tween 60/500 ml. On the average the suspension contained 100 ( $\pm$  15) conidia/ml. One ml of the prepared suspension was mixed with 20 ml of sterile tap water and filtered through millipore membrane filters of 4 cm/diam with 0.45  $\mu$  pore size.

In the case of conidial survival, membranes containing filtered conidia were exposed to  $-40$  and  $-15^{\circ}$  C. Each week, during four weeks of exposure, one filter, with each isolate, was placed on PDA (Difco) medium and incubated at  $28^{\circ}$  C until visible mycelial growth was developed and then the survival ability was recorded. In the case of the germination tests, the membrane filters containing conidia were placed, in duplicates, on PDA medium and incubated at different temperatures as indicated in Table 4. This experiment was repeated twice and the results that are summarized in Table 4 show that temperatures of 40 or 15 below zero, after four weeks of exposure, had practically no effect on the viability of conidia. However, the results of germination experiments show that conidia of all isolates tested did not germinate at 6 and  $34^{\circ}$  C during 6 days of incubation. Furthermore, from the data presented it is apparent that *B. bassiana* isolate "Tim" was more sensitive to  $10^{\circ}$  C than the isolate "Insect". Whereas conidia of both isolates of *M. anisopliae* germinated normally at this temperature. All

isolates incubated at temperature 14°, room (19-21), and 26° C during 6 days of incubation produced good mycelial growth and *M. anisopliae* isolate "8-P-1" began to sporulate.

Compatibility and integrated activity of fungi and chemical insecticides against adult weevils.

The experiments on toxicity of chemical insecticides to conidia of entomogenous fungi indicated slight reduction on germination of *B. bassiana* conidia by concentrations  $10^{-2}$ ,  $10^{-3}$  of Gardona, phoxim and Dursban, but these concentrations had no effect on conidia of *M. anisopliae*. Furthermore, the activity of mixtures of chemical insecticides and conidia of entomogenous fungi against adults of *P. strobi* is shown in Table 5. From the data presented it is apparent that the chemical insecticides in mixtures with fungi accelerated their lethal time ( $LT_{50}$ ) as compared with the  $LT_{50}$  of fungi alone. It is of interest to note that concentrations of  $10^{-5}$  or less of chemical insecticides had no effect on adult weevils but when they were combined with conidia of entomogenous fungi,  $LT_{50}$  was obtained 3, 4, and 5 days sooner than with conidia alone. Also, the concentration of conidia used eventually killed all test insects when used alone. It was also observed that newly emerged adults were considerably more susceptible to fungus attack than were more mature adults.

Although the mechanism of enhanced activity of entomogenous fungi in mixture with low doses of chemical insecticides against insect pests is not clearly understood, it was suggested, by some

insect pathologists (Steinhouse, 1958; Vago, 1959) that insects, like any other organisms, are more susceptible to disease under stress, produced by environmental conditions such as extreme temperatures, scarcity of food or overpopulation. Furthermore, in the opinion of some contemporary workers, application of low dosages of chemical insecticides also act as stressors (Benz, 1971). Morris (1972) has also shown that sublethal doses of certain chemical insecticide reduce the lethal time of Bacillus thuringiensis against some forest insect species. The question of sublethal doses of chemical insecticides as stressor needs further investigation.

#### SUMMARY AND CONCLUSIONS

The results of experiments on pathogenicity of *Beauveria* and *Metarrhizium* to larvae, pupae, and adults of *Pissodes strobi* (Peck) show that:

1. Larvae and pupae of *P. strobi* are very susceptible to *B. bassiana* and *M. anisopliae* isolates and 100% kill was obtained, in 48 hours for larvae and 72 hours for pupae. Adults were more resistant.
2. The exposure for four weeks of conidia of both pathogens to  $-15^{\circ}$  and  $-40^{\circ}$  C had no effect on their viability.
3. The conidia of both species did not germinate during 6 days of incubation at 6 and  $34^{\circ}$  C, and isolates of *B. bassiana* were more sensitive to  $10^{\circ}$  C than isolates of *M. anisopliae*.
4. All isolates at temperature  $14^{\circ}$  C, room (19-21), and  $26^{\circ}$  C, during 6 days of incubation produced good growth and isolate 8-P-1 of *M. anisopliae* began to sporulate at  $26^{\circ}$  C.
5. Chemical insecticides Gardona, phoxim, and Dursban in concentrations  $10^{-2}$  and  $10^{-3}$  exhibited a weak toxicity to conidia of

Table I - The effect of composition of medium on virulence of *M. anisopliae* for *P. strobi* larvae.

