Molecular biological quantification of the causal agent of common bunt in wheat

M Kochanova, M Marek, E Prokinova, P Rysanek
University of Agriculture in Prague, Kamycka 129, 16521 Prague 6 Suchdol, Czech Republic
Email: kochanova@af.czu.cz

INTRODUCTION

Hyphae of the causal agent of common bunt (*Tilletia caries*, syn. *T. tritici*) penetrate the tissue of wheat during the growing phase, but the infection process is latent until plants reach full maturity. It then transforms grains into sori filled with *Tilletia* teliospores. *Tilletia* produces strongly smelling trimethylamin, which prevents grain being used for food or from being stored. The presence of common bunt lowers the harvest quality index and leads to total loss of the crop; this means loss of all costs incurred in growing the crop (including fertilizer and other treatments, and harvest) and its disposal.

Basic precautions against bunt include the use of resistant wheat cultivars. These already exist in the world collection and the others are constantly being bred because the pathogen constantly overcomes plant resistance (Wilcoxson & Saari 1996). Optimal inoculation methods and plant infection by bunts are tested. All methods need visual confirmation of the presence of bunt at full maturity, and comparisons of the number of infected and non-infected plants (Gaudet *et al.*, 1989; Leijerstam, 1991; Goates, 1996; Blazkova & Bartos, 2002). None currently use molecular diagnostics or other modern methods for reducing the time for resistance testing. In the future, molecular biological methods could be rapid and a precise solution to several months of plant cultivation. Using polymerase chain reaction (PCR), the genus *Tilletia* has already been detected. Using quantitative PCR it would be possible to quantify pathogen at the early growth stage, and the amount present in a plant would demonstrate resistance grading of the cultivar.

Sequences from *Tilletia* mycelium DNA and wheat DNA were cloned to a bacterial vector, and used together with TET-labelled probes in Real-Time PCR for quantification of mycelium in plant tissue of two wheat varieties with different resistance to *Tilletia*.

METHODS

Biological material was *Tilletia caries* mycelium, wheat plants (at the three-node growth stage) infected by *T. caries* and uninfected wheat plants.

Mycelium DNA was extracted by using CTAB DNA extraction protocol, amplified by Tilf (5′–CACAAGACTACGGAGGGGTG–3′) (Kochanova *et al.*, 2004) and Tc-R (5–ATGCCACATTTCTCCTACTATTATCCA–′3′) (McNeil *et al.*, 2004) primers and visualisated on agarose gels. Obtained PCR products were extracted from agarose gels, purified and cloned to plasmid, and used to reaction as standards in concentrations of 10–10^8 molecules.

Apical meristems from plants infected by *T. caries* and non–infected plants were isolated, and DNA was extracted by using CTAB protocol extraction and used as samples.

Mastermix for qPCR reaction included DyNamo kit (Finnzymes), probe Tc-Pr, primers Tc-F, Tc-R (McNeil *et al.*, 2004).

RESULTS AND DISCUSSION

Standards have concentrations of 10^2–10^8 plasmids. Experimental plant samples have C(t) values of 27.70 to 27.87, which means 1.41×10^6 to 2.3×10^5 DNA copies/microgram of infected tissue (C(t), log DNA copies, DNA copies/microgram). Standards prepared in this way can be used for quantitation of mycelium in plant tissue. Although there is no information on the number of sequence copies used in the cells, it is possible to compare the obtained values with the amounts in plant tissue.

Early bunt diagnostics can be used by breeders to confirm infection. In the past, the plant tissue method (Hansen, 1959; Swinburne, 1963) and the PCR method were developed. These easy methods merely confirm the presence or absence of mycelium in the plant. Real-Time PCR can determine the amount of mycelium in the plant tissue. For this reason, standard production is decribed in our protocol.

REFERENCES

Blazkova V; Bartos P (2002). Virulence pattern of European bunt samples (*Tilletia tritici* and *T. laevis*) and sources of resistance. *Cereal Research Communications* **30**, 335-342.

Gaudet D A; Puchalski B J; Entz T (1989). Effect of environment on efficacy of seed treatment fungicides for control of common bunt in spring and winter wheat. *Pesticide Science* **26**, 241-252.

Goates B J (1996). Common bunt and dwarf bunt. In: R D Wilcoxson & E E Saari (eds), *Bunt and smut diseases of wheat: concepts and methods of disease management*, pp. 12-25. CIMMYT: Mexico.

Hansen F (1959). Anatomische Untersuchungen über Eindringen und Ausbreitung von *Tilletia*-Arten in Getreidepflanzen in Abhängigkeit vom Entwicklungszustand der Wirtspflanze. *Phytopathologische Zeitschrift* **34**, 169-208.

Kochanova M; Zouhar M; Prokinova E; Rysanek P (2004). Detection of *Tilletia controversa* and *Tilletia caries* by PCR method. *Plant, Soil and Environment* **50**, 75-77.

Leijerstam B (1991). Virulence pattern in bunt of wheat in Sweden. *Sveriges Ütsädesförenings Tidsskrifter* 101**,** 85-88.

McNeil M; Roberts A M I; Cockerell V; Mulholland V (2004). Real-time PCR assay for quantification of *Tilletia caries* contamination of UK wheat seed. *Plant Pathology* **53**, 741–750.

