LABORATORY BIOASSAYS OF ENTOMOPATHOGENIC OR POTENTIALLY PLANT GROWTH PROMOTING FUNGAL STRAINS FOR THE CONTROL OF CABBAGE ROOT FLY (*Delia radicum* L.) AND THEIR RHIZOSPHERE COMPETENCE

Jaka RAZINGER[1], Matthias LUTZ[2], Hans-Josef SCHROERS[3], Gregor UREK[4], Jürg GRUNDER [5]

[1,3,4] Kmetijski inštitut Slovenije, Oddelek za varstvo rastlin, Ljubljana
[1,2,5] Zuriška univerza za aplikativne znanosti, Wädenswil, Switzerland

ABSTRACT

Entomopathogenicity of 9 entomopathogenic and/or potentially plant growth promoting fungal species was assessed against cabbage root fly (CRF) in soil and in-vitro laboratory bioassays. 18 different isolates were tested. All isolates tested were infective to one or more life stages of CRF (eggs, larvae, pupae or imago). Abbott's corrected mortality in soil experiments ranged from 4.5 ± 13.6 % to 58.4 ± 15.0 % and in the in-vitro experiments from 8.7±5.0 % to 47.6±9.0 %. The 7 most pathogenic isolates (*T. atroviride*, *T. konigiopsis, T. gamsii*, *B. bassiana*, *M. anisopliae* (2 isolates) and *C. solani* f. *nigrovirens*) were further tested for their rhizosphere competence and abilities to grow as endophytes. The preliminary results showed that rhizosphere competence varied slightly but endophytism considerably, possibly due to the ecological preferences of the different fungal species.

Keywords: biological control, biopesticide, Diptera, pest

IZVLEČEK

LABORATORIJSKI POSKUSI ENTOMOPATOGENIH ALI POTENCIALNO RAST SPODBUJAJOČIH GLIV ZA ZATIRANJE KAPUSOVE MUHE (*Delia radicum* L.) IN NJIHOVA RIZOSFERNA KOMPETENCA

Ocenjevali smo entomopatogeni potencial devetih entomopatogenih ali potencialno rast spodbujajočih vrst gliv za zatiranje kapusove muhe (KM). Preskušali smo 9 vrst gliv, skupno 18 različnih izolatov. Vsi testirani izolati so uspešno okuževali enega ali več razvojnih stadijev KM (jajčeca, ličinke, bube ali image). Smrtnost po Abbottovemu popravku v substratnih poskusih je bila od 4,5 ± 13,6 % do 58,4 ± 15,0 %, v *in-vitro* poskusih pa od 8,7 ± 5,0 % do 57,6 ± 12,0%. Sedem najbolj patogenih izolatov (*T. atroviride, T. koningiopsis, T. gamsii, B. bassiana, M. anisopliae* (2 izolata) in *C. solani*) je bilo dodatno testiranih za njihovo rizosferno prilagojenost in endofitskost. Prvi rezultati nakazujejo, da se rizosferna prilagojenost malo razlikuje, endofitskost pa precej, verjetno zaradi specifičnih okoljskih zahtev različnih izolatov gliv.

Ključne besede: biotično varstvo, biopesticid, Diptera, škodljivec

[1] dr., Hacquetova ulica 17, SI-1000 Ljubljana in Campus Grueental P.O.Box 335, CH-8820 Wädenswil, Švica; e-mail: jaka.razinger@kis.si
[2] dr., Campus Grueental P.O.Box 335, CH-8820 Wädenswil, Švica
[3] dr. rer. nat., Hacquetova ulica 17, SI-1000 Ljubljana
[4] doc. dr., prav tam
[5] dr., Campus Grueental P.O.Box 335, CH-8820 Wädenswil, Švica

1 INTRODUCTION

Cabbage, cauliflower and other brassicaceous plants are attacked by many insect pests (Klingen *et al*., 2002). Specifically the cabbage root flies (CRF) *Delia radicum* and *Delia floralis* present major threats for many *Brassica* crops in Europe (Vanninen *et al*., 1999). No sustainable control strategies are currently available however, several papers report that larvae of *Delia* sp. can be biologically controlled using entomopathogenic fungi (Vanninen *et al*., 1999; Klingen *et al*., 2002; Bruck *et al*., 2005).
Entomopathogenic fungi kill dipterous insects after they are ingested or through infection via external contact (Thomas and Read, 2007; Toledo *et al*., 2007). Both mechanisms imply that an effective biological control agent should live in juxtaposition of either the crop or the insect pest. The aim of the study was to screen aggressiveness of the various isolates to CRF, to evaluate their rhizosphere competence and possible endophytic lifestyle, and to check if the fungal isolates have an effect on plant growth and survival.

2 MATERIALS AND METHODS

2.1 *Delia radicum* rearing

CRF were reared according to protocols kindly provided by Dr. M. Hommes (Julius Kühn Institute, Braunschweig, Germany) and Harris and Svec (1966) with minor modifications.

