The effect of composting on *Synchytrium endobioticum*, the organism causing potato wart disease

S Steinmöller, C Büttner
Humboldt University Berlin, Lentzeallee 55, D-14195 Berlin, Germany
Email: s.steinmoeller@bba.de

P Müller
BBA, Stahnsdorfer Damm 81, 14532 Kleinmachnow, Germany

INTRODUCTION

During the fiscal year 2004/2005 c. 6.2 million tonnes of potatoes were processed industrially in Germany (Hambloch *et al.*, 2005). During this processing different wastes accumulate which are potentially suitable for use on arable farmland (Steinmöller, 2003). Potato processing waste might be contaminated with *Synchytrium endobioticum*, the causal agent of potato wart disease. For this reason, waste must be sanitized before being applied to agricultural fields. The German Biowaste Ordinance sets out composting as an appropriate measure to sanitize waste, in addition to fermentation and pasteurization.

Given parameters for composting processes are 2 wk at temperatures around 55°C and 1 wk at around 65°C. So far, no research has been carried out to estimate the suitability of this processes to eradicate relevant quarantine organisms, such as *S. endobioticum*, in potato wastes.

The present study is aimed at finding out whether *S. endobioticum* can be totally eradicated by composting.

METHODS

Quartz sand contaminated with resting spores of potato wart disease pathotype 1 was introduced through special carriers into the substrate to be composted. The substrate was a mixture of pulp and garden compost, at a ratio of 2:1. The carriers consisted of PE-canes (volume 120 ml), where lid and base had been removed. Instead, a poly-ethylene gauze with a pore size of 17 µm was attached with a PE-ring. Additional adhesive tape was used to secure the steadiness of the carriers.

Examinations of the eligibility of this carriers showed that resting spores of the pathogen were satisfactorily contained in the carrier. The heading of the substrate in the carriers was comparable to the surrounding substrates.

Composting was conducted in two 60-litre composters. The first run lasted for 2 wk and for 2 months, respectively. Temperatures were held below 50°C. Further composting runs lasted for 12 and 21 days. Temperatures reached 65°C during that time.

Carriers containing contaminated quartz sand were arranged on three levels in the composters. Altogether, 27 carriers were used per run and composter.

To evaluate the experiment, resting spores of the causal agent were recovered from the composted substrate using a sieve washer and then examined under the microscope. Under the microscope, definitive differentiation between viable and dead resting spores is extremely difficult (Langerfeld, 1984). For this reason, only completely empty resting spores were considered dead.

These examinations were paralleled by a bioassay on potato tubers (tube test). Therefore, potato tubers with a small sprout were fixed under a plastic tube. Subsequently, the tube was filled with the composted substrate and incubated at 15°C and 16 h light. After 3 months the potato tubes were examined for newly grown proliferations.

RESULTS

During the microscopical examinations, viable resting spores were found after 2 wk and 2 months composting with temperatures held under 50°C.

The percentage of completely emptied (dead) resting spores enhanced after composting for 2 months compared favourably with composting for 2 weeks.

The bioassay resulted in the development of sporadic warts on the test plants after 2 wk of composting, whereas no warts could be found after 2 months composting. However, results from the bioassay are of unreliable because it is very difficult to standardize the test. Only a few control plants in untreated contaminated quartz sand showed warts.

Evaluation of composting for 12 and 21 days held above 65°C is still under way.

REFERENCES

Hambloch C ; Menth H; Elfgen S; Stelzer M (2005). *ZMP-Marktbilanz Kartoffeln 2005.*

Langerfeld E (1984). *Synchytrium endobioticum* (Schilb.) Perc.. Zusammenfassende Darstellung des Erregers des Kartoffelkrebses anhand von Literaturberichten. *Mitteilungen der Biologischen Bundesanstalt für Land- u. Forstwirtschaft* **219**, 1-142.

Steinmöller S (2003). Bewertung des Risikos der Verschleppung von Quarantäneschadorganismen mit Abfällen aus den kartoffelverarbeitenden Betrieben, *Abschlussbericht für das Forschungsvorhaben des BMELV 02HS028.*

The efficacious fauna of carabids (Coleoptera: Carabidae) from apple plantations in north-eastern Romania

M Talmaciu, N Talmaciu
University of Agricultural Sciences and Veterinary Medicine Iassy, Street Aleea Sadoveanu M., no 3, Iasi, ZIP: 700490, Romania
E-mail: ntalmaciu@yahoo.com

A Diaconu
Biological Research Institute of Iasi, 20-A, Carol I Bd., 700505, Iasi, Romania

INTRODUCTION

In agricultural environments, and even in forest areas, carabid beetles are extremely important ecological indicators, reacting immediately to the interference of man. Pesticides, for example, may cause paralysis or even the death of the adult insects and their larvae shortly after their application.

