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Short Communication

Entomopathogenic fungi in cornfields and their potential to manage larval western corn rootworm *Diabrotica virgifera virgifera*

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ABSTRACT

Entomopathogenic ascomycete fungi are ubiquitous in soil and on phylloplanes, and are important natural enemies of many soil-borne arthropods including larval western corn rootworm, *Diabrotica virgifera virgifera*, which is a major pest of corn. We measured the prevalence of *Beauveria bassiana* and *Metarhizium anisopliae* sensu lato in ten cornfields in Iowa, USA by baiting with larval insects. *B. bassiana* and *M. anisopliae* s.l. were present in 60% ± 6.3% and 55% ± 6.4% of soil samples, respectively. Subsequent laboratory bioassays found that some *M. anisopliae* s.l. strains collected from cornfields killed a greater proportion of *D.v. virgifera* larvae than a standard commercial strain.

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1. Introduction

Western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae), is a major pest of corn in the United States and Europe, with larvae that live in the soil and feed on corn roots (Gray et al., 2009). Current management relies in part on corn that produces toxins of *Bacillus thuringiensis*; however, development of Bt resistance and predominant use of conventional corn hybrids in Europe requires the exploration of other management tactics (Gray et al., 2009; Gassmann et al., 2011). Entomopathogenic fungi, especially hypocreallean Ascomycetes, occur widely in the soil (Scheepmaker and Butt, 2010) and kill a variety of insect species (Hajek and St. Leger, 1994), including larval and pupal *D.v. virgifera* (Kuhlmann and van der Burgt, 1998; Pilz et al., 2008). In a two-year field study, we sampled cornfields in Iowa for the natural occurrence of entomopathogenic fungi *Metarhizium anisopliae* sensu lato (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Hypocreales: Cordycipitacea). We also measured mortality of *D.v. virgifera* larvae when challenged with naturally occurring *M. anisopliae* s.l. and *B. bassiana* strains isolated from cornfields, and with two commercial strains of these pathogens.

2. Materials and methods

2.1. Survey

Between 9 and 18 October, 2008 and between 23 September and 18 October, 2009, soil samples were obtained from five cornfields in Iowa that were planted to corn for at least two years; different fields on the same five farms were sampled in both years, for a total of ten fields. Six samples (each separated by ≥15 m) were collected from each field, with a sample consisting of the root mass and surrounding soil from one corn plant (ca. 5 L). Soil samples were sifted through a 1.5 cm sieve and stored at 4 °C. Three insect species were used for baiting entomopathogens: *Diabrotica virgifera virgifera*, *Galleria mellonella* and *Tenebrio molitor*. Baiting was conducted between 24 October and 7 November 2008, and between 21 October 2009 and 16 January 2010 (with more time used in 2009 because of initial contamination of *G. mellonella* with *B. bassiana*). *T. molitor* larvae were obtained commercially (Jax Outdoor Gear and Earl May Nursery & Garden Center, Ames, Iowa). Fifth instar *G. mellonella* and early third instar *D.v. virgifera* larvae were reared in the laboratory following King and Hartley (1985) and Jackson (1985), respectively. The *D.v. virgifera* larvae were from a non-diapausing laboratory strain obtained from United States Department of Agriculture's North Central Agricultural Research Laboratory.

Each soil sample was divided among three 500 ml containers (Reynolds Del-Pak 16 oz Container, Johnson Paper & Supply Co. Minneapolis, Minnesota). Containers with *D.v. virgifera* received

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50 ml of soil and containers with *G. mellonella* and *T. molitor* received 150 ml. Ten pieces of corn root, 1 cm long, were added from each sample, soil was moistened with deionized water following Goettel and Inglis (1997), and six insects of a single species were placed in each container. For each species, 36 insects were exposed to soil from each of the five sites per year, for a total of 360 insects per species and 1080 insects per year. Controls were run simultaneously with soil samples, and each 500 ml control container consisted of six insects exposed to either autoclaved field soil or moistened paper towels. In 2008, for each insect species, two replicates were established for each type of control, for a total of 72 control insects. In 2009, 10 replicates were run for each insect species by type of control, for a total of 357 control insects.

Containers were held in an incubator (27 °C, 0/24 L/D) and checked every 2 to 3 d for a total of 11 d. Dead larvae were placed on modified White traps (Kaya and Stock, 1997), which allowed fungal infections to become expressed. Fungi were identified by morphological characteristics (Brady, 1979; Samson et al., 1988) and confirmed by culturing on semi-selective media (Goettel and Inglis, 1997), with *M. anisopliae* s.l. cultured on the medium of Veen and Ferron (1966) and *B. bassiana* on the medium of Doberski and Tribe (1980).

2.2. Bioassays

Bioassays used three strains each of *M. anisopliae* s.l. and *B. bassiana*. *B. bassiana* strains D2008 and D2009 were isolated from *D.v. virgifera* larval cadavers at site D in 2008 and 2009, respectively (Fig. 1A and 1B). Strain GHA was derived from the commercial product BotaniGard ES (Laverlam International, Butte, Montana). *M. anisopliae* s.l. strains C20091 and C20092 were each isolated from individual *D.v. virgifera* larval cadavers at site C in 2009 (Fig. 1D), and *M. brunneum* (F52 strain) (=*M. anisopliae* sensu lato) was derived from the commercial product Met52 EC (Novozymes Biologicals, Salem, Virginia). Strains obtained from *D.v. virgifera* were isolated by transferring external spores from the cadavers to selective media for further culturing (Goettel and Inglis, 1997).