Swinburne T R (1963). Infection of wheat by *Tilletia caries*, the causal organism of bunt. *Transactions of the British Mycological Society* **46**,145-156.

Wilcoxson R D; Saari E E (eds) (1996). *Bunt and smut diseases of wheat: concepts and methods of disease management*. CIMMYT: Mexico.

Approaches to cultivating prospective strains of mycoherbicide producers in liquid media

L Kolombet
Federal Medico-Biological Agency, 102A Lenin St., Serpukhov, Moscow Region, 142253, Russia
Email: kolombet@toxicbio.ru

N Kosareva, E Bystrova, S Besaeva, S Jigletsova
SRCAM, Obolensk, Serpukhov District, Moscow Region, 142279, Russia

INTRODUCTION

The development of low-cost methods for mass production of spores is an important step for the commercialization of a bioherbicide (Stowell *et al.*, 1989, Boyette *et al.*, 1991).

Canada thistle (*Cirsium arvense*) is considered to be one of the world's most damaging weeds in temperate zones and is a problem on at least 27 crops in 37 contries (Bailey, 2004). By screening of the collection of phytopathogenic fungi to infect Canada thistle, A O Berestetsky (unpublished) isolated the M-8 strain of *Ascochyta sonchi* as a prospective candidate for development as a mycoherbicide.

This report is devoted to the study of different nutrient medium components in respect to accumulation of mycelium biomass of the *A. sonchi* M-8 strain and its conidia in submerged cultivation.

METHODS

To form liquid nutrient media, the following complex organic components were used: soybean flour, yeast extract, peptone, trypton, fish flour hydrolysate and molasses (a by-product of sugar production), as well as chemical compounds such as glucose, phosphoric monosubstituted potassium, magnesium sulfate, sodium chloride and ammonium sulphate.

To inoculate the nutrient medium, a 2-day inoculate was prepared on the medium containing soybean flour and yeast extract. The nutrient medium was inoculated with a piece of culture grown on a solid nutrient medium. Test flasks were inoculated with 5% inoculums. Inoculated flasks were incubated on an orbital shaker at 220 rpm under ambient laboratory conditions (at $24 \pm 3^{o}C$). The growth and the development of the *A. sonchi* culture was the studied for 10 days. During the course of growth, pH, biomass dry weight (assessed by drying to a constant weight at $105^{o}C$) and the micro-morphological state of the fungi were studied. The efficacy of weed (Canada thistle) control by the mycelium and by the submerged liquid conidia cultures was determined according to method previously described by Berestetsky *et al.* (2005).

RESULTS

During the course of fungal growth a change occurred in the pH of the cultural liquid from 6.2 up to 4.5 (on the 2nd and 3rd day of the growth) on all of the studied media; thereafter, a smooth increase in pH up to 9.5 (by the 10th day of the growth) occurred. Subsequently, up to the 15th day of growth, the pH of the cultural liquid did not change

Maximal dry fungi biomass weight on the medium with soybean flour and yeast extract amounted to 29.4 g/litre in four days. The extent of infection on Canada thistle plants on the 14th day following spraying with a suspension of the mycelium (i.e. the M-8 strain of *A. sonchi*) diluted by 1:100 amounted to 74.0 ± 9.4%.

The medium with molasses as a base and cultivation parameters were selected so that submerged conidia of the M-8 strain of *A. sonchi*, with a titer of 3.3×10^7 con/ml, would be obtained on the 5th day of growth. The processing of the Canada thistle leaves with the suspension lead to infection of the plants on the 14th day (85.8 ± 14.2%). Similar results were obtained where the leaves of Canada thistle were processed with the suspension of conidia grown with the aid of a surface technique for 3 weeks.

DISCUSSION AND CONCLUSIONS

Following submerged cultivation of *A. sonchi*, conidia (as surface conidia) possessing biological activity against Canada thistle were obtained. The selected media and cultivation conditions also allow submerged conidia to be obtained, and these are not inferior to surface conidia in respect of their efficacy. Spore production in submerged liquid culture is the preferred technique for mass production of the biocontrol agents, because the technology is readily available and the scale-up process from the research phase to the development phase is relatively easy. The studies are being done to increase the productivity of cultivation media and to develop formulations that would preserve the viability and activity of spores obtained by submerged cultivation. The work formed part of ISTC project No. 2939.

REFERENCES

Bailey K (2004). Microbial weed control: an off-beat application of plant pathology. *Canadian Journal of Plant Pathology* **26**, 239-244.

Berestetskiy A O; Gagkaeva T Y; Gannibal Ph B; Kungurtseva O V; Mitina G V; Yuzikhin O S; Bilder I V; Levitin M M (2005). Evaluation of fungal pathogens for biocontrol of Cirsium arvense. *Proceedings of the 13th EWRS Symposium*, Abstract 007.

Boyette C D; Quimby P C Jr; Connick W J; Daigle D J; Fulgham F E (1991). Progress in the production, formulation and application of mycoherbicide. In: D O TeBeest (ed), *Microbial control of weeds*, pp. 209-222. Chapman & Hall: New York.