Media Used	No. of 1 larvae/ experiment	Percent Mortality at Day			
		1	2	3	4
		Post-inoculation			
Plate count agar (Difco)	20	0	100	--	--
PDA (Difco)	20	0	40	80	100
Malt peptone Muller-Kogler (1968)	20	0	40	100	--
Soybean mash (Timonin 1937)	20	0	60	90	10
Sabouroud's dextrose agar	20	0	50	100	--
Control	20	0	0	5	0

<sup>1</sup> Experiments were repeated 3 times.

Table 2. - Percent mortality of *P. strobi* larvae caused by isolates of different fungi.

Fungus isolates	No. larvae <sup>1</sup> experiment	Percent Mortality at Day			
		1	2	3	4
		Post-inoculation			
<i>B. bassiana</i> (55)	20	*0	90	100	--
"(Forest)"	20	*0	100	--	--
"(Tim)"	20	*0	100	--	--
"(71453)"	20	*0	80	100	--
"(HB-BR)"	20	*0	5	50	100
<i>M. anisopliae</i> (8-P-1)	20	*0	100	--	--
"(34970)"	20	*0	80	100	--
Control	20	0	5	0	0

<sup>1</sup> Experiments were repeated 4 times

\* larvae very sluggish.

Table 3. Mortality of *P. strobi* pupae as caused by various fungi

Fungi	No. pupae <sup>1</sup> / experiment	Percent Mortality at Day				
		1	2	3	4	5
		Post-Inoculation				
B. bassiana (55)	20	0	70	90	100	--
"       "       (Forest)	20	0	60	100	--	--
"       "       (Tim.)	20	0	80	90	100	--
"       "       (71453)	20	0	50	70	90	100
"       "       (HB-BR)	20	0	40	80	90	100
M. anisopliae (8-P-1)	20	0	60	100	--	--
"       "       (34470)	20	0	50	70	80	100
Control	20	0	0	10	0	* 0

<sup>1</sup> Experiments were repeated 4 times.

\* Three adults were emerged.

Table 4 - Effect of temperature on germination and survival of entomogenous fungi conidia

Fungus Species	Temperature ° C							
	-40	-15	+6	+10	+14	room	+26	+34
	Survival			* Germination				
B. bassiana (Tim)	3	3	0	2	4	4	4	0
" " (Insect)	4	3	0	3	4	4	4	0
M. anisopliae (8-P-1) 8-P-1	4	4	0	4	4	4	Sp	0
" " (34470)	3	3	0	4	4	4	4	0

Results are average of two experiments.

Survival after 4 weeks; germination after 6 days of exposure

4 = normal; 3 = slight reduction; 2: considerable reduction; 0 = no germination; Sp = sporulated.

TABLE 5. Integrated activity of chemical insecticide and fungi against adult *P. strobi*

Fungi	Insecticides and concentration	No. insects/ treatment	Mi	Mf LT <sub>50</sub>	Mf + Mi LT <sub>50</sub>
<i>B. bassiana</i> (55)	DURSBAN <sup>R</sup>	10 <sup>-4</sup>	20	75	12
		10 <sup>-5</sup>	20	0	12
		10 <sup>-6</sup>	20	0	12
	GARDONA <sup>R</sup>	10 <sup>-4</sup>	20	5	12
		10 <sup>-5</sup>	20	0	12
		10 <sup>-6</sup>	20	0	12
	METHOXYCHLOR	10 <sup>-4</sup>	20	10	12
		10 <sup>-5</sup>	20	0	12
		10 <sup>-6</sup>	20	0	12
	PHOXIM	10 <sup>-4</sup>	20	100	12
		10 <sup>-5</sup>	20	0	12
		10 <sup>-6</sup>	20	0	12
<i>M. anisopliae</i> (8-P-1)	DURSBAN <sup>R</sup>	10 <sup>-4</sup>	20	75	13
		10 <sup>-5</sup>	20	0	13
		10 <sup>-6</sup>	20	0	13
	GARDONA <sup>R</sup>	10 <sup>-4</sup>	20	4	13
		10 <sup>-5</sup>	20	0	13
		10 <sup>-6</sup>	20	0	13
	METHOXYCHLOR	10 <sup>-4</sup>	20	10	13
		10 <sup>-5</sup>	20	0	13
		10 <sup>-6</sup>	20	0	13
	PHOXIM	10 <sup>-4</sup>	20	100	13
		10 <sup>-5</sup>	20	0	13
		10 <sup>-6</sup>	20	0	13
	UNTREATED (CONTROL)	20	0	0	1 insect died during 13 days incubation

Mi = Percentage mortality caused by insecticide

Mf = Number of days to 50% mortality caused by fungus.

