

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2013 – 2015**

TITLE: Auto-dissemination strategy for management of red clover seed crop pest with insect pathogens

RESEARCH LEADER: Sujaya Rao

COOPERATORS: Growers in the Willamette Valley

SUMMARY:

The clover root borer is a major pest of red clover seed production in the Willamette Valley. Adult and larvae feed internally within roots, pest infestation leads to a reduction in seed yield in the subsequent year, and consequently, the crop can only be grown economically for only two years. The pest is a challenge to control due to its subterranean life cycle. Organochlorine insecticides which controlled the pest earlier are banned due to negative impacts on the environment resulting from their persistence in the environment, and No new insecticides are labeled for the pest. The long term objective is to develop a biological control management strategy for the clover root borer. Specifically, in this proposal, we planned to focus on insect pathogenic fungi, and assess their virulence, and assess the potential for spread by auto-dissemination of the pathogen between individual clover root borers. Surveys were conducted in red clover seed production fields, and by collection of infected adults and use of the soil baiting method, we isolated and identified two strains of two species of insect pathogenic fungi. In a laboratory bioassay, these naturally occurring fungi were found to be as effective as commercial sources in killing the pest under laboratory conditions. The study also documented that, at high doses the insect pathogenic fungus *Beauveria bassiana* can transfer horizontally between clover root borer adults under laboratory conditions. Thus, based on the studies conducted, insect pathogenic fungi are present in red clover seed production fields, they can be as effective as commercially available insect pathogens, and the pathogens can be spread by auto-dissemination of fungal spores between clover root borer adults. The study suggests that some level of natural mortality of clover root borers may be occurring, and this could be augmented. The horizontal transfer of fungal spores documented in the study is critical for development of an auto-dissemination strategy for management of clover root borers.

OBJECTIVES: Our overall goal is to develop a biological control management strategy for the clover root borer. Specifically, in this proposal, we planned to focus on insect pathogenic fungi, and assess their virulence, and assess the potential for spread by auto-dissemination of the pathogen between individual clover root borers

PROCEDURES:

For assessing virulence of various pathogen species, we collected naturally occurring pathogens and compared their virulence with those of commercial products obtained from USDA labs.

Unhealthy red clover plants were collected from seed production fields in the Willamette Valley. Plant samples were placed in Berlese funnels for collection of adult clover root borer beetles. Pathogens observed growing on dead beetles were isolated and plated on appropriate media. In addition, soil baiting method using wax worms was used for isolating pathogens from soil samples. Two strains of two species of insect pathogenic fungi, *Beauveria bassiana* and *Isaria fumosorosea* were isolated from the fields (Fig. 1). The 4 strains of insect pathogenic fungi collected from the field and 2 commercial products, *Metarhizium anisopliae* and *Isaria fumosorosea*, were evaluated for their virulences against the clover root borer. Ten adult beetles per petridish were dipped in fungal spore solutions, 10^8 spores/ml. The trial was conducted as a randomized block design with six replicates. The number of infected CRB was recorded daily for two weeks. All insect pathogenic fungi tested caused more than 70% mortality of clover root borer adults (Fig 2.).

A second study was conducted to determine the extent of horizontal transfer of pathogenic fungi between adult clover root borers for assessing their efficacy in spreading the pathogen. In a laboratory bioassay, individual clover root borer adults dipped in suspensions of fungal spores at two concentrations were exposed to 10 untreated adults each in 5 replications, and the numbers of beetles that died were recorded on a daily basis. After 21 days, 67 % of beetles died with the higher concentration while 25% died with the lower concentration. To determine if the pathogen is transferred to the next generation, the clover root borers needed to be reared. There is no diet available for rearing clover root borers, and hence we attempted to rear the pest on red clover roots. Feeding and mating behaviors were observed but the females did not lay eggs.

For development of an auto-dissemination tactic, first the target pest has to be attracted to a device. We evaluated a diversity of traps including Lindgren traps for their efficacy in drawing clover root borers. We placed diverse clover volatiles in the traps for attracting the beetles. None of the traps were found to be effective.



Fig 1. Clover root borer adult infected by insect pathogenic fungus isolated from the field.