Stowell L J; Nette K; Heath B; Shutter R (1989). Fermentation alternatives for commercial production of a mycoherbicide. In: A L Demain; G A Somkuti; J C Hunter-Cevera & H W Rossmoore (eds), *Novel microbial products for medicine and agriculture*, pp. 219-227. Society for Industrial Microbiology: Fairfax.

Competitive tests of herbicides on spring wheat in western Siberia

A Kondratov, V Korobov, L Korobova, A Belyaeva, N Shadrina
Novosibirsk state agrarian university, Dobrolyubov str. 160, Novosibirsk, Russia
Email: nich.@nsau.edu.ru

V Altuhov
Interregional Association «Sibirskoe soglashenie», Uritskiy str. 19, Novosibirsk, Russia

INTRODUCTION

In Western Siberia different types of herbicides are used for the weed control in crops of spring wheat. Nevertheless, not all the preparations on the market meet the quality requirements or soil-climatic peculiarities of the region. At the same, grants for purchase pesticides. To effectively use grants allocated and to buy the best pesticides their selection is necessary on the basis of a comparative estimation of efficiency in field.

METHODS

The competitive herbicide tests were done in 2004–2005, at basic farms of the Novosibirsk agrarian university 'Lesnaya Polyana' and 'Kwant', located in the northern forest-steppe zone of the Novosibirsk region. Such companies as DuPont (USA), Bayer CropScience (Germany), Syngenta (Switzerland) and the Russian Siberian Agrarian Holding Company (SAHO), Alsico Agroprom, Shchelkovo Agrohim, TD Agrohimprom, Altaihimprom, Sibbiofarm, Biohimzaschita, took part in these tests. The experiments were done on farm-scale sowings. The experimental areas were 1.1 and 1.4 ha. Fields of long-term fallow land (of a mid-loamy, leached black soil) were chosen to provide a high infestation background of weeds. The chemicals were applied by serial tractor sprayer SAHO-2000-18. The weeds in experiments were assessed before tillage, 3–4 weeks after tillage, before harvesting and in the autumn after wheat harvesting. Aerial photography of the experimental area was added to the terrestrial weed stock. To appreciate the aftereffect of herbicides in the crop rotation, an electronic card with marked allotments was created with the help of a satellite GPS-navigator

RESULTS

The total number of weeds before the tillage at experimental allotments was 137/m^2 in 2004, and 122/m^2 in 2005. Yellow thistle (*Cirsium setosum*), field sow-thistle (*Sonchus arvensis*), trailing bindweed (*Convolvulus arvensis*), peavine grass (*Lathyrus sativus*), knotweed (*Fallopia convolvulus*) and spurges (*Euphorbia* spp.) dominated among dicotyledonous weeds, and wild oat (*Avena fatua*) and millet broomcorm (*Panicum miliaceum ruderale*) among monocotyledonous ones. Wild oat was the dominant background weed in 2004, with thistle and sow-thistle dominating in 2005.

The results of the two-year experiments showed that the best biological effectiveness was provided by graminicide tank mixtures based on fenoxaprop-P-ethyl (at 0.5–0.8 litres/ha as Puma Super 100, Lastic, Ovsugen and Gepard Extra), the sulfonylureas amidosulfurone + iodosulfuron-methyl-sodium (at 150 g/ha as Secator) and metsulfuron-methyl (at 8 g/ha as Magnum; at 6 g/ha as Singer), and by dicamba + chlorsulfurone (at 0.15 litres/ha as Phenisan), chlorsulfoxim + chlorsulfurone (at 80 g/ha as Cross) and MCPA (at 0.5 litres/ha as Lintaplant and Agrytox). The exploitation of a graminicide tank mixture of clodinafop (at 0.3 litres/ha as Topic) with dicamba (at 0.15 litres/ha as Banvel) and triasulfuron (at 10 g/ha as Logran) provided a high level of weed destruction. Tank mixtures suppressed the complex of dicotyledonous weeds by 91.8–93.6% and monocotyledonous ones by 90.8–97.6%.
Among the chemicals and tank mixtures investigated against the complex of dicotyledonous weeds, an efficiency level of more than 80% was shown by 2,4-D heavy ethers (at 0.7 litres/ha as Elant), 2,4-D heavy ethers + dicamba (at 0.75 litres/ha as Elant Premium), 2,4-D heavy ethers + triasulfuron (at 0.8 litres/ha + 10 g/ha as Tresor), tribenuron-methyl + dicamba (at 10 g/ha + 0.15 litres/ha as Granstar Super), metsulfuron-methyl + dicamba (7 g/ha + 0.1 litresl/ha as Dimesol), metsulfuron-methyl (at 10 g/ha as Laren and Metalt), 2,4-D + dicamba (0.5 litres/ha as Dialen Super), tank mixtures 2,4-D heavy ethers (at 0.4 litres/ha as Octapon Extra) with metsulfuron-methyl (at 5.0 g/ha). The protective feature of most of the experimental herbicides and tank mixtures was evident up to harvest. An especially long-term aftereffect on thistle and sow-thistle was given by herbicides on the basis of metsulfuron-methyl. This aftereffect was also evident in the following year.
The experimental herbicides provided high crop safety in 2004, wheat harvest being 1.17–1.47 t/ha. In 2004, in conditions of high infestation by wild oat at sowing, tank mixtures with graminicides resulted in a considerable increase in yield. In 2005 the increase in the harvest was achieved by sulfonylureas.

