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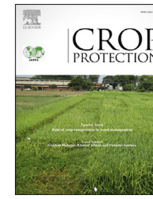

# Evaluation of low risk methods for managing *Delia radicum*, cabbage root fly, in broccoli production

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ABSTRACT

The cabbage root fly (CRF; *Delia radicum*) is currently difficult to control as insufficient plant protection products are available, especially in organic farming. In this study the influence of two strains of the entomopathogenic fungi *Metarhizium brunneum* and *Beauveria bassiana* (Hypocreales), straw mulch, and nitrogen lime were investigated in regard to CRF and root damage reduction in field trials. Spinosad-based insecticide was used as a positive control, because of its known efficacy against CRF. Furthermore, rhizosphere competence and endophytism, mineral uptake, chlorophyll *a* fluorescence, and growth parameters of *M. brunneum* treated plants were examined under field and/or greenhouse conditions. Neither fungi nor straw mulch or nitrogen lime decreased CRF pressure significantly, but spinosad did. *M. brunneum* colonized the rhizosphere of broccoli plants but did not show endophytic characteristics. A significant increase of Mg and N concentrations was detected in *Metarhizium*-treated plants when grown in the greenhouse whereas no different nutritional values in plant tissue were observed in field grown plants. Plants from the greenhouse also exhibited higher maximum quantum efficiency of PS2 photochemistry and electron transport rate as well as promoted growth when inoculated with *M. brunneum*. We conclude that for brassicas where the CRF damaged parts are not consumed (e.g. cauliflower) the tested low risk CRF management tools might be adequate. But for brassicas, where CRF directly damages the produce (e.g. turnips), treatments having greater impact on CRF (e.g. spinosad) should be considered.



## 1. Introduction

Cabbage root fly (CRF; *Delia radicum* L.) is one of the major root-feeding pests in *Brassica* vegetables in the north temperate region (Finch, 1993; Ferry et al., 2009). Adult females lay eggs in the soil at or near the plant base of wild or domesticated cruciferous plants (de Jong and Staedler, 1999; Jensen et al., 2002). After egg hatch, the larvae feed on plant roots and can affect plant development by disrupting water and nutrient transport (Broatch et al., 2006). Plants infested with CRF larvae show yellowing, stunting and slow growth (Natwick, 2009). Losses of up to 100% (Ferry et al., 2009) are possible and young plants are especially at risk (Bligaard, 1999). Indirect damage to plants occurs when larvae feed on tissue not used for human consumption while direct damage applies when the harvested parts of plants are affected. The latter occurs when roots of radish, swedes or turnips are infested (Finch, 1993) and when crops show slight feeding damage rendering them unmarketable (Bruck et al., 2005). In the past, control of CRF was mainly based on organophosphate and carbamate insecticides (Chandler and Davidson, 2005). However, the number of registered insecticides is declining or their application is limited. In Germany, insecticide products with the active ingredients spinosad and clothianidin are currently registered for CRF control in cabbage crops. In Slovenia there are no registered chemical control agents available. Control of CRF is especially difficult in organic plant production as no synthetic insecticides are allowed. According to EU regulations, spinosad can be used in organic production systems, however, its use has been rejected by some organic farming

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associations.

Considerable effort has thus been invested in the development of alternative integrated CRF management strategies utilizing entomopathogenic fungi (Inglis et al., 2001), principally due to their low non-target impact and low impact on environmental safety (Liu, 2012). Particularly *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin as well as *Metarhizium brunneum* Petch (Hypocreales: Clavicipitaceae), which may have been misidentified in the past as *M. anisopliae*, were investigated in laboratory and greenhouse experiments (Bruck et al., 2005; Chandler and Davidson, 2005; Myrand et al., 2015; Razinger et al., 2014a, 2014b; Vänninen et al., 1999a). These fungi are often applied innundatively to the soil so they may infect insect pests once they appear (Bruck, 2005). Both fungi are ubiquitous insect pathogens and infect and kill dipterous insects via external contact or after ingestion by their insect host (Lacey et al., 1988; Thomas and Read, 2007). Some strains may also enhance plant growth and have been discussed as beneficial rhizosphere colonizers or endophytes (Pava-Ripoll et al., 2011; Razinger et al., 2014a; Vega et al., 2009). Plant growth promotion by *M. anisopliae* was reported for maize (Kabaluk and Ericsson, 2007), tomato (García et al., 2011), and cowpea (Ngakou et al., 2007). Following work by Vega et al. (2009), who suspected enhanced provision of mineral nutrients, we hypothesized that any significant changes in plant growth or higher levels of plant physiological processes, e.g. photosynthesis, would have its origin in changes in mineral uptake.

Nitrogen lime and straw applications are frequently mentioned as alternative methods for CRF management. Cyanamid is released on contact with soil water (AlzChem, 2016) and is known to possess lethal effects on insects of different orders (Ritter et al., 2014). Applying straw on the soil surface uses similar principles to intercropping but without possible competition with the main culture (Hellqvist, 1996). The procedure is aimed at affecting the optical orientation of CRF females and thus reducing oviposition (Hofsvang, 1991).

