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INFECTIVITY OF INSECT PATHOGENS AGAINST *NEOCHETINA EICHHORNIAE*, A BIOLOGICAL CONTROL AGENT OF WATERHYACINTH

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ABSTRACT

Several insect pathogens were tested for infectivity against *Neochetina eichhorniae* Warner (Coleoptera:Curculionidae), a biological control agent for waterhyacinth, *Eichhornia crassipes* (Mart.) Solms. No mortality resulted from exposure to 21 strains of *Bacillus thuringiensis*. No significant mortality resulted from exposure to 2 strains of *Metarhizium anisopliae*. Although treatment of adult weevils with 6 strains of *Beauvaria bassiana* produced no significant mortality, 2 strains (BbAFA - 35%, Bb 5647 - 65%) did result in statistically significant mortality. Exposure to 2 strains of *Steinernema carpocapsae* resulted in 60-70% mortality. Our results indicate limited potential for these insect pathogens to control *N. eichhorniae* in resource recovery or wastewater treatment facilities.

RESUMEN

Se probó la infectividad de varios patógenos de insectos hacia *Neochetina eichorniae* Warner (Coleoptera: Curculionidae), un agente de control biológico de *Eichornia crassipes* (Mart.) Solms. No hubo mortandad cuando expuestos a 21 tipos de *Bacillus thuringiensis*. Cuando expuestos a 2 tipos de *Metarhizium anisoplia*, no caulsaron una mortalidad significante. Aunque el tratamiento de picudos con 6 tipos de *Beauvaria bassiana* no produjo una mortalidad significante, 2 tipos (BbAFA - 35% Bb 5647 - 65%) producieron una mortalidad estadísticamente significativa. Exposición a 2 tipos de *Steinernema carpocapsae* resultó en una mortalidad de 60-70%. Nuestros resultados indican un potencial limitado para estos patógenos de insectos para controlar *N. eichorniae* en recuperación de recursos o en facilidades de tratamiento de aguas de desecho.

The concept of using biological control to reduce or eliminate a population of released biocontrol agents is not unique. It originates in the context of conflict of interest, a frequent adjunct to weed control efforts (Huffaker 1964). Typically, conflicts arise at the time of a proposed phytophage introduction, when one interest perceives the target

plant as a pest and others view it as beneficial (Drea 1973). In other cases a newly introduced predator can incidentally supress an already established, non-target plant feeder (Anneke et al. 1969, Batra 1981). These conflicts can often be resolved by accumulating more precise economic, ecological and aesthetic data (Andres 1981), and finally by assigning monetary values to the beneficial and harmful aspects involved (DeLoach 1978) and making an objective evaluation on that basis.

The conflict of interest concerning waterhyacinth *Eichhornia crassipes* (Mart.) Solms arose when it became evident that in some situations use of waterhyacinth can generate revenue (Thyagarajan 1984). However, biocontrol and use of waterhyacinth need not be incompatible (Bennett 1982). A weed management strategy can be devised whereby undesireable attributes of waterhyacinth are suppressed while benefits are still exploited (Wright & Center 1984).

In Florida, two weevils were released for biocontrol of waterhyacinth in the 1970's, when *E. crassipes* was the most important aquatic weed problem in the state. Since that time *Neochetina eichhorniae* Warner and *N. bruchi* Hustache have dispersed widely and are now found throughout areas of waterhyacinth infestation. Their ubiquitous distribution became evident recently with the development of both wastewater treatment and resource recovery projects that use waterhyacinth. Weed populations kept in culture in at least one wastewater treatment facility appeared to suffer heavy damage due to waterhyacinth weevil feeding. Anything which would impair the growth rate of these plants in culture is undesireable. It occurred to us that an alternative to broad spectrum insecticide treatment might be the use of a pathogen or parasite specific to the weevils which would act quickly and then lose infectivity under field conditions. The purpose of this study was to test several potential candidates for control of *N. eichhorniae* in specific locations such as wastewater treatment and resource recovery facilities.

