POTENTIAL FOR MANAGEMENT OF THE CLOVER ROOT BORER PEST IN RED CLOVER SEED PRODUCTION FIELDS USING INSECT PATHOGENIC FUNGI

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Introduction

The clover root borer (CRB, also known as clover crown borer) is a major pest of red clover seed production in the Willamette Valley. It was inadvertently introduced into the U.S. more than 100 years ago (Rockwood, 1926). The larvae and adults feed internally within roots. As a result, nutrient and water transport within the plant are disrupted, and infested plants turn brown, wilt, and die (Rockwood, 1926). Koehler et al. (1961) reported that about 40% of first-harvest-year plants are infested by the end of the first season, and by the second harvest year almost all plants are infested. Hence, a third year of seed production is generally not economical. Thus, the impact of the clover root borer is considerable, and effective management is critical for economical red clover production.

CRB is a challenge to control due to its predominantly subterranean life cycle. Adults are present above ground briefly in spring when they emerge, mate, and then migrate to new plants in the same or in different fields (Rockwood, 1926). Subsequent development occurs below ground. Females lay eggs in niches in clover roots, and the emerging larvae burrow within roots, feed on root tissue, develop slowly over the summer, and pupate inside the clover root. Adults emerge by late summer but remain below ground. The pest has one generation per year.

No pesticide is effective in suppressing CRB larvae and adults within roots. In a field trial conducted in 2011, four insecticides labeled for red clover seed crops were evaluated, but none caused significant mortality compared with the controls (Rao et al., 2012). Foliar applications of organochlorine insecticides such as aldrin, dieldrin, heptachlor, and lindane, which had long residual action, were effective against adults present above ground in spring (Gyrisco and Marshall, 1950). However, the persistence of these compounds in soil and groundwater, along with their low water solubility, which resulted in their accumulation in fatty tissue of non-target organisms, including humans and wildlife, led to a ban on their use in the 1970s. Currently no pest suppression strategy exists for CRB.

Insect pathogenic fungi, known as entomopathogenic fungi, have potential for management of subterranean pests (Brownbridge et al., 2006; Pilz et al., 2007). Rockwood (1926) reported one fungus, *Beauveria globulifera*, in association with CRB, but no further information is available on associations of this or other entomopathogenic fungi with CRB. The objectives of this study were to: (1) determine the presence of naturally occurring entomopathogenic fungi in red clover seed fields, and (2) assess virulence of naturally occurring and commercial entomopathogenic fungi against CRB.

Material and Methods

For all studies, CRB adults were collected from red clover seed production fields in the Willamette Valley. Roots of infested plants were transported to a laboratory, where they were cut longitudinally and placed in Berlese funnels overnight for collection of adults.

Objective 1. Determine the presence of naturally occurring entomopathogenic fungi in red clover seed fields

In 2013, during a preliminary survey, five field-collected CRB adults were observed to be infected with a fungus. The fungus was transferred to fungal media, Potato Dextrose Agar (PDA) for isolation and growth for subsequent identification. For further detection of entomopathogenic fungi, the soil around roots from individual plants was collected, placed in separate petri dishes and baited with five waxworms (*Galleria mellonella*). Waxworms were used as surrogate hosts instead of CRB due to their higher sensivity and larger suface area in baiting entomopathogenic fungi from soil. After two weeks, infected waxworms were removed, rinsed with water, and incubated in separate dishes on wet filter paper. Fungi from the infected waxworms were transferred to PDA media for fungal development and identification as described above. Confirmation of the fungal identities using molecular techniques is in progress.

In 2014, four red clover seed production fields were surveyed for further detection of naturally occurring entomopathogenic fungi. At three randomly selected locations in each field, a 4 meter x 4 meter grid was placed on the ground. Twenty-five plants from each grid were randomly dug up, bagged, and transported

Table 1. Entomopathogenic fungi evaluated for pathogenicity against CRB.

Species	Host or source	Type
Isaria fumosorosea (FE 9901)	White fly, Natural Industries	Commercial
Metarhizium anisopliae (F52)	Taenure granular bioinsecticide	Commercial
Beauveria bassiana A	Infected CRB	Field isolated
Beauveria bassiana B	Soil, Galleria baiting, red clover field	Field isolated
Isaria fumosorosea A	Soil, Galleria baiting, red clover field	Field isolated
Isaria fumosorosea B	Soil, Galleria baiting, red clover field	Field isolated
Control	Tween 80 (0.03% solution)	—

to the lab. The soil baiting method previously desribed was used for detection of entomopathogenic fungi. Soil was collected from around the roots of 10 randomly selected red clover plants at each location. Each of the 10 soil samples was placed in a petri dish and exposed to 5 waxworms. After two weeks, waxworms that died were removed and processed as previously described for fungal development. Identification of the fungi is in progress.

Objective 2. Assess the virulence of entomopathogenic fungi against CRB

Fungi evaluated in this study included the naturally occurring entomopathogenic fungi collected from red clover seed production fields (Objective 1) and two commercially available products, *Metarhizium anisopliae* and *Isaria fumosorosea* (Table 1). Field-collected adult beetles, 10 per petri dish, were dipped in fungal spore solutions (10^8 spores/ml) for five to seven seconds and then maintained in an incubator (22°C ± 1°C; 70–75% relative humidity). Spore solutions were dissolved in sterile water with the addition of Tween 80 (0.03%), which served as a surfactant for lowering the water tension for thorough mixing of the spores. CRB adults dipped in Tween 80 (0.03% solution) were used as controls. Parts of red clover roots were added to each dish for beetles to feed on. The experiment was set up as a randomized block design with six replications. Daily observations were made for two weeks on numbers of CRB adults that became infected with entomopathogenic fungi.