METHODS

The studies were conducted in the 2006 growing season, with 10 ha of intensive apple orchards were investigated ecologically. Carabid beetles were collected (in a plantation belonging to S.C.D.P. Falticeni) from May to October, using 'Barber' soil traps. The experimental ground variants were: V_1 – ecological (90% *Trifolium repens* + 10% *Lotus corniculatus*); V_2 – ecological wood pulp; and V_3 – chemically treated. The resultant material was brought into the laboratory, where carabids were extracted and determined to species.

RESULTS

During the research period (2006), observations were made upon the structure, abundance and dynamics of the collected carabids, which belonged to 24 species. We also present the value of ecological parmeters (abundance, constance, dominance and importance), and Table 1 provides information on the species most frequently encountered.

At the first variation 1, abundance (A) represents the number of the samples collected, of which *Pseudophonus rufipes* (39 examples), *Carabus violaceus* (29 examples) and *Harpalus aeneus* (23 examples) were commonest.

For these species, constancy (C) ranged from 5.6% to 50%. Depending on the values of indicator C, the species collected are classified as either 'accessory' (represented by *Carabus violaceus*, *Pseudophonus rufipes*, *Harpalus aeneus*, *Abax carinatus*, *Pterostichus nigrita* and *Cicindela germanica*) and 'accidentally' (represented by *Amara aenea*).

Table 1. The major ecological indices of the dominant carabids collected from apple orchards in north-eastern Romania.

Species	Ecological parameters											
	A			C			D			W		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
Pseudophonus rufipes	39	123	14	50.0	40.7	22.2	31.0	43.9	14.2	12.1	17.9	3.2
Carabus violaceus	29	60	11	50.0	46.3	27.5	23.0	21.4	11.1	11.5	9.9	3.1
Harpalus aeneus	23	5	-	44.4	5.6	-	18.3	1.8	-	8.1	0.1	-
Amara aenea	5	10	1	11.1	11.1	5.6	4.0	3.6	1.0	0.4	0.4	0.1
Abax carinatus	5	12	18	27.8	13.0	44.5	4.0	4.3	18.2	1.1	0.6	8.1
Cicindela germanica	3	4	-	16.7	7.4	-	2.4	1.4	-	0.4	0.1	-
Pterostichus nigrita	3	2	7	16.7	1.9	16.7	2.4	0.7	7.0	0.4	0.0	1.2

Species collected had dominance (D) values ranging from 2.4% to 30%. Depending of the value which have every species collected, these are distributed thus: ‘eudominate’ species, with a domination value over 10% (*Pseudophonus rufipes*, *Carabus violaceus*, *Harpalus aeneus*); and ‘subdominate’ species, with domination indices ranging from 2.1% to 5% (*Cicindela germanica*, *Pterostichus niger*, *Pterostichus nigrita*, *Abax carinatus* and *Amara aenea*).

The indices with the ecological importance (W), for the species collected ranged from 0.4% to 12.1%. Depending on these values, the species collected were typed as: ‘residential’ (*Carabus violaceus* and *Pseudophonus rufipes*; ‘accompanying’ (*Abax carinatus* and *Harpalus aeneus*); or ‘accidental’ (*Pterostichus nigrita*, *Cicindela germanica* and *Amara aenea*).

DISCUSSION AND CONCLUSIONS

The carabid fauna in apple orchards is a very important ecological indicator, reacting immediately to the intervention of man (for example, the application of insecticides against pests). In the observed apple orchards, the variant V_3 had fewer ecological parameters than the variants V_1 and V_2, some species being absent.

Nano-structured silica – physically active insecticides for urban environments

Ch Ulrichs, T Mucha-Pelzer, S Entenmann, I Mewis
Humboldt University Berlin, Lentzeallee 55, D-14195 Berlin, Germany
Email: christian.ulrichs@agrar.hu-berlin.de

A Goswami
Indian Statistical Institute, 203 B. T. Road, Kolkata 700 108, West Bengal, India

INTRODUCTION

Insecticides, when applied in large quantities in urban communities pose a potential threat to human health. Many synthetic-derived pesticides are highly effective against pests for which they are registered, have good residual activity and are fairly cheap. However, the danger of synthetic pesticides lies in their toxicity to different life forms and their persistence in the environment. Consequently, there is renewed interest in using natