WOZNIAK, CHRIS A., and ANN C. SMIGOCKI, USDA, Agricultural Research Service, Molecular Plant Pathology Laboratory, Beltsville Agricultural Research Center, Beltsville, MD.
Development of a fungal biopesticide for management of the sugarbeet root maggot.

ABSTRACT

A natural pathogen of the SBRM has been patented and is under development as a biological control agent. The imperfect fungus *Syngliocladium tetanopsis* has been isolated from several sites in the Red River Valley of ND and MN. *S. tetanopsis* was isolated from infected third instar root maggots and cultured axenically on OatM medium (per L: 30 g rolled oats, 3.0 mL light olive oil, 30 mg cholesterol, 12 g Bacto-agar) at 23 to 25 °C. To date thirty-seven isolates have been purified, cultured and forwarded to two commercial interests and another ARS lab researching this potential biopesticide. Conidiospores were collected in 0.85 % saline from culture plates and used to inoculate 120 g sterile sand at 3 x 10^3, 3 x 10^4, 3 x 10^5, and 3 x 10^6 viable spores / plate. Spores were enumerated on a haemocytometer, then examined for viability using fluorescein diacetate (FDA). Epifluorescence microscopy was used to detect fluorescing spores using a standard fluorescein filter set. Only viable spores were considered in the final count before application to bioassays. A saline control was used for comparison. SBRM larvae (n = 118 or 120 / treatment) incubated with conidiospores in sand (10 / dish) for 7 days at 24 °C were washed and moved to filter paper in a closed dish to observe the infection process. *In vitro* bioassays of third instar SBRM challenged with conidiospores of *S. tetanopsis* strain NRRL 21853 resulted in 4 % (3 x 10^3 spores / plate) to 47 % (3 x 10^6 spores / plate) mortality at 25 days and 14 % (3 x 10^3 spores / plate) to 86 % (3 x 10^6 spores / plate) at 55 days post-inoculation, increasing with increasing spore load. Control treatments resulted in 4 and 6 % mortality at 25 and 55 days post-inoculation, respectively. In a similar experiment with strain NRRL 21854 from Hillsboro, ND, mortality at 3 x 10^5 viable spores / plate was 92 and 96 % at 23 and 53 days post-inoculation. Control mortality was 9 and 34 % at these same time points.

SBRM larvae were readily infected with this entomopathogen and were typically killed within 14 to 28 days following contact with conidiospores. Rates of infection were higher and time to sporulation shorter for challenged first instar larvae cultured on a gellan gum base and reared from surface disinfested eggs (gnotobiotics). Sporulation of *S. tetanopsis* on cadavers of first instars was noted as early as seven days after inoculation in some experiments. Production of synnemata was observed on infected third instar SBRM, but never on first instars, most likely due to their small size.

Fruit (*Drosophila melanogaster*) and House (*Musca domestica*) fly challenges were performed on first and second instar larvae (15 or 25 / dish; n = 75 to 125) by directly inoculating larvae with conidiospore suspensions (3 x 10^5 / mL) and wetting of food (chopped liver - house fly; banana - fruit fly) and substrate surfaces (filter paper) to ensure > 3 x 10^5 spores / dish. Larvae were incubated at 27 °C and observed until pupation. Fruit flies reared on banana slices or on artificial media indicated no evidence of infection when challenged with conidiospore suspensions of *S. tetanopsis*. Control plates treated with 0.85 % saline showed no difference in survival, larval activity or pupation. Similarly, house fly larvae reared on chopped liver or rehydrated calf's liver did not demonstrate any detrimental effects following incubation and contact with spores. For

both species, inoculated and control cultures were allowed to proceed through pupation until emergence of adults. Attempts to infect these other dipterans (larvae), namely house flies and fruit flies, indicate that these species are not within the host range for the isolates of *S. tetanopsis* evaluated (NRRL 21853, NRRL 30031). Previous bioassays with four coleopteran, one lepidopteran and one neuropteran species also demonstrated a lack of pathogenicity. The apparent host range of this fungus is very narrow. Further experiments are underway to look at other dipteran species that may be of economic importance.

Current efforts are aimed at enhancing the time to sporulation in culture and spore viability. Oat based carbon and nitrogen sources (*i.e.*, oatmeal, oat bran, commercial cereals) have been modified with vegetable oil and cholesterol to achieve proper cultural nutrition. Supplemental nitrogen sources (*i.e.*, casein hydrolysate, tryptone, peptone) have been found to enhance conidial germination, however, they have been detrimental to spore production and have negative morphogenetic effects on culture development

The ability of *S. tetanopsis* to infect SBRM larvae (and adults to a lesser extent), persist in the soils of the Red River Valley (ND / MN), and be easily cultured, suggest that this pathogen has potential as a biological control agent for management of this destructive insect. The proper delivery system could provide for an effective alternative to current granular insecticides or as an amendment to these treatments under suitable field conditions. The parameters that influence this fungus in the soil and its efficacy as a biopesticide are only poorly understood presently. Further research will include an assessment of SBRM larval mortality and sugarbeet yield (tonnage, % sucrose, root damage ratings).