The use of chemicals and tank mixtures was most effective in 2005. The cost-efficiency level (50% and more) was shown by tribenuron-methyl + dicamba, metsulfuron-methyl and by the mixture of 2,4-D heavy ethers with metsulfuron-methyl and that of triasulfuron with clodinafop. In 2004 this cost-efficiency level was achieved by the tank mixture of metsulfuron-methyl with fenoxaprop-P-ethyl.

In some cases it was effective to use herbicides with antidepressants based on *Bacillus subtilis*. Thus:

- Bactophit (at 2.0 litres/ha), combined with the graminicide fenoxaprop-P-ethyl, increased wheat yields by 0.95 t/ha;

- Novosil (at 0.03 litres/ha), combined with fenoxaprop-P-ethyl, increased wheat yields by 0.67 t/ha, compared with a yield increase of 0.5 t/ha for the herbicide alone. Also, the cost-efficiency of fenoxaprop-P-ethyl was increased by 2.5 and 1.5 times.

- Larixin (at 0.03 litres/ha), increased the cost-efficiency of tank mixtures of triasulfuron with clodinafop, 2,4-D + dicamba with fenoxaprop-P-ethyl and that of the herbicide metsulfuron-methyl + dicamba by 1.6–1.7 times.

Forecasting systems

M Kraatz
Sächsische Landesanstalt für Landwirtschaft, Referat Pflanzenschutz, Stübelallee 2, D-1307 Dresden, Germany
Email: Michael.Kraatz@smul.sachsen.de

INTRODUCTION

Weather-based forcasting models used in plant protection practice of Saxony for many important, strongly weather dependent pests are essential tools for decision making in agricultural crops. These systems and models use weather data from the agro-meteorological network of Saxony as input, and predict the dates of first occurrence, simulate the development of pests or calculate recent infection or epidemic disease pressure. Their results serve as the main input for warning services. Warning services are provided by the official crop protection service of Saxony, and its information is transmitted to the farmers by fax services and via the internet. As a consequence plant protection product (pesticide) use can be reduced or optimised.

CONCEPT

The most important condition for running forecasting systems successfully is the input of correct meteorological data. Saxony possesses more then 30 agro-meteorological measuring stations. The data are automatically collected by modem every day. We use the program AgmedaWin to manage and distribute the meteorological data. The program is a flexible tool to import, administrate, check and represent the data from meteorological stations. The stations are dispersed all over the state of Saxony, according to the intensity of agriculture and horticulture. The meteorological data are used as input for the forecasting models, to predict the dates of first occurrence, simulate the development of pests or to calculate recent infection or epidemic disease pressure. Widely accepted models are SIMCERC for eyespot disease (*Pseudocercosporella herpotrichoides*), SIMPHYT for potato late blight (*Phytophthora infestans*), SIMLEP for Colorado beetle (*Leptinotarsa decemlineata*) in potato, SkleroPro for Sclerotinia stem root in oilseed rape (*Sclerotinia sclerotiorum*) and CERCBET for Cercospora leaf spot (*Cercospora beticola*) in sugar beet. Before using the results of simulation in agricultural practice the models are validated under Saxonian conditions.

EXAMPLE

The SIMPHYT model for potato late blight is used in a warning service in Saxony.

The first part of this Model (SIMPHYT I) is used for regional forecasting of the date of first appearance of *Phytophthora infestans* on potato (Gutsche & Kluge, 1996); another part (SIMPHYT III) simulates epidemic pressure of late blight, and is used to give recommendations on spraying intervals (Gutsche *et al*., 1999).

The results of the SIMPHYT models are combined with field data (collected by monitoring) from the potato-growing regions in Saxony and regional advice, and integrated within an internet information system on integrated crop production (ISIP).

The main contents of the information system are as follows:

- The weather data from the meteorological stations owned by the governmental crop protection services, and required to run the SIMPHYT models – collected daily by modem, checked and transferred to the ISIP system.

- The results from monitoring in 17 surveyed potato fields – done at regular intervals and transferred via internet to ISIP.

- Regional advices and recommendations concerning start of spraying schedule, spraying intervals or other comments – transferred via internet to ISIP.

- Also, the grower himself can do interactive calculations of appropriate plot-specific spraying intervals on the basis of a few, simple data inserted into ISIP.

All this information, collated and simplified, enables the grower to optimise his strategy of late blight control.

REFERENCES

Gutsche V; Jörg E; Kleinhenz B (1999). Phytophthora-Prognose mit SIMPHYT III. *Kartoffelbau* **50**, 128-135.

Gutsche V; Kluge E (1996). SIMPHYT I und II . *Mitteilungen aus der Biologischen Bundesanstalt* **321**, 332.