Bioassays used sieved (0.6 mm mesh), air-dried field soil. The GHA strain (*B. bassiana*) was produced using solid substrate fermentation (Jaronski and Jackson, 2012) and represented the sixth passage since culturing in insects. Previously, this strain had no change in efficacy after 15 *in vitro* cycles (Brownbridge et al., 2001). The remaining five strains were cultured on *G. mellonella* larvae. Live larvae were infected by dipping into a concentrated spore solution (>1 × 10^6 spores per ml) for 5 s, incubating in sealed Petri dishes in the dark at 27° C until the insects died (3–5 d), and placing the cadaver in a modified White trap. Conidia were harvested by vortexing three to seven conidia-covered cadavers with 8 ml of 0.1% Tween for 2 min, sonicating 10 min, and vortexing. Conidial viability was determined and soil was prepared by mixing each fungal solution with soil to achieve a uniform distribution of inoculum at the desired concentration and 25% water holding capacity following MacDonald and Ellis (1990). Bioassays were conducted in 45 ml containers (Souffle Cup, Solo Cup Company, Highland Park, Illinois) containing three germinated corn seedlings, 30 g of moistened soil and six second instar *D.v. virgifera* larvae. Containers were covered with fine mesh and a vented lid, and placed between two trays lined with moist paper towels. *M. anisopliae* s.l. was applied at 6.1 × 10^5 and 6.1 × 10^6 conidia per g of soil and *B. bassiana* was applied at 1.33 × 10^6 and 1.33 × 10^7 conidia per g of soil, with the high concentrations selected to achieve 50% mortality based on preliminary experiments with F52 and GHA. Ten days after larvae were placed in bioassay containers, soil and corn roots were carefully inspected, and the number of live larvae counted. Six replicates were run (except the high concentration of F52, which had four replicates), with each replicate consisting of three containers per strain per concentration, for a total of 210 containers and 1260 larvae. Six control containers, which were identical to experimental containers but did not receive fungal spores,

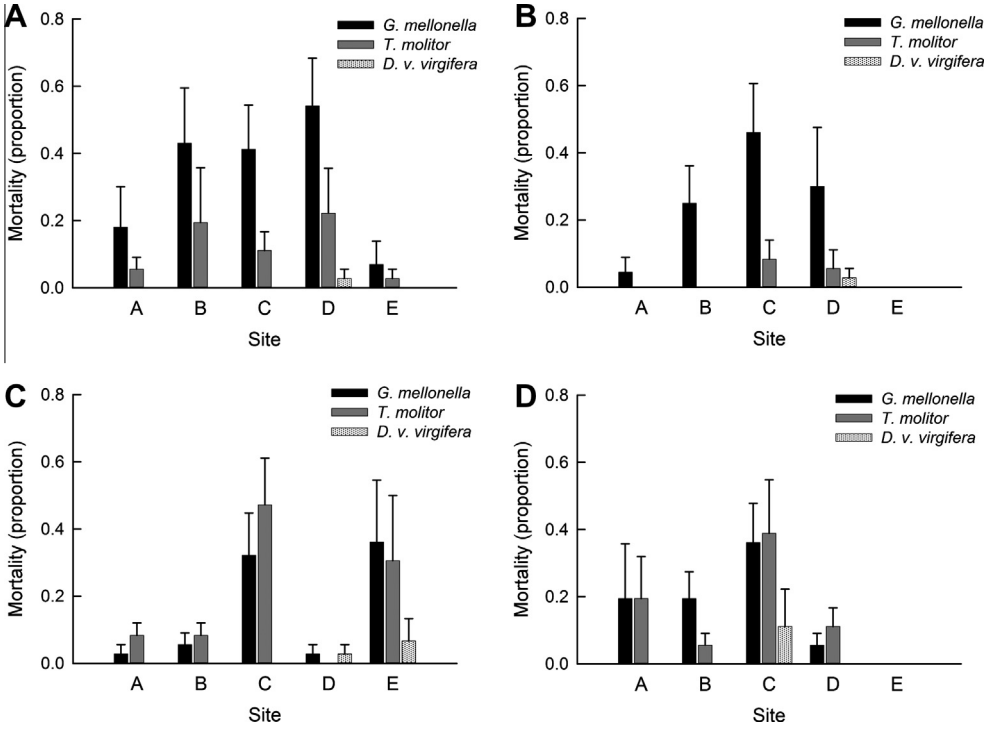

Fig. 1. Mortality from entomopathogenic fungi. Data are present for *B. bassiana* in (A) 2008 and (B) 2009, and for *M. anisopliae* sensu lato in (C) 2008 and (D) 2009. Bar heights are sample means and error bars are the standard error of the mean. Letters on the *x*-axis indicate site (i.e., farm) sampled. The *y*-axis describes the average proportion of insects killed by each pathogen.