THREE FUNGI TESTED AGAINST THE LOVEBUG, *PLECIA NEARCTICA*, IN FLORIDA[1]

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ABSTRACT

Reinfectivity studies were conducted on late instar lovebug larvae, *Plecia nearctica* Hardy (Diptera-Bibionidae), inoculated with *Tolypocladium cylindrosporum* Gams, *Metarhizium anisopliae* (Metch.) Sor. and *Beauveria bassiana* (Bals.) Vuill. which were isolated and identified from dead larvae. Two tests demonstrated that each fungus apparently affected larval mortality; however, data analyses indicated that only *B. bassiana* caused significant mortality levels (27% to 33%). Problems related to reinfectivity tests are discussed. Nine additional fungi are reported from dead and moribund larvae collected in the field.

Each year in Florida, large numbers of adult lovebugs, *Plecia nearctica* Hardy (Diptera-Bibionidae), emerge from grass litter where they develop for 3 to 9 months through the larval and pupal stages. Adults become a nuisance and a traffic hazard during May and September when their bodies coat windshields an opaque white as they are struck by motor vehicles.

Seasonal sampling revealed a decrease in larval population numbers in the spring of 1973 and a subsequent reduction of adults in the May flight. Samples of larvae taken prior to emergence demonstrated a high percentage of late instars apparently killed by fungi.

Kish et al. (1974) reported 7 fungi isolated from dead larvae and adults. Since then 9 additional species have been identified from lovebug cadavers found during larval surveys. Reinfectivity studies were initiated to test 3 of the most frequently encountered fungi, *Beauveria bassiana* (Bals.) Vuill. *Metarhizium anisopliae* (Metch.) Sor., and *Tolypocladium cylindrosporum* Gams, for pathogenicity to lovebug larvae.

METHODS AND MATERIALS

Larvae used for testing were collected from various locations in Alachua County, Florida. Larvae for each replicate were taken from the same locality, rinsed thoroughly with sterile water and placed in Petri plates (25 larvae per plate) containing a steam sterilized, soil-detritus mixture.

Axenic cultures of the fungi were established by isolating conidia or hyphae from infected lovebug larvae collected in the field. Fungal cultures were maintained on Sabouraud Maltose Agar containing 1% yeast extract. Inoculum utilized for the tests was collected from the first pure culture obtained from the field collected larvae.

Inoculations were made by dusting the fungus directly onto the integument. Although the inoculum concentration was not standardized, an approximately equivalent amount was applied for each test.

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TABLE 1.—LARVAL MORTALITY CAUSED BY 3 FUNGI TESTED AGAINST THE LOVEBUG.

	Percent Infection					
	Test Larvae*		Control Larvae**		Contaminants	
	Test 1	Test II	Test I	Test II	Test I	Test II
Tolypocladium	8	0	4.6	0	1 (*Beauveria bassiana*)	1 (*Beauveria bassiana*)
					5 (*Metarhizium anisopliae*)	
Metarhizium	0	17	1	11		35 *Tolypocladium cylindrosporum*)
Beauveria	27	33	0	0		

* 75 larvae per test
** 150 larvae per test

Inoculated larvae were maintained in the Petri plates under ambient laboratory conditions of light and temperature throughout the test period.

Two tests with 3 replicates each were completed for each fungus. One replicate consisted of 25 larvae in 1 plate. Controls, which consisted of 6 replicates per test, were established by the previously described methods except the inoculum was omitted.

The plates were periodically observed for dead and moribund larvae. Soil was kept moist with sterile water. Cumulative counts of larval mortality caused by fungi were made during the test period which lasted 35 days. Larvae and adults apparently infected with fungi were removed immediately and the fungus was identified.

RESULTS

Infection levels for each test are presented in Table 1. Exposure to *B. bassiana* resulted in infection levels of 27% and 33% for tests I and II, respectively. Results for the other 2 fungi were inconsistent and infection levels were lower. Some larvae became infected with more than the test organism and these fungi are listed in Table 1.