Results and Discussion

Naturally occurring entomopathogenic fungi in red clover fields

A single fungus was isolated from five field-collected CRB adults in 2013. Based on morphological characters, the fungus was identified as *Beauveria bassiana*. Of the 20 waxworms used as soil baits in 2013, 8 (40%) died due to fungal infection. Based on the morphology of the spores, spore-producing structures, and fungal colony characteristics, the fungi isolated from these waxworms were identified as belonging to two strains of entomopathogenic fungi, *Beauveria bassiana* (62.5%) and *Isaria fumosorosea* (37.5 %) (Table 2). In all, 13 entomopathogenic fungi were isolated from the red clover field surveyed in 2013 (Table 2). In 2014, out of the 600 waxworms used as baits, 147 (24.5%) died as a result of entomopathogenic fungal infection. The infection rate ranged from 15.3 to 28.7% across the four fields. Isolation and identification of the fungi are in progress.

Table 2. Naturally occurring entomopathogenic fungi isolated from the red clover field surveyed in 2013.

Species	Source	# isolates
Beauveria bassiana A	Soil	4
Beauveria bassiana B	5 dead CRB and soil	6
Isaria fumosorosea A	Soil	2
Isaria fumosorosea B	Soil	1

Virulence of entomopathogenic fungi against CRB

Fungal spores were observed on the mouth, thorax, and anal regions of CRB adults exposed to entomopathogenic fungi (Figure 1). All six fungal treatments caused significantly greater CRB mortality compared with the controls (Figure 2). Within seven days of exposure to fungal treatments, more than 50% mortality of CRB adults was observed, and by two weeks mortality increased to 70% or more (Figure 2).

Overall, the study documented the presence of two naturally occurring entomopathogenic fungi, *B. bassiana* and *I. fumosorosea*, in red clover seed production fields in western Oregon. Additionally, the study documented that field-isolated entomopathogenic fungi had similar levels of virulence compared to commercial products. Thus, *Beauveria bassiana*, *Isaria fumosorosea,* and *Metarhizium anisopliae* have potential as biological control agents for CRB. Further research is needed to determine the extent to which the naturally occurring entomopathogenic fungi are present in Willamette Valley red clover seed production fields, and to evaluate the efficacy of strains of the fungi used in the current study in suppressing CRB populations in the field.

References

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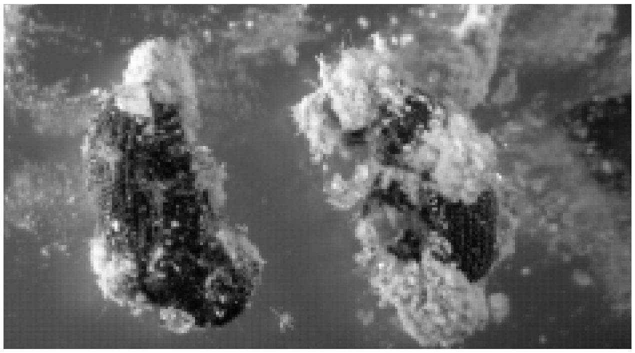

Figure 1. Fungal spores emerging from various body parts of CRB adults after exposure to an isolate of entomopathogenic fungus.

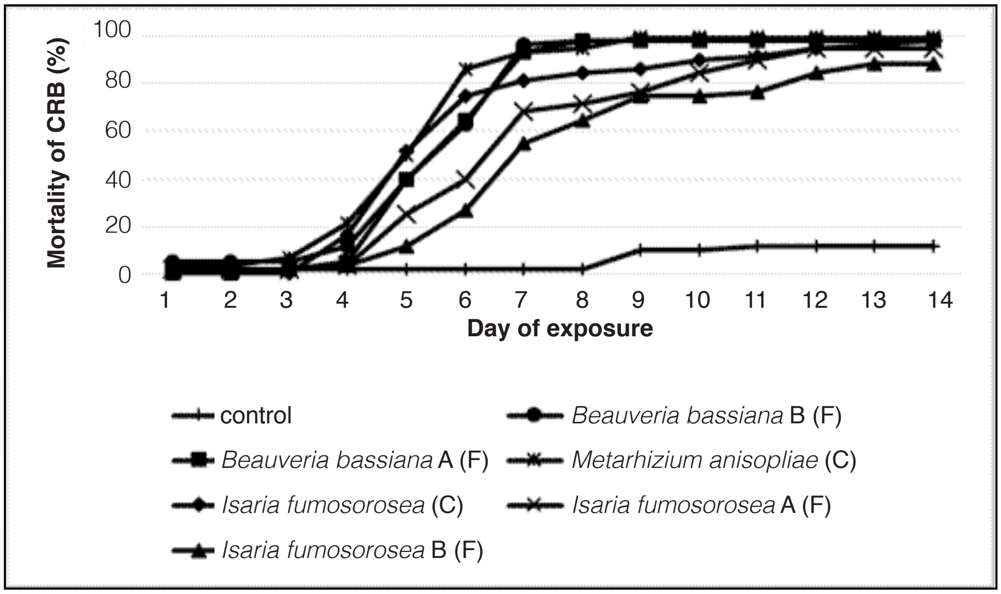

Figure 2. Mortality (%) of CRB adults exposed to spores of field isolates and commercial strains of entomopathogenic fungi. C = Commercial product; F = Field collected.