2.2 Entomopathogenic fungi collection and growing

Nine entomopathogenic or potentially plant growth promoting fungal species were used in the *in-vitro* and soil laboratory bioassays. The fungal strains were isolated from various substrata in Slovenia. The isolates are being kept in the mycological collection of the Agricultural Institute of Slovenia. Altogether, 18 different isolates were tested: *Trichoderma atroviride* (isolates 1872 and 1873), *T. koningiopsis* (1874), *T. gamsii* (1875, 1876 and 1879), *Beauveria brongniartii* (1877), *B. bassiana* (1174 and 1878), *Metarhizium robertsii* (1880), *M. anisopliae* (1154, 1704, 1858 and 1868), *Purpureocillium lilacinum* (1796 and 1797) and *Clonostachys solani* f. *nigrovirens* (1828 and 1860).

2.3 Entomopathogenicity tests

The *in-vitro* tests were designed to screen pathogenicity of the various fungal isolates to CRF. 50 µl of 1 x 10^8 viable conidia ml^{-1} suspension was directly applied to 10 CRF eggs. 5 replicates per treatment with 10 eggs per individual test vessel were made. Experiments were observed after 7 (egg hatching) and 14 (live larvae counting) days.
The soil experiments mimicked natural exposure pathways of the various insect life stages to the fungal strains. Spore concentrations used in soil tests were comparable to economic rates for in-furrow application (3.85 x 10^6 spores/g dry soil). Experiments were observed after 14 (rutabaga replacement) and 35 (pupae counting) days.

2.4 Rhizosphere competence experiments

7 most entomopathogenic isolates were further tested for their rhizosphere competence and endophytism in cauliflower (*Brassica oleracea* Botrytis group). Rhizosphere competence was assessed by placing root pieces, washed 3-times with sterile water, on selective media. Endophytism was assessed as EPF growing out of 3-times washed and surface sterilized root pieces.

2.5 Biostimulating effects

Plant weight and mortality was assessed at experiment endpoint to provide information on possible biostimulating effects of the fungal inoculums.

3 RESULTS AND DISCUSSION

3.1 Pathogenicity of the EPF isolates

All isolates tested were infective to one or more of the life stages of cabbage root fly - CRF (eggs, larvae, imago or pupae). Abbott's corrected mortality (Abbott, 1925) ranged from 8.7 ± 5.0 % to 57.6 ± 12.0% in the short term in-vitro experiments and 4.5 ± 13.6 % to 58.4 ± 15.0 % in the soil experiments. For a graphical representation of the results see Figure **1**.

Figure 1: Infection of various cabbage root fly life stages by some of the entomopathogenic fungi tested. Top left: *M. anisopliae* (1868) infected eggs. Top right: *B. bassiana* (1174) infected larva. Bottom left: *M. robertsii* (1880) infected pupa. Bottom right: *M. anisopliae* (1868) infected imago.

Some isolates performed better in *in-vitro* tests, whereas some performed better in soil tests. This is probably because of different ecological preferences of the tested fungi (Scheepmaker and Butt, 2010; Pava-Ripoll et al., 2011). 7 most pathogenic EPF isolates were further tested for their rhizosphere competence and potential biostimulating effects. The selected isolates were: *M. anisopliae* (1154 and 1868), *B. bassiana* (1174), *C. solani* (1828), *T. atroviride* (1873), *T. konigiopsis* (1874) and *T. gamsii* (1876).

3.2 Rhizosphere competence and endophytism

All tested fungal species colonized the rhizoplane (the immediate root surface). All tested fungal species except *M. anisopliae* (1154), *B. bassiana* (1174) and *C. solani* (1828) exhibited endophytic behavior – they were successfully isolated from within plant tissue. All *Trichoderma* spp. isolates (1873, 1874 and 1876) always inhabited plant tissue. *Trichoderma* spp. isolates 1874 and 1876 inhabited all plant tissues (Figure 2).

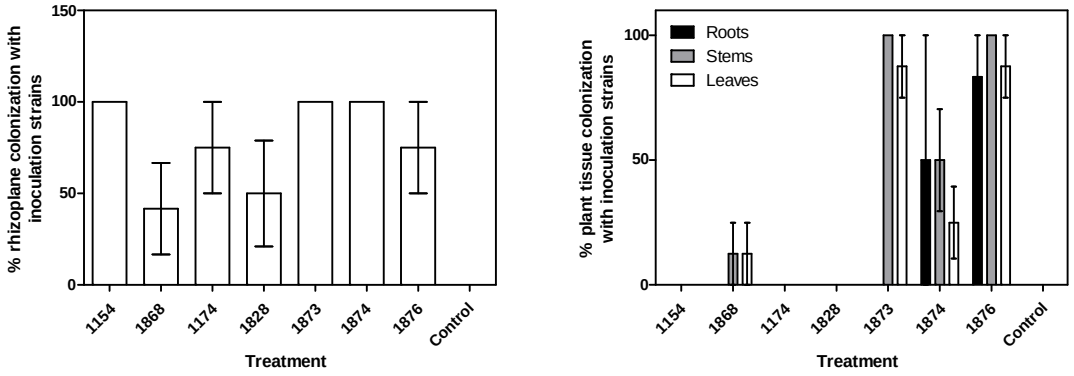

Figure 2: Rhizoplane colonization (left) and endophytism (right) of the seven most entomopathogenic fungal isolates. The isolates tested were: *M. anisopliae* (1154 and 1868), *B. bassiana* (1174), *C. solani* (1828), *T. atroviride* (1873), *T. konigiopsis* (1874) and *T. gamsii* (1876).

