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Use of *Beauveria bassiana* and *Metarhizium anisopliae* for Fruit Fly Control: A Novel Approach

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INTRODUCTION

Current social and environmental problems associated to insecticide use for fruit fly control, either by aerial or ground applications on foliage for adult control or to the soil for larvae or new-emerged adult control (Saul et al. 1983, Penrose 1993), have motivated the search for biological control alternatives, including enotmopathogenic bacteria, nematodes and fungi (Toledo 2002).

Beauveria bassiana (Bals.) and *Metarhizium anisopliae* (Met.) Sorokin are two species of entomopathogenic fungi, belonging to the Hyphomycetes group, that are natural inhabitants of soil, where they are found infecting a wide range of insect species that spend at least one stage of their life cycle in the soil. They are also found in agricultural crops as epizooties on defoliator lepidopteran larval populations.

The main infection route is through the integument, although they can also be ingested and enter the organism through the digestive tract, or through the trachea, or wounds (Madelin 1963).

The conidial phase (spores) of a large number of strains of both species, coming from different geographic regions, have been assessed, under laboratory conditions, for control of different fruit fly species and on different life history stages (García et al. 1984, Espin et al. 1989, Campos 2000, Castillo et al. 2000, Lezama-Gutiérrez et al. 2000, De la Rosa et al. 2002, Ekesi et al. 2002). Most of these studies have determined the dose-response curve for different strains. The most common method used has been the immersion of any insect stage (larva, pupa or adult) in a conidia solution (De la Rosa et al. 2002), although topic, oral or contact applications have also been tested (Toledo et al. 2003a, Toledo et al. 2007).

Results obtained, so far, indicate that there are some strains from both fungi species with great potential to infect and kill adult fruit flies. However, studies on practical field applications are scare and do not provide the elements required for an efficient practical use of these biological control agents.

Application of conidia to the soil have been suggested as a method to use these entomopathogenic fungi for fruit fly control (Dimbi et al. 2003, Lezama-Gutiérrez et al. 2000). This method has been shown to be an effective

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way to infect new-emerged adults (Ekesi et al. 2005), and the entomopathogenic fungi might survive better under soil conditions (Gaugler et al. 1989). However, the amount of conidia required, and the interactions with biotic and abiotic factors under the diverse soil conditions must be evaluated, since these might strongly affect the efficacy of this application method. In addition, the risk of side effects on non-target organisms must be assessed (i.e. Ekesi et al. 2005).

Howse and Underwood (2000) proposed the use of electrostatic charged powders to spread a killing agent within a pest population. In developing methods to test this novel technique (Toledo et al. 2003a), Jenifer Knapp (personal communication) brought the idea of using sterile flies as vectors to spread the spores of slow killing entomopathogenic fungi within wild populations of fruit flies.

Here, we summarize some of our results from different laboratory and field cage experiments aimed to assess the potential of these entomopathogenic fungi as practical fruit fly biocontrol agents, using sterile flies as vectors.

MATERIALS AND METHODS

Insects and Environmental Conditions. Two species of fruits flies were used in our experiments: the Mexican fruit fly, *Anastrepha ludens* (Loew) and the Mediterranean fruit fly, *Ceratitis capitata* (Wied). To reduce biological variability, all insects used in our studies were obtained as larvae or pupae from the standard mass-rearing process at the MOSCAMED / MOSCAFRUT facility, located in Metapa, Chiapas, Mexico.

Pupae were placed in 30 x 30 x 30 cm glass cages, one side of the cage was covered with synthetic mesh that allowed air circulation and handling. Flies were provided with food (a 3:1 sugar: enzymatic yeast hydrolyzate dry mixture) and water (entomological vials with a cotton wick). Between 4 and 7 days old, males and females were collected from these emergence cages and placed in groups of 25 pairs in 10 cm diameter x 23 cm height plastic containers, for the different bioassays.

Laboratory conditions where the flies were maintained were: 26 ± 1 ºC temperature, 70% de relative humidity and 12: 12 h (L:D) photoperiod.