Influence of entomopathogenic hyphomycetes and bacteria (*Pseudomonas* sp.) on locusts

V Kryukov, V Khodyrev, V Glupov
Institute of Systematic and Ecology of Animals SB RAS, Frunze Street 11, Novosibirsk, 630091, Russia
Email: krukoff@mail.ru

M Levchenko, G Lednev
All-Russia Institute of Plant Protection, Podbelskogo highway 3, Pushkin, 196608, Russia

INTRODUCTION

The possibility of using entomopathogenic hyphomycetes (*Beauveria bassiana* and *Metarhizium anisopliae*) for management of locust populations has been shown by many researchers (Lomer *et al*., 2001). However, mycoses are characterized by their long latency time. Index LT_{90} usually varies from 7 to 34 or more days. Some authors (Bajan, 1973; Логинов & Павлюшин, 1987) have indicated a shortening of latency time and increased mortality of various insects when using a mixture of microorganisms from close or distant taxons. In this work we studied different species and strains of entomopathogenic fungi and bacteria which were virulent to locusts (*Locusta migratoria*, *Calliptamus barbarus* and a complex of species in the tribe Dociostaurini).

METHODS

Entomopathogenic hyphomycetes were isolated from dead locusts collected in the steppe zone of Western Siberia. The bacteria (*Pseudomonas* sp.) were isolated from a laboratory population of crickets (*Gryllus bimaculatus*) at the Institute of Systematic and Ecology of Animals. The nymphs were infected by once washing in the aqueous suspension of the conidia and/or bacterial cells. The dilution of fungal and bacterial suspensions was varied from 5×10^5 to 5×10^7 conidia or cells per ml. The nymphs were placed in the 700 ml plastic hatcheries which were then covered with cloth. Each treatment was replicated four times. For each replication 5–10 nymphs were used. The mortality was measured daily for 13–17 days.

RESULTS AND DISCUSSION

Following infection of *L. migratoria* with *B. bassiana* and *M. anisopliae*, a 5-day latency time was observed. Subsequently, there was rapid nymphal mortality: thus, mortality of *L. migratoria* nymphs was 95–100% 12–15 days after inoculation; similarly, mortality of *L. migratoria* nymphs was c. 30–50% 3–6 days after infection with *Pseudomonas* sp. Subsequent mortality of the locusts was not observed. With synchronous inoculation of *L. migratoria* with fungi and bacteria, nymphal mortality was more rapid than with monoinfections (Table 1); LT_{50} was about 3 days. Very similar dynamics of mortality occurred for Dociostaurini and *C. barbarus* infected with fungi and bacteria.

Individuals which died in the first few days of the experiment had typical symptoms of bacteriosis. Those that died subsequently were mummified: typical of mycosis. Microbiological analysis of the dead insects shown that co-existence of both pathogens in the infected locusts is possible. To determinate the antagonism of the fungi and *Pseudomonas* on the synthetic nutrient medium the blocking method was used. The fungi did not influence the growth of the bacteria, and *Pseudomonas* had little (an insignificant) effect on that of the fungi. Zones of growth-inhibition (when using blocks of 12 mm diameter) were 4.5 mm for *B. bassiana* and 7.5 mm for *M. anisopliae*.

.

The most effective concentrations for the concurrent use of fungi and bacteria were 1×10^7 and 5×10^6, respectively (for 2nd- and 3rd-instar nymphs of Dociostaurini and *C. barbarus*) and 1×10^7 for fungi and 5×10^7 for bacteria (for 5th-instar nymphs of *L. migratoria*). Increasing the concentration of one or both pathogens two- or five-fold, levelled all differences in the dynamics of nymphal death resulting from bacteriomycosis and monoinfections. After the dilution of the pathogens we observed a decrease in the additive effect.

Two main factors can have an additive effect, depending on the composition of the pathogens present. Firstly, bacterial gut infection can lead to poisoning and subsequent death of the insects. Secondly, bacterial infection can reduce of growth, limit eating and delay moulting. These conditions favour the germination of fungal hyphae into the cuticle and the haemolymph, and also stimulate mycosis in the insects. Our data demonstrate that a mixture of bacteria (*Pseudomonas* sp.) and hyphomycete fungi may be unique for producing a combined preparation for the regulation of locust populations.

Table 1. Dynamics of the mortality of fifth instar nymphs of *Locusta migratoria* infected with *B. bassiana* (1×10^7 conidia/ml) and *Pseudomonas* sp. (5×10^7 cells/ml)

Treatment	Mortality in days (%)					
	3	5	7	9	11	13
B. bassiana	5 ± 5	15 ± 5	50 ± 15	90 ± 1	100	100
Pseudomonas sp.	28 ± 5	33 ± 5	45 ± 3	50 ± 4	53 ± 5	55 ± 3
B. bassiana + *Pseudomonas* sp.	55 ± 4	70 ± 1	75 ± 5	85 ± 6	95 ± 5	100
Control (water)	0	2.5 ± 2	7,5 ± 2	12.5 ± 5	12.5 ± 5	20 ± 7

REFERENCES

Bajan C (1973). Changes in the pathogenicity of the entomopathogenic fungi under the influence of the method of culture and infection. *Ekologiya Polska* **46**, 715-729.

Lomer C J; Bateman R P; Johnson D L; Lagewald J; Thomas M (2001). Biological control of locusts and grasshoppers. *Annual Review of Entomology* **46,** 667-702.