Reduction in pesticide dependency and associated environmental and health-related risks is an integral part of the European Union's agenda for agriculture and was addressed in the context of the European Project PURE (Pesticide Use-and-Risk reduction in European farming systems with Integrated Pest Management, http://www.pure-ipm.eu). Field studies were conducted at the Agricultural Institute of Slovenia (AIS) and Julius Kühn-Institute (JKI), Germany, investigating alternative CRF management tools, including applications of nitrogen lime, straw and biopesticides in cruciferous crops such as broccoli. Furthermore, rhizoplane colonization and endophytism of selected fungi and their plant-growth promoting effect was evaluated under greenhouse and field conditions. The aims of the studies reported here were to assess whether these low risk CRF management tools offer sufficient protection to broccoli plants, and to investigate plant-microbe-insect interactions.

## 2. Material and methods

### 2.1. Fungi collection and growing

The *M. brunneum* strain 1154 was isolated from soil in Slovenia. The isolate was kept in the mycological collection of the Agricultural Institute of Slovenia. The fungal isolate was grown on potato dextrose agar (PDA, Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Fungal cultures were incubated at 24 ± 1 °C in darkness for 14 d. Conidial suspensions were prepared by adding 20 ml of sterile 0.05% Tween 80 (Sigma–Aldrich Chemie GmbH, Buchs, Switzerland) to one PDA plate of sporulating colonies that were then scraped with a sterile objective glass in a laminar flow chamber. Retrieved suspensions were collected in 50 ml centrifuge tubes (Vitaris AG, Baar, Switzerland). Tubes were vortexed vigorously for 30 s and shaken on an orbital shaker (650 RPM, 30 min). This produced a suspension of approximately $10^8$ conidia $ml^{-1}$. Conidial viability was assessed by plating 100 µl of a 10-fold dilution of the original suspensions onto malt extract agar (MEA, Sigma–Aldrich Chemie GmbH, Buchs, Switzerland). The plates were incubated at 24 ± 1 °C. After 24 h they were covered with coverslips (20 × 40 mm) and observed under a microscope. Conidia were considered viable if the germination tube was at least twice as long as the diameter of the conidium. Test suspensions of the desired concentrations were prepared after the viability assessment of the spores using a haemocytometer (Faust Laborbedarf AG, Schaffhausen, Switzerland).

### 2.2. Field experiments and CRF pressure

Similar protocols were adopted in field experiments based on broccoli cultivar 'Montop' (Slovenia) and 'Belstar' (Germany) in summer of 2013. Young plantlets were transplanted on experimental fields on 26 April 2013 in Slovenia (AIS experimental station Jablje, near Ljubljana) and 19 July 2013 in Germany (JKI experimental station Ahlum, near Braunschweig). The in-row distance of plants in Slovenia was 70 cm and the inter-row distance 40 cm on lifted beds, whereas in Germany 50 cm in-row and 40 cm inter-row distances were used. Experimental fields were divided into six beds at five plots in Slovenia and four beds at four plots in Germany according to a randomized block design. In Germany experiments were conducted on organic farmland. Therefore no fertilizer was applied and weeds were controlled mechanically if necessary. In Slovenia 600 kg/ha NPK (5:16:24) and 400 kg/ha KCl (60%) were added before transplanting. At hoeing (15 May 2013) an additional amount of 200 kg/ha N fertilizer (27% - half as $NO_3$ and half as $NH_4$) were added except for the nitrogen lime treatment, where 500 kg/ha nitrogen lime was applied (see below). Another fertilisation with 200 kg/ha of N fertilizer (27%) was performed on all plots on 7 June 2013. Weed control was performed with hoeing and Glyphosate (Touchdown®) only between beds on 22 May 2013. Other herbicide treatments were avoided to minimize their effect on biopesticide treatments. The egg-laying of cabbage root flies was monitored twice a week with 25 felt traps (Freuler and Fischer, 1982) until five weeks after transplanting in Germany, and once a week with 20 felt traps in Slovenia.

### 2.3. Treatments

Plants were treated with spinosad (product Laser®, Dow AgroSciences, Vienna, Austria or SpinTor®, Dow AgroSciences, Munich, Germany) and *B. bassiana* (product Naturalis®, Andermatt Biocontrol AG, Grossdietwil, Switzerland, based on strain ATCC 74040) one day before transplanting. In Slovenia Laser® (a.i. spinosad, 22.75%) was applied at rates of 24 ml/1000 plants (5.46 mg a.i./plant), whereas in Germany SpinTor® (a.i. spinosad, 44% a.i.) was used at rates of 12 ml/1000 plants (5.76 mg a.i./plant). Insecticides were applied by pipetting 3 ml of 125-fold diluted Laser® per plant in Slovenia, or as a drench treatment of 250-fold diluted SpinTor® in Germany. In both countries 5 ml of $4.6 \times 10^6$ viable conidia/ml ($1.15 \times 10^7$ viable conidia/plant) of *B. bassiana* were pipetted around the stem of each plant. *M. brunneum* (strain HJS 1154) was applied two days prior to transplanting by pipetting 2 ml of $1.15 \times 10^7$ viable conidia per ml suspension ($2.30 \times 10^7$ viable conidia /plant) around the stem of each plant. In Slovenia the effect of 500 kg/ha Nitrogen lime (PERLKA®) and the establishment of a straw mulch soil cover at first hoeing (15 May 2013) were also tested.