LT<sub>50</sub>

Mf + Mi = Number of days to 50% mortality caused by fungus in combination with  
LT<sub>50</sub> chemical insecticide.

LT<sub>50</sub> = lethal time for 50% mortality (estimated)

All experiments were repeated twice.

*B. bassiana* but had no effect on conidia of *M. anisopliae*.

6. Chemical insecticides when mixed with conidia enhanced the virulence of the pathogen by reducing its  $LT_{50}$  by 3-5 days.

#### ACKNOWLEDGEMENTS

Authors are grateful to Dr. James J. Fettes, Director, Chemical Control Research Institute, Canadian Department of Environment for interest and encouragement in the project and for providing financial assistance.

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Fig. II. Adults of P. Strobi after death were placed in sterile tubes (Fig. II-2) containing some water. As a rule, the surface growth of pathogen first appeared at the end of proboscis and between the segments of chitinous cover (arrows). Later the fungi covered nearly the whole body and profusely sporulated (Arrows F:II-1 and 3); Fig. II, 1 and 3 B. bassiana; Fig. II 4 and 6, M. anisopliae. Fig. II-5 untreated dead insect (control) did not develop mycelial growth.

Fig. I. Surface sterilized larvae incubated on PDA (Difco) medium developed mycelial growth and sporulation characteristic of B. bassiana (Fig. I-1) and M. anisopliae (Fig. I-2). Bacteria growth is also developed from some larvae (Fig. I-1 arrows), but they originated from non-spore-forming bacteria.