A Chi Square analysis with a 2 × 2 contingency table compared each treatment to the control (Table 2). Results of both tests were combined for this analysis. Because larvae began pupating and adults emerging before

TABLE 2.—CHI SQUARE 2 × 2 CONTINGENCY TABLE ANALYSIS OF INFECTION LEVELS OF 3 FUNGI TESTED AGAINST THE LOVEBUG[1].

Treatment	X^2 of Infection Level[2]
Beauveria bassiana (larvae)	9.17**
Beauveria bassiana (adults)	11.10***
Metarhizium anisopliae[3]	n.s.
Tolypocladium cylindrosporum[3]	n.s.

[1]Data from both tests are combined

[2]X^2 values are calculated by the formula

$$X^2 = \frac{(\text{Observed}-\text{Expected})^2}{\text{Expected}}$$

Where: Observed is the number infected by the treatment
Expected is the number infected in the controls

[3]X^2 values for both larvae and adults were not significant

** $P < .01$

*** $P < .001$

n.s. not significant

the end of the test, the number of infected larvae and adults was compared to the non-infected control population. Numbers of both adults and larvae infected by *B. bassiana* were significantly greater. Infection levels of both larvae and adults inoculated with *T. cylindrosporum* and *M. anisopliae* were not significant.

All the fungi isolated and identified from dead larvae and adults to the present time are listed in Table 3.

TABLE 3.–FUNGI ISOLATED FROM LOVEBUG CADAVERS IN FLORIDA SINCE 1973.

Entomogenous taxa	Suspected Entomopathogens	Nematode Predator	Others
Beauveria bassiana	*Tolypocladium cylindrosporum*	*Arthrobotrys oligospora*	*Eupenicillium brefeldianum*
Beauveria brongniartii	*Verticillium psalliotae*		*Cladorhinum spp.*
Metarhizium anisopliae	*Isaria spp.*		*Cunninghamella spp.*
Paecilomyces javanicus			*Mucor spp.*
Paecilomyces ramosis			*Curvularia spp.*
Conidiobolus coronatus			*Fusarium spp.*

Discussion

Beauveria bassiana appeared to be pathogenic to the lovebug and to cause significant mortality among larvae and adults. Although larvae inoculated with *M. anisopliae* and *T. cylindrosporum* did become infected, levels were quite low.

Several problems exist concerning interpretations of the data. Larvae used in this test were not reared under stringent, aseptic laboratory conditions, which could have altered the results. Although larvae were dipped into mixtures of fungicides in other tests, contaminants persisted, probably surviving in gut and fecal material. Additionally, at best, only *B. bassiana* showed evidence of potential as a control agent. Many field experiments which have tested fungi, including *B. bassiana*, as biological control agents, were failures or had minimal success owing largely to our ignorance of the dynamics of the biotic and meteorological influences existing for any given insect-pathogen relationship.

Among the additional fungal species reported from lovebugs in Table 3 are 3 entomogenous forms: *Beauveria brongniartii* (Sacc.) Petch, closely related to *B. bassiana* and an effective insect pathogen; and 2 members of the genus *Paecilomyces, P. javanicus* (Friederichs & Bally) Brown & Smith and *P. ramosis* Samson & Evans. *Paecilomyces javanicus* has been reported as an effective natural control agent of scale insects of citrus in Florida, (Watson and Berger 1937) and in Puerto Rico, (Wolcott 1955).

Additional information concerning the nutritional needs of the lovebug is needed in order that these insects can be reared and tested under more aseptic conditions.

Literature Cited

Hetrick, L. A. 1970. Biology of the "Lovebug", *Plecia nearctica* (Diptera: Bibionidae). Fla. Ent. 53:23-6.

Kish, L. P., G. E. Allen, J. W. Kimbrough, and L. C. Kuitert. 1974. A survey of fungi associated with the lovebug, *Plecia nearctica*, in Florida. Fla. Ent. 57:281-4.

Watson, J. R., and E. W. Berger. 1937. Citrus insects and their control. Fla. Agr. Ext. Ser., p. 13-61.

Wolcott, G. N. 1955. Experiences with entomogenous fungi in Puerto Rico. Puerto Rico Sta. Bull. 130.