Логинов Е В; Павлюшин В А (1987). Типы синергизма при бактериально-грибной смешанной инфекции личинок большой пчелиной огневки. В: *Интегрированная защита растений от вредителей*. Новосибирск. С. pp. 123-134.

Optimised application of plant protection products for control of Colorado beetle (*Leptinotarsa decemlineata*) in organic farming

S Kühne
BBA, Stahnsdorfer Damm 81, D-14532 Kleinmachnow, Germany
Email: s.kuehne@bba.de

T Reelfs, F Ellmer
Humboldt Universität zu Berlin, A.-Thaer-Weg 5, D-14195 Berlin, Germany

B Kleinhenz
ZEPP, Rüdesheimerstraße 60-68, D-55545 Bad Kreuznach, Germany

INTRODUCTION

The Colorado beetle is one of the most important pests of potato. The beetle is constantly able to adapt to different climatic conditions and is a significant quarantine pest for much of the world where the bulk of potatoes are grown, for example China and Korea. The effect of various plant protection products based on neem (NeemAzal-T/S), pyrethrum/rape oil (Spruzit Neu) and *Bacillus thuringiensis* var. *tenebrionis* - *B.t.t.* (Novodor FC) against this pest were compared in an organic farming field experiment during 2006.

METHODS

Field experiments (organically certificated under an EU Directive) were done in Germany (Land Brandenburg), involving eight treatments (Table 1) with four replicates: plot size 6 × 17 m. Site factors: sandy silt soil; temperate climate, with an annual average temperature of 8.4°C; annual average precipitation 526 mm; vegetative period from April to September.

Table 1. Treatments for control of Colorado beetle

Treatment and dose rate(s)		Application costs
1.	Untreated control	0 €/ha
2.	pyrethrum/rape oil @ 8 litres/ha + pyrethrum/rape oil @ 8 litres/ha (12 days apart)	173 €/ha
3.	neem @ 2.5 litres/ha	147 €/ha
4.	*B.t.t* @ 5 litres/ha	92 €/ha
5.	neem @ 2.5 litres/ha + pyrethrum/rape oil @ 8 litres/ha (2 days apart)	233 €/ha
6.	*B.t.t* @ 5 litres/ha + pyrethrum/rape oil @ 8 litres/ha (2 days apart)	178 €/ha
7.	neem @ 2.5 litres/ha + *B.t.t.*@ 1.7 litres/ha* (together)	174 €/ha
8.	neem @ 1.5 litres/ha* + *B.t.t.*@ 5 litres/ha (2 days apart)	184 €/ha

* reduced application rate

RESULTS

In all plots, most larvae were found from the end of June to mid-July (average 20 larvae/plant in the control). The most effective treatment against the Colorado beetle was the combined application of neem and B.t.t (Treatment 7 and 8) (Table 2). Here, defoliation (loss of plant material) caused by leaf intake of the pest was least (compared with the control). Defoliation was correlated to tuber yield (without treatment 5). In treatment 8 the yield was significant better (42 dt/ha) than in the control. In three years of field experiments, application of pyrethrum/rape oil has shown no significant effect on the reduction of Colorado beetles.

Table 2. Defoliation (loss of plant material) caused by leaf intake of the Colorado beetle, efficiency factor relative to defoliation, tuber yield and additional profit (profit/ha minus application costs in €/ha) * $\alpha = 0.05$ Dunnett

Treatment	Defoliation (%) 22 days after first pesticide treatment	Efficiency factor (%)	Yield in dt/ha	Additional profit (€/ha)
1	45	-	217	-
2	38	16	235	256
3	19*	57	237	318
4	25	45	234	304
5	13*	71	216	-262
6	26	43	227	45
7	10*	77	235	255
8	9*	80	259*	826

DISCUSSION AND CONCLUSION

Plant protection products are used as a last option for the control of pests in organic farming (Zehnder *et al.*, 2007). It can be economic to apply neem and *B.t.t.* in combination against Colorado beetle, because significant additional profit can result, compared with untreated controls. However, efficiency declines with increasing age of the pest larvae. Therefore, timing of treatments should be based on accurate pest assessment, enabling the optimal date of application to be established.

The inadequate effect of pyrethrum/rape oil in our experiments was probably due to a general resistance of Colorado beetle to pyrethroids.

REFERENCES

Zehnder G; Gurr G M; Kühne S; Wade M R; Wratten S D; Wyss E (2007). Arthropod pest management in organic crops. *Annual Review of Entomology* **52**, 57-80.