Conidia Viability Tests. Before conducing the pathogenicity bioassays, the conidia viability for the different strains and products was verified. We used the microculture method (Jiménez 1992). Small aliquots of the fungal solution, previously homogenized in a vortex, were placed on a slide with Saboraud Dextrose Agar (SDA) as growth media. Viability was determined by the presence of germination tubes. A given strain was considered viable if more than 90% of the conidia showed germination tubes.

Mean Letal Time (LT_{50}). A 1% concentration stock solution was prepared, using 1 g of conidia diluted in 100 ml sterile distil water. Glycerin was used as dispersal agent. The solution was homogenized with a magnetic agitator.

From this stock solution, additional solutions were prepared (for example: 0.1, 0.01, and 0.001%) for the different bioassays. For each concentration, including the control, a minimum of 5 replicates were done to fulfill the statistical requirements for this type of studies (Ibarra y Federeci 1994). Each replicate had the same number of treated insects. For the control the same number of insects was used and these were treated with sterile distil water.

Once treated, the flies were placed in glass cages with food and water (as described above) and maintained at 26 ºC y 70% de R. H.. Mortality was recorded daily until the last fly died. Dead insects were placed in humid chambers to promote fungal mycelia development and confirm that dead was caused by fungal infection.

Mean Lethal Concentration (LC_{50}). A concentration series was evaluated (i.e. 0.001, 0.003, 0.006, 0.01, 0.06, 0.1, 0.6, 1.0%, and the control). To determine the number of conidia in each solution, five recordings from samples taken from each concentration were made, using a hemocytometer.

For each concentration, 1.0 or 2.0 ml of the suspension was applied with a pipette to the unit where fruit fly adults were inoculated. The number of replicates was determined as required for this type of bioassays (Ibarra and Federeci 1994). Sample handling and fungal infection was confirmed as described above.

Field Cage Tests. Those strains or products that showed potential for fruit fly control under laboratory conditions, were selected for field cage tests. The product used was from the Local Committee on Plant Protection (LCPP), and sterile flies were inoculated with the dry conidia, two or three hours before the test. In order to facilitate their management during the inoculation process, insects were placed in test tubes, and were placed in a refrigerator at 3º C for 5 minutes to render them lethargic. Cooled flies were then placed in groups of 50 individuals in Petri dishes containing 0.5 g of conidia and were gently shaken for 1 minute. Treated and untreated flies were released inside 3 m diameter x 2 m height field cages, with small potted tress inside. The number of matings, as well as the duration of each copula was recorded. After mating, the flies were placed individually into 8 cm diam x 12.5 cm height clear plastic containers with food and water and daily mortality was recorded over a period of 20 consecutive days. At the end of the test, all fruit flies that do not mating were collected and placed in the same type of cages. Mortality was recorded daily and dead insects were placed in humid chamber to confirm that fungal infection was the cause of dead. Mortality due to direct fungal transmission through mating and indirect transmission due to male-male interactions or courtship was estimated.

Data Analysis. Abbott correction (Abbott 1925) was used when mortality was recorded in the control. Afterwards, for LC_{50}, data were analyzed by Probit analysis (SAS Institute 2002). For LT_{50}, means were compared by 95% fiducial limits to determine if differences in the time required to kill 50% of the sample were significant. Data from field cage tests were analyzed by analysis of variance ANOVA (SAS Institute 2002).

RESULTS

Laboratory bioassays showed that several strains of *M. anisopliae* (Ma3, Ma5 y Ma wild) and *B. bassiana* from the ECOSUR strain collection were highly virulent to adults of *A. ludens* and *C. capitata*. Also, two *B. bassiana* commercially available products showed high adult virulence, the one produced by the Local Plan Protection Committee (LPPC) and Bassianil® (Table 1).

We found no effect of these strains or products when applied to larvae or pupae. Similar results were found by Dimbi et al. (2003).

Transmission using sterile flies as vectors showed promising results. High female mortality was observed for both, *A. ludens* and *C. capitata,* when they mate with infected males. High male mortality was also observed through male-male lek interactions. Male - female transmission through courtship and mating attempts was also observed, although female mortality was not as high as when they actually mate. Table 2 shows the data from field cage studies with sterile medflies.