METHODS

Neochetina eichhorniae adults were used in this study because *N. eichhorniae* is the most common of the two insects, and because adults are the only life history stage vulnerable to application of an external control agent (eggs and larvae live within plant tissue; pupae are sealed in cases on the roots). Adult weevils were collected in the field prior to each experiment. A 150mm x 25mm plastic petri dish fitted with moistened filter paper was supplied with adult insects. Control dishes received a large freshly-cut waterhyacinth leaf which had been dipped into or sprayed with distilled water. Experimental dishes were supplied with a similar leaf which had been dipped into or sprayed with the test pathogen until it was completely covered on both surfaces.

Initial tests with 21 strains of *Bacillus thuringiensis* consisted of one experimental dish per strain and 3 control dishes, with 10 insects per dish. All subsequent trials utilized 5 replicates of the experimental pathogen and 3 replicates of the control, each with 5 insects per dish. Assay dishes were incubated at room temperature for 14 days and checked daily to detect weevil mortality. At 2-day intervals leaves were removed from experimental and control dishes, the dishes were cleaned, and fresh untreated leaves were added. Insects were thus exposed for 2 days to the pathogens. Cadavers were removed and kept at room temperature and high humidity in individual covered dishes. They were periodically examined to verify the presence of test pathogens (eg fungal hyphae) and confirm the cause of death.

A total of 21 serotypes or strains from 10 different *Bacillus thuringiensis* (*B. t.*) subspecies were tested against *N. eichhorniae*. These strains included: *B. t. aizawai* (HD 111, 248), *B. t. alesti* (HD 4, 44, 84), *B. t. darmstadiensis* (HD 200, 601), *B. t. entomocidus* (HD 110), *B. t. israelensis* (HD 656), *B. t. kenyal* (HD 62, 291), *B. t.*

kurstaki (HD 21), *B. t. sandiego*, *B. t. tenebrionis*, *B. t. tolwarthi* (HD 121), *B. t. thuringiensis* (HD 2, 18, 50), and two unidentified *B. t.* strains (HD 326, 490). Stock cultures of all except 2 strains were provided by the USDA Cotton Research Laboratory, Brownsville TX. *B. t. sandiego* was obtained from Mycogen Corporation (San Diego, CA) as M-One Insecticide formulation. *B. t. tenebrionis* was accessed from the German Collection of Microorganisms, D3400, Gottingen, Germany.

Spore-crystal preparations of the various *B. t.* isolates were produced in 125 ml of medium B-4 (Dulmage et al. 1981) on shakers for 72 h at 28° C and harvested by centrifugation at 4000 g for 30 min. Resulting pellets were suspended in 5% lactose solution followed by the addition of 4 volumes of acetone. Precipitated spore-crystal preparations were collected on Whatman #1 filter paper and air dried overnight. Spore-crystal powders were sonicated into suspension at a rate of 15 mg dry powder/ml phosphate-buffered saline (pH 7.0). The leaves were dipped into the bacterial suspensions and allowed to air dry before adding test weevils. The *B. t.* beta-exotoxin was tested for pathogen-icity using Abbott Laboratories preparation of thuringiensin, available as ABG-6162A concentrate (3% active ingredient). Waterthyacinth leaves were sprayed with a 1:10 or 1:100 aequous dilution and allowed to air dry as described above.