Pathogenic micromycetes of *Cirsium arvense* and selection of species for biological control

M Levitin, A Berestetsky, I Bilder, T Gagkaeva, Ph Gannibal, E Gasich, L Khlopunova
All-Russian Inst. of Plant Protection, sh. Podbelskogo, 3, Saint Petersburg, 196608, Russia
E-mail: mif@rol.ru

INTRODUCTION

Canada thistle (*Cirsium arvense*) is spread over the territory of Russia and neighbouring countries. This plant is one of the main weeds in field crops. A large spectrum of chemical herbicides is being used against this weed. The research on biological control was begun in Russia in the early 20th century, using the rust fungus *Puccinia suaveolens* (Potapov, 1925). However, this fungus has not found a broad application as a biological herbicide. In the USA *Sclerotinia sclerotiorum* has been used against Canada thistle (Brosten & Sands, 1986). However, wide specialization of *S. sclerotiorum* did not allow using it as biocontrol agent. The aim of our research is to analyse the specific composition of micromycete parasites of Canada thistle and to select species perspective for creation of biological herbicides. Studies of weed mycoflora were initiated at our laboratory in 1993. Different weeds and wild plants were collected in the European part of Russia (Gasich *et al*., 1999). In Berestetsky (1997) results of the study micromycetes of Canada thistle and allied species in the European part of Russia are presented. The present article describes the results of research conducted not only in the European but also in the Asian part of Russia and in some neighbouring countries.

The research was funded by the International Science and Technology Centre (Project No. 2939).

METHODS

Canada thistles were collected in 2005–2006 in the South-west regions of Russia (North Caucasus), in the Central and North-west regions, in the Far East and in neighbouring countries – Kirgizia and Moldova. Affected parts of plants were washed in water, disinfected by 0.1% solution of AgNO3 and placed on nutrition media in Petri dishes. Isolated fungi were identified according to Gerlach & Nirenberg (1982), Teternikova-Babajan (1987), Braun (1995) and Mel'nik (2000). In some cases molecular diagnostic methods were used. Pathogenicity was evaluated in laboratory conditions using disks cut out of Canada thistle or on plants in greenhouses. Depending on a fungus species, spore suspensions containing 105 to 107 conidia/ml or a fungus grown on grain substrates were used as inoculums. Pathogenicity was assessed by the number of affected plants and the degree of damage.

RESULTS AND DISCUSSION

Fusarium and Alternaria species most frequently occur in South-west Russia. Amongst Fusarium species F. avenaceum, F. sporotrichioides and F. solani were predominant; amongst Alternaria – A. tenuissima and complex species of A. infectoria. In central region Septoria cirsii and Ascochyta sonchi were predominant. Ramularia cynarae, Puccinia suaveolens, Fusarium spp. and Alternaria spp. were spread throughout the North-west of Russia. F. avenaceum, F. sporotrichioides and F. solani made up 80% of all selected Fusarium species.

Amongst Canada thistle samples selected in the Far East, eight fungal species were identified: Ramularia cynarae, Puccinia punctiformis, Phyllosticta cirsii, Septoria cirsii, Stagonospora cirsii, Phoma exigua var. exigua, Botrytis cinerea and Alternaria tenuissima; of these, Septoria cirsii was predominant. In Kirgizia, Alternaria cirsinoxia was found on Canada thistle for the first time. A collection of pure fungal cultures isolated from Canada thistle (containing 157 strains of 20 micromycetes species) was created. Species of Septoria, Fusarium, Ascochyta and Alternaria are most widely represented in this collection.

Estimation of pathogenic properties of some fungal species was carried out in laboratory-scale and greenhouse experiments. When the Canada thistle plants were inoculated with spore suspension of the Ascochyta sonhi M-8 strain, containing 2 × 106 conidia/ml, 7 days after inoculation 100% of the leaf surface was damaged and 40% of the plants died.

Alternaria cirsinoxia C-363 strain exhibited pathogenicity 2–3 days after inoculation with a spore suspension. In 4–5 days, leaf disks were completely lost and, on their surface, weak sporogenesis was observed. A suspension concentration of 5 × 104 conidia/ml was most effective. This fungal strain also demonstrated pathogenicity when whole plants were inoculated. In the study of this pathogen, damage symptoms were observed only on Canada thistle and slightly on woolly burdock (Arctium tomentosum). The disease did not develop on perennial sow-thistle (Sonchus arvensis), ground elder (Aegopodium podagraria) or cocksfoot (Dactylis glomerata). Some F. solani and F. oxysporum strains were also characterized by their narrow specialization, and affected only Canada thistle. These strains did not infect perennial sow-thistle or wheat and had only a weak effect on salad crops and tomato. Pathogenicity of F. solani and F. oxysporum was greater on Canada thistle (from which these strains were isolated) than on other plants. These data suggest narrow specialization of the studied strains.

Assessment of the pathogenicity of strains of Phoma exigua var. exigua (= Ascochyta sonchi), Alternaria cirsinoxia, Fusarium solani and F. oxysporum was also conducted, and those indicating promise for development as mycoherbicides have been selected.

REFERENCES

Berestetsky A O (1997). Mycobiota of Cirsium arvense and allied species over the territory of the European part of Russia. Mycology & Phytopathology 31, 39-45.

Braun U A (1995). Monograph of Cercosporella, Ramularia and allied genera (Phytopathogenic Hyphomycetes), 1. IHW-Verlag..

Brosten B S; Sands D C (1986). Field trials of Sclerotinia sclerotiorum to control Canada thistle (Cirsium arvense). Weed Science 4, 377-380.

Gasich E L; Titova Yu A; Berestetsky AO; Zharov V. R (1999). Micromycetes of weeds in European part of Russia. Research results 1993-1998. VIZR: Saint Petersburg.

Gerlach W; Nirenberg H I (1982). The genus Fusarium – a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Berlin-Dahlem.