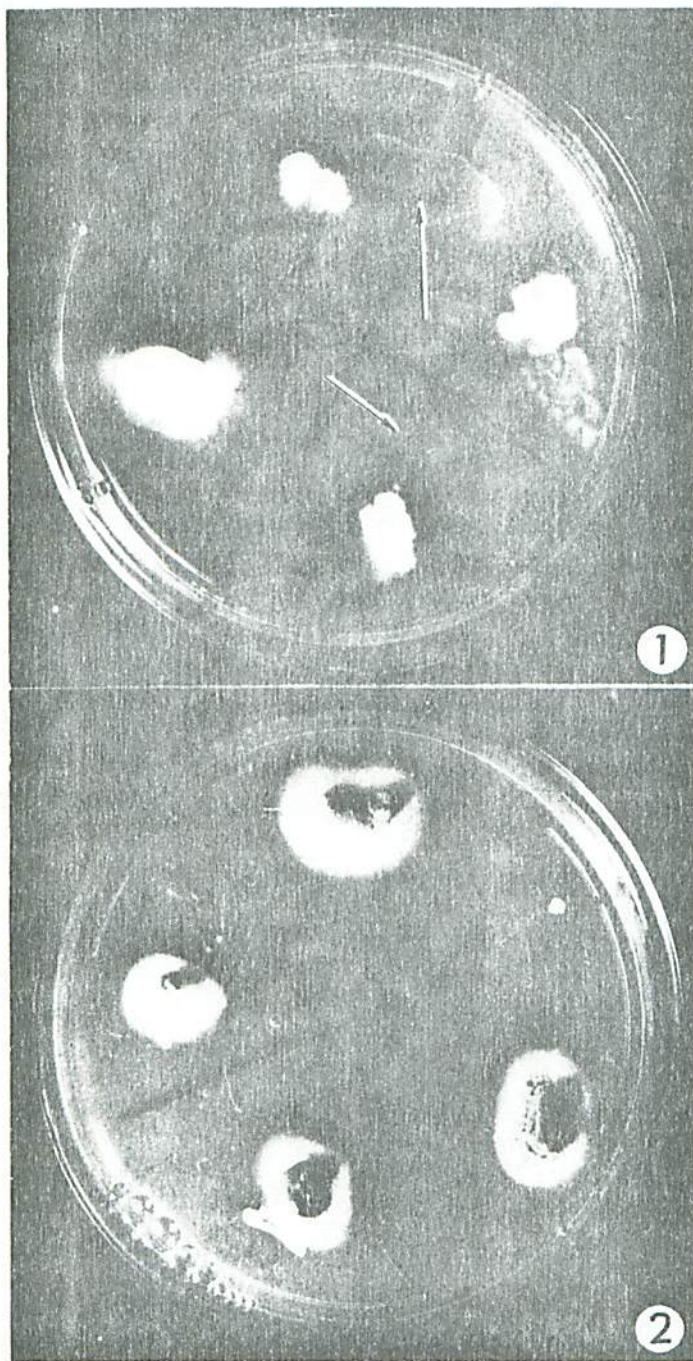


Fig. I

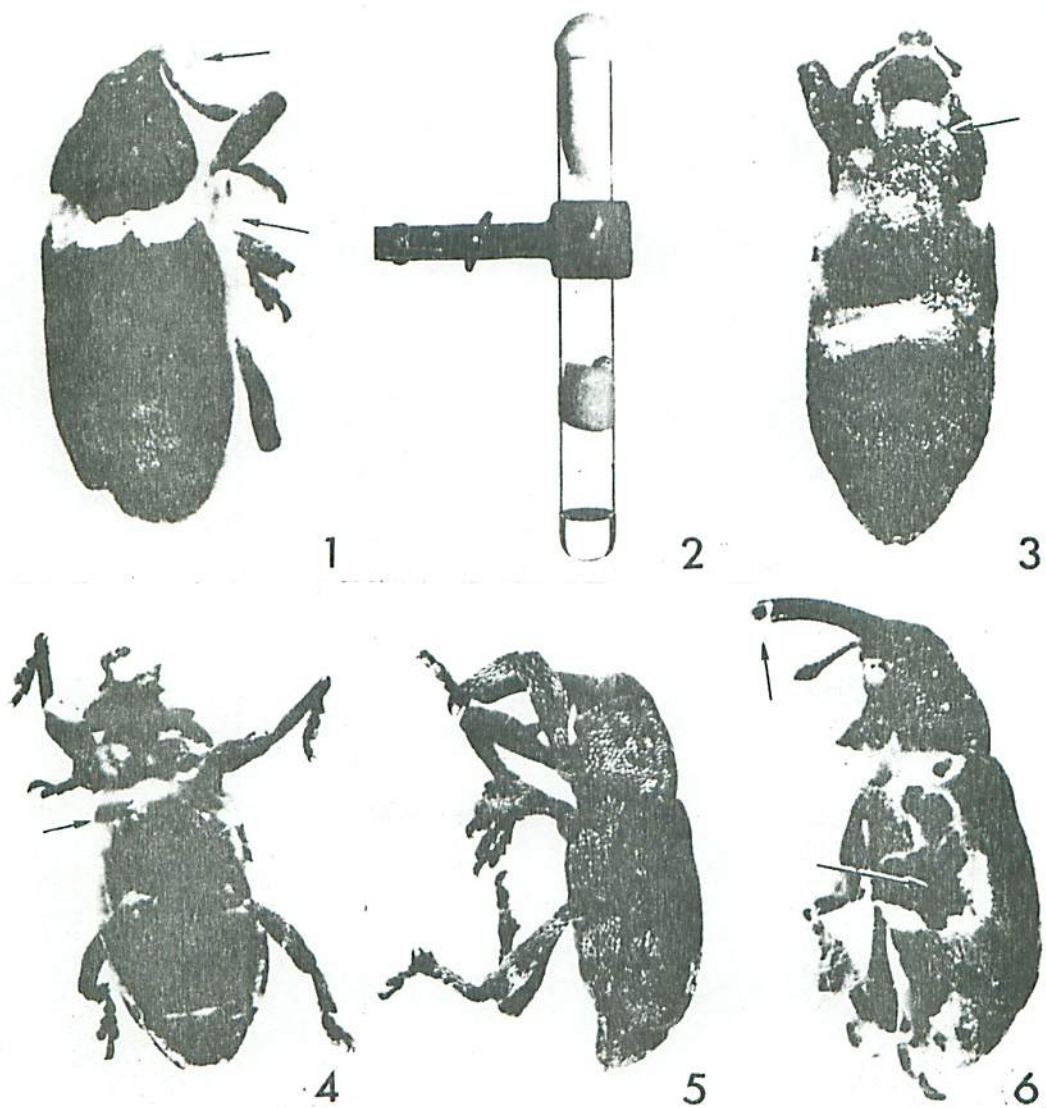


Fig. II