An *N. eichhorniae* weevil adult found dead in the field in December 1986 proved to be infected with the pathogen *Metarhizium anisopliae*. This fungus was isolated and cultivated on yeast extract-glucose-basal minerals (YGM) agar plates (Poprawski et al. 1988). Within a 2 week incubation at 24 °C this isolate sporulated. Progeny spores were scraped from plates and suspended in a 0.1% Tween 80 solution. The spore suspension (1.0×10^8 spores/ml) was applied to waterhyacinth leaves with an airbrush (Paasche Airbrush Co.) until the leaf surface was completey covered. A second strain of *M. anisopliae* (Strain 35-79) isolated from *Otiorhynchus sulcatus* (Fab.) was obtained from the Institute of Horticultural Research, Littlehampton, U. K. (Adrian Gillespie) and propagated on YGM agar plates. Spores were collected, suspended in 1% Triton-X-100 (1.4×10^7 spores/ml) and sprayed on waterhyacinth leaves with an airbrush. To conduct a second trial of this strain, spores were reisolated from cadavers and applied at a rate of 2.7×10^7 spores/ml. Aliquots of spore suspensions were applied to YGM plates and germination rates were assessed at 24h post-inoculation.

Nine strains of *Beauveria bassiana* (Bb) were tested against *N. eichhorniae* adults. A commercial preparation (MYD 583C) provided by Abbott Laboratory was tested at rates of 10^3 to 10^8 spores/ml. The other 8 strains were propagated on YGM media and spores were harvested as described previously. Spore suspensions were sprayed onto the surface of waterhyacinth leaves at the following concentrations: BbAFA (from *Artipus floridanus* Horn) 1×10^7, 1.94×10^9; BbCBW 79-28 (from *Araecenus fasciculatus* (DeGeer)) 1×10^7, 2.13×10^9; Bb 143 (from *Sitona* sp.) 4.10×10^8; Bb 737 (from Pentatomidae) 1.39×10^9; BbRS 804 (from Elateridae) 1×10^7, 3.34×10^8; BbRS 252 (from *Leptinotarsa decemlineata* Say) 1×10^7, 1.53×10^9; UFLBb 5477 (from *Scapteriscus vicinus* Scudder) 1×10^7, 1.62×10^9; and UFLBb 5647 (from *Scapteriscus acletus* Rhen and Hebard) 1×10^7, 1.71×10^9. Viability of spore preparations (except MYD 583C) was assessed as described previously for the *M. anisopliae* isolates.

Two strains (Breton, All) of the nematode *Steinernema carpocapsae* isolated from Lepidoptera were tested against *N. eichhorniae*. The nematodes were obtained from stock cultures maintained by Grover Smart, Department of Entomology and Nematology, University of Florida in Gainesville. Both strains were applied to moistened filter paper at a rate of 6,000 infective juveniles per petri dish (17.6 cm^2). Fresh waterhyacinth leaves were immediately placed on the surface of the treated filter paper, after which adult weevils were added.

Experimental mortality was corrected for control mortality using Abbott's Formula (Abbott 1925, Neal 1975). Mean % mortality in the various treatments was tested for significance using Student's T-test ($P \leq 0.05$) (SAS Institute 1985).

Results and Discussion

No adult weevil mortality resulted from exposure to any strain of *B. thuringiensis* tested (Table 1). Tests of the beta-exotoxin of *B. t.* also failed to produce significant mortality although this material did appear to be a feeding deterrent. Based on qualitative examination, both size and frequency of weevil feeding scars seemed to be less on leaves sprayed with thuringiensin compared to control leaves sprayed with water. While this indicates little hope for use of existing strains of *B. t.* as a waterhyacinth weevil control, it is in agreement with other studies of *B. t. israeliensis* for blackfly control which have demonstrated no significant decrease in populations of other filterfeeding organisms present in the aquatic habitats treated with this larvicide (Colbo & Undeen 1980). Our results suggest that the presence of commercially-used *B. t.* strains in water as a result of runoff or drift poses little danger to *N. eichhorniae* populations which have been established for waterhyacinth management.

Germination of harvested *M. anisopliae* as well as all *B. bassiana* spore preparations plated in PYGM agar was >90% at 24 h post-innoculation. No mortality resulted from exposure in the lab to *M. anisopliae* isolated from a dead *N. eichhorniae* weevil collected in the field. Upon exposure to the *M. anisopliae* strain from *O. sulcatus*, waterhyacinth weevils suffered 27% mortality, but this was not statistically significant. A second trial with serial passaged *M. anisopliae* cultures yielded no significant mortality, suggesting rapid attenuation of this strain under in vitro conditions.