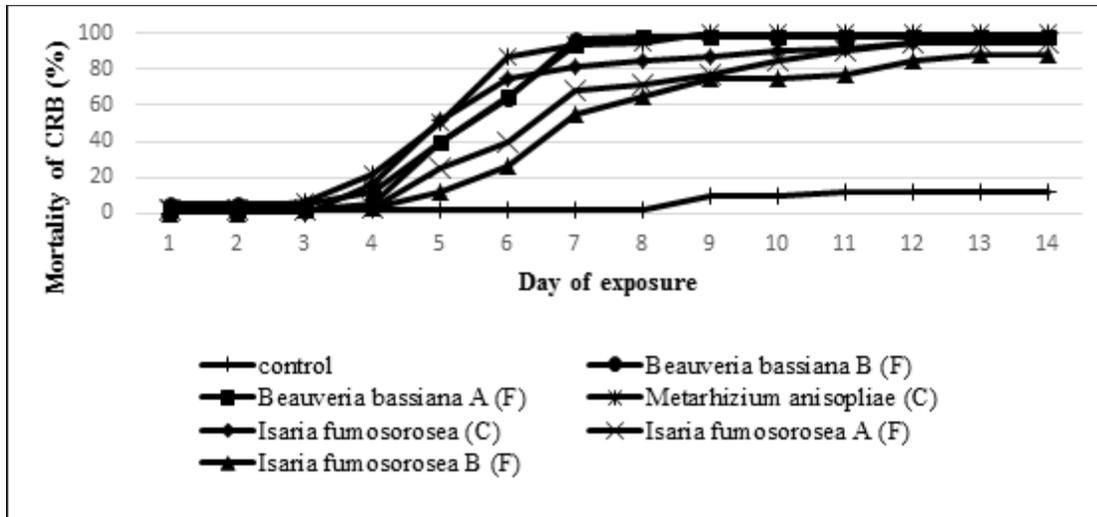


Fig.2. The virulence of entomopathogenic fungi against CRB. Mortality (%) of adult CRB exposed to spores of field-isolated and commercial strains. (C) = Commercial product; (F) = Field collected

SIGNIFICANT ACCOMPLISHMENTS:

The study documented that insect pathogens are present in the soil in red clover seed production fields. We detected and identified the presence of two strains of two species. The naturally occurring insect pathogenic fungi were found to be as virulent as the commercial products. Thus, clover root borers are likely being exposed to insect pathogenic fungi and some level of natural mortality is occurring in production fields. The study also documented that, at high doses, the insect pathogenic fungus *Beauveria bassiana* can transfer horizontally between clover root borer adults under laboratory conditions. Thus insect pathogenic fungi can spread by auto-dissemination of fungal spores between clover root borer adults. Thus, based on the study, some level of natural mortality of clover root borers may be occurring, and this could be augmented. The pathogens can spread between individual clover root borer adults and this will facilitate control of the pest.

Publications:

Lestari, A. and Rao, S. 2015. Entomopathogenic fungi as a potential biocontrol strategy for clover root borer management. In preparation for: Seed Production Research Seed Production Research, OSU

Presentations:

Lestari, A. and Rao, S. 2014. Managing a unique bark beetle pest with entomopathogens.

Entomological Society of America national meeting, November, Portland, OR:

Rickard, L. Rao, S. and Lestari, A. 2014. Horizontal transfer of *Beauveria* fungus between clover root borer beetle adults. OSU Undergraduate Poster

BENEFITS & IMPACT:

Currently, there is no insecticide registered for the clover root borer and no other management tactics are available. As a result red clover seed fields can be in production for a maximum of 3 years even though red clover is a perennial. The presence of naturally occurring insect pathogens in production fields that killed clover root borer adults under laboratory conditions suggest that some level of natural mortality may be occurring and could be augmented. The horizontal transfer of fungal spores documented in the study is critical for development of an auto-dissemination strategy for management of clover root borers.

ADDITIONAL FUNDING RECEIVED:

1. An undergraduate received an OSU URISC grant (Undergraduate Research, Innovation, Scholarship & Creativity) (\$ 1,800) for her proposal on “Innovative microbial management for key Oregon crop pest”.
2. A graduate student received a USDA-Western SARE graduate grant (\$ 12,859) for her proposal on “Managing a challenging subterranean clover pest: biological control using insect pathogens”.

A proposal for \$ 500 K submitted to USDA-AFRI (PI) entitled “Integrating semiochemical induced behavior with microbial control for management of challenging subterranean bark beetle agricultural pest” was not funded due to inadequate preliminary data.

FUTURE FUNDING:

Based on USDA-AFRI grant proposal reviewer responses, a lot more preliminary data needs to be collected before a proposal on this topic will be competitive at a national level. Strong stakeholder support is critical given the limited significance of the pest (clover root borer is a key red clover seed crop pest but only the Willamette Valley) and limited regions where red clover is raised for seed. Granting agencies prefer to fund proposals that can have a broad impact in terms of commodities and/or geographic region.