Mel'nik V A (2000). Key to the fungi of the genus Ascochyta Lib. (Coelomycetes). Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 379, 1-193.

Potapov A I (1925). Biological control of perennial sow thistle. Irkutsk.

Teternikova-Babajan D N (1987). The fungi of genus Septoria. Erevan.

The effect of AMF inoculation on growth and disease resistance of field cotton, field pepper and potted marigold

X-q Long, W-d Cui, R Yang
403 Nanchang Road, Urumqi, Xinjiang 830091, P. R. China
Email: longxq_xj@yahoo.com.cn

INTRODUCTION

The arbuscular micorrhizal fungi (AMF) are regarded as a beneficial factor for host plants, promoting growth and improving disease resistance. AMF can colonize up to 90% of vascular plants on earth, to form arbuscular micorrhizae. Because AMF hyphae can extend into tiny pores in soil aggregates, the AM formation greatly enhances the efficient root absorptive area to absorb more water and mineral nutrition, and therefore promotes growth, improves drought resistance (ZHEN Shu-cai *et al.*, 2005) and increases the economic yield of host plants (Ortas *et al.*, 2003). Further, AMF provide protection for host plant root systems from detrimental soil pathogens (Liu R-J *et al.*, 2000) and nematodes, and increase the survival rate following transplantation (Idoia, 2004; YU Zhuo-ling *et al.*, 2005).

METHODS

Two AMF inoculants of *Glomus mosseae* and *G. etunicatum* were introduced to field cotton (Xinjiang upland cotton precocious variant 12). The inoculants were added to soil close to seeds immediately after sowing, at a rate of 20–30 spores per seed. Two timings of *Verticillium* wilt development and one timing of yield prediction were investigated, on 16 August, 27 August and 1 September 2005, respectively.

The same two *Glomus* inoculants were introduced to field pepper (Xinjiang pepper variant 3). They were added under the seeds at a rate of 40–50 spores per seed when raising seedlings in the greenhouse; afterwards, the seedlings were transplanted into the field. Two timings of yield prediction and one timing of *Phytophthora* blight development were investigated, on 19 July and 18 August 2005.

Two AMF inoculants of *G. mosseae* and *G. versiforme* were introduced to pot marigold (*Tagetes erect*). The inoculants were added under the seeds at a rate of 30–40 spores per seed when raising seedlings in the greenhouse; afterwards, the seedlings were transplanted into plastic pots. Shoot length, stem diameter, leaf number and bud diameter were investigated on 30 May and 19 June 2005.

RESULTS

In the cotton experiment, the treatment *G. etunicatum* reduced the disease rate and index by 47.8% and 56.6% (at the 125th day) compared with the control, whereas the treatment

G. mosseae improved the lint predictive yield by 48% compared with the control (118.7 kg/Mu and 80.2 kg/Mu, respectively (1 Mu = 666.7 m^2).

In the pepper experiment, the treatment *G. etunicatum* improved single plant yield by 116.4% compared with the control (1.87 kg and 0.86 kg, respectively) and reduced *Phytophthora* blight rate by 41.0% (at the 156^{th} day) compared with the control.

In the marigold experiment, both treatments improved shoot length, stem diameter and leaf number significantly compared with the control. In the first 40 days both treatments delayed blossoming; in the following 20 days both improved blossoming (8.5, 7.2 and 5.8 buds for the *G. mosseae* treatment, *G. versiforme* treatment and control, respectively)..

DISCUSSION AND CONCLUSIONS

In the three experiments, the inoculation of three kinds of AMF strains generally introduced significant effects on improving plant growth, yield and resistance to disease. In the cotton experiment, the root sampling and microscope investigation (Trouvelot, 1986) showed that the root colonization was positively related to lint predictive yield. Data on bud number in the marigold experiment suggested that the AMF inoculation prolonged the marigold's vegetative growth and, subsequently, improved reproductive growth. The different AMF strains showed different effects. Further studies on the mechanism of AMF effect and condition for its stable function are needed for future wider application in practice.

REFERENCES

Idoia G (2004). Effectiveness of three Glomus species in protecting pepper (*Capsicum annuum* L.) against verticillium wilt. *Biological Control* **31**, 296-305.

Liu R-J *et al.* (2000). *Arbuscular Mycorrhizal Fungi and the Application*. Beijing: Science Press. [In Chinese.]

Ortas I; Sari, N; Akpinar, C (2003). Effect of mycorrhizal inoculation and soil fumigation on the yield and nutrient uptake of some Solanaceas crops (tomato, eggplant and pepper) under field conditions. *Horticulture Crop Science, Plant Protection, Agricoltura Mediterranea* **133**, 249-258.

Trouvelot A (1986). *Physiological and Genetical Aspects of Mycorrhizae*. INRA Press: Paris.

YU Zhuo-ling *et al.* (2005). Study on effect of plant absorbency and tolerance for VA mycorrhiza. *Guangdong Agricultural Sciences* **3**, 44-47. [In Chinese.]

ZHEN Shu-cai *et al.* (2005). Effects of VA Mycorrhiza (VAM) on nutrient acquisition and transmission of plants. *Journal of Southwest Forestry College* **25**, 72-75. [In Chinese.]