3.3 Biostimulating effects

B. bassiana (1174) increased plantlet germination and / or survival. Both isolates of *M. anisopliae* (1154 and 1868) and *T. atroviride* (1873) increased biomass production (Figure 3).

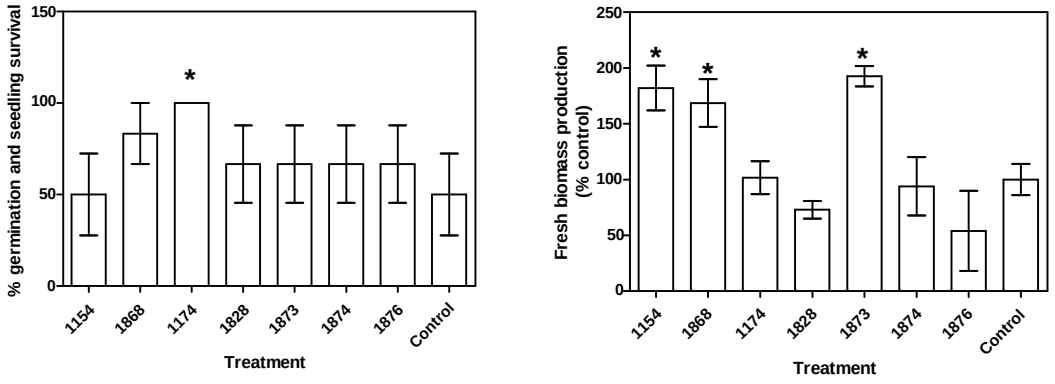

Figure 3: The effect of fungal isolates on germination and seedling survival (left) and fresh biomass production (right). The isolates tested were: *M. anisopliae* (1154 and 1868), *B. bassiana* (1174), *C. solani* (1828), *T. atroviride* (1873), *T. koningiopsis* (1874) and *T. gamsii* (1876).

4 CONCLUSIONS

All 18 fungal isolates tested were infective to one or several pest life stages (eggs, larvae, pupae or flies). The 7 most pathogenic isolates were all found to colonize the rhizoplane successfully – most of them were also able to colonize plant tissue. Additionally, *B. bassiana* (1174) significantly increased germination and seedling survival whereas *M. anisopliae* (isolates 1154, 1868) and *T. atroviride* (1873) significantly increased fresh biomass

production. These isolates (1174, 1154, 1868 and 1873) could be possibly considered as an environmentally friendly alternative for CRF control in conventional or organic farming systems.

5 LITERATURE

Abbott W.S., 1925. A Method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265-267.

Bruck D.J., Snelling J.E., Dreves A.J., Jaronski S.T., 2005. Laboratory bioassays of entomopathogenic fungi for control of *Delia radicum* (L.) larvae. Journal of Invertebrate Pathology 89, 179–183.

Harris C.R.; Svec H.J., 1966. Mass rearing of the Cabbage maggot under controlled environmental conditions, with observations on the biology of cyclodiene-susceptible and resistant strains. Journal of Economic Entomology 59, 569-573.

Klingen I., Hajek A., Meadow R., Renwick J.A.A., 2002. Effect of brassicaceous plants on the survival and infectivity of insect pathogenic fungi. BioControl 47: 411–425.

Pava-Ripoll M, Angelini C, Fang W, Wang S, Posada FJ, St Leger R., 2011. The rhizosphere-competent entomopathogen *Metarhizium anisopliae* expresses a specific subset of genes in plant root exudate. Microbiology 157, 47-55.

Scheepmaker J.W.A. & Butt T.M., 2010. Natural and released inoculum levels of entomopathogenic fungal biocontrol agents in soil in relation to risk assessment and in accordance with EU regulations. Biocontrol Science and Technology 20, 503-552.

Thomas M.B., Read A.F., 2007. Can fungal biopesticides control malaria? Nature Reviews Microbiology 5, 377 – 383.

Toledo A.V., Virla E., Humber R.A., Paradell S.L., Lastra C.C., 2006. First record of *Clonostachys rosea* (Ascomycota: Hypocreales) as an entomopathogenic fungus of *Oncometopia tucumana* and *Sonesimia grossa* (Hemiptera: Cicadellidae) in Argentina. Journal of Invertebrate Pathology 92, 7-10.

Vänninen I., Hokkanen H., Tyni-Juslin J., 1999. Attempts to control cabbage root flies *Delia radicum* L. and *Delia floralis* (Fall.) (Dipt., Anthomyiidae) with entomopathogenic fungi: laboratory and greenhouse tests. Journal of Applied Entomology 123, 107-113.