DISCUSSION

Mortality levels produced in our studies are within the range reported for other fungal strains and other species of fruit flies. For example, Dimbi et al. (2003) found from 7 to

Table 1.- Adult mortality in two species of fruit flies treated with conidia suspension of two entomopathogenic fungi species.

Fungal strai Code§	Fruit fly species	Concentration Conidia/ml	Mortality*	LT_{50} (days)
Bb16	*A. ludens*	5.13×10^5	100.0	2.82
Bb26	*A. ludens*	1.05×10^8	98.0	3.74
Bb18	*A. ludens*	1.40×10^8	86.0	3.94
Ma nat. (6)	*A. ludens*	6.15×10^8	89.4	4.07
Ma3	*A. ludens*	7.95×10^8	78.5	4.43
Ma5	*A. ludens*	3.48×10^8	75.2	4.95
Bb25	*C. capitata*	1.70×10^8	91.9	4.01
Bb6	*C. capitata*	4.50×10^8	91.2	3.83
Bb26	*C.capitata*	2.00×10^8	87.4	4.22

§ Codes from ECOSUR strain collection
* Mortality corrected by Abbot formula.

100% mortality in adults of *C. capitata* and *C. rosa var. fasciventris* treated with *B. bassiana*. Castillo et al. (2000) reported 100% mortality in *C. capitata* treated with 1×10^6 con/ml of *M. anisopliae*. Other strains of this fungi species have been found to be virulent to adults and immatures of *Anastrepha fraterculus* (Wied.) (Rodrigues-Destéfano et al. 2005). Muñoz (2000) evaluated 16 strains of *B. bassiana* against *C. capitata* adults and found mortality levels between 20 and 98.7%. Similarly, Campos (2000) and De la Rosa et al. (2002), testing *M. anisopliae* and *B. bassiana* for the control of *A. ludens* adults reported a mortality range between 82 and 100%. Most workers have not found virulent strains for immature stages.

The LT_{50} produced by the virulent strains and products tested were similar to those previously reported for other insect pests (Davidson and Chandler 2005). These laboratory bioassays were useful to identify and select the strains and products that were worth to test under field cage conditions.

Our results from field cage conditions confirm that there is horizontal transmission and suggest that the use of sterile flies as vectors seems to be a feasible application method. Released sexually mature inoculated sterile flies will transmit the fungi through mating

Table 2.- Horizontal transmission of two *B. bassiana* (Bassianil®, and LCPP) products on adults of *Ceratitis capitata* in field cage tests.

Product	Means of Transmission	Mortality (%) ♂♂	Sporulation (%) ♂♂	Mortality (%) ♀♀	Sporulation (%) (♀♀)
Bassianil	Mating	93.2	91.9	41.6	33.5
LCPP	Mating	86.2	84.1	57.0	39.3
Bassianil	Indirect	94.2	76.6	14.2	8.8
LCPP	Indirect	83.5	80.7	33.8	14.6

LCPP = Local Committee on Plant Protection.

(direct), courtship and male-male interactions (indirect) to the wild population (Toledo et al. 2003a, 2003b, 2007).

Field survival of sterile flies will be a key element to determine the feasibility of this approach. If under current Sterile Insect Technique (SIT) programs, sterile flies live for more than 4-5 days after release and after this time, males continue mating and inducing sterility into the wild population, it will not be advisable to release infected sterile flies that will only live and mate during 4-5 days after release. But if sterile males only live a very short time, or they are only capable to inseminate a few females after release because of sperm depletion, then the capability of fungal transmission might represent a feasible alternative to improve pest control. For this approach, fungal strains with long lethal times will be preferred over those with short lethal times.

We believe that the use of sterile flies as fungi vectors is a feasible approach that deserves further attention. The amount of conidia required and the side effects on non-target organisms will be minimized. For further development, the performance of sterile males under current SIT programs conditions must be compared with the performance under this new approach, considering both effects, sterility induction and mortality produced by fungal transmission.

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