Exposure to the *B. bassiana* strains yielded a range of mortality reponses. The commercial formulation (MYD 583C) provided by Abbott Laboratories resulted in no mortality at any of the concentrations tested (1×10^4 - 1×10^8 spores/ml). Although

TABLE 1. Mortality of *Neochetina eichhorniae* adults exposed to selected insect pathogens.

Pathogen	Test Concentration	% Mortality ± SEM[1]
Bacillus thuringiensis		
21 strains (spore + crystal)	15 mg/ml	0
exotoxin	1:10 H_2O	13.3 ± 6.6
	1:100H_2O	0
Metarhizium anisopliae		
strain from *N. eichhorniae*	1.0×10^8 spores/ml	0
strain 35-79	1.4×10^7	27.1 ± 23.1
strain 35-79 (second passage)	2.7×10^7	0
Beauveria bassiana		
Abbott Laboratories	1.0×10^8	0
Bb 143	4.1×10^8	25.0 ± 15.0
BbAFA	2.1×10^9	35.0 ± 5.0[2]
BbCBW7928	2.2×10^9	35.0 ± 25.0
BbRS 252	1.7×10^9	40.0 ± 30.0
BbRS 804	4.5×10^9	5.0 ± 5.0
Bb 5477	1.8×10^9	20.0 ± 20.0
Bb 5647	2.0×10^9	65.0 ± 5.0[2]
Steinernema carpocapsae		
ALL strain	6000/17.6 cm^2	60.0 ± 14.1[2]
Breton strain	6000/17.6 cm^2	76.7 ± 6.1[2]

[1]% mortality values have been corrected for control mortality.
[2]indicates significant mortality at $P \leq 0.05$ by Student's T-test.

mortality from strains Bb 5477, Bb 143, BbCBW 7928, and BbRs 252 ranged from 20.0 - 40.0%, none of these values were statistically significant. The only strains to produce significant mortality were BbAFA (35.0 ± 5.0%) and Bb 5647 (65.0 ± 5.0%). The concentration of *B. bassiana* ($\geq 2.0 \times 10^9$ conidia/ml) producing this mortality represents an extremely high rate which may not be economically feasible to use.

Trials with 2 strains of nematodes resulted in the highest significant mortality of weevil adults overall. The ALL strain produced 60.0 ± 14.1% mortality, whereas exposure to the Breton strain resulted in mortality of 76.7 ± 6.1% within 8 days.

The objective of this research was to survey the relative susceptibility of *N. eichhorniae* weevils to various entomopathogens. The results of the assays demonstrated that *N. eichhorniae* weevils are resistant to many entomopathogens currently being studied for their microbial control potential. However, small-scale field trials are warranted with the Bb 5647 strain of *B. bassiana* and the two strains of nematodes to determine if they can maintain infectivity under more typical environmental conditions. The aquatic habitat offers a high relative humidity, especially in the late afternoon and evening, which is an important factor in maintaining viability of nematodes (Gaugler 1981). Waterhyacinth weevils feed on leaves and petioles primarily after dark, so they would be likely to come in contact with pathogens applied late in the day to the leaf surfaces. The question of field rates and cost effectiveness remains to be determined.

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AN ANNOTATED CHECKLIST OF THE COCCOIDEA OF BERMUDA

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ABSTRACT

The scale insects (Coccoidea) are a particularly important group economically in Bermuda; 85 species having been recorded (although at least 5 records are doubtful). Of these, two species killed nearly all the mature indigenous cedar in the late 1940s and 1950s. This checklist contains all previously known records and also extensive new information collected during 1987-89. Information summarized for each species includes: 1. number of plant families and genera on which it has been recorded; 2. current status in Bermuda; 3. a brief history (where relevant) including attempts at biological control; and 4. museums where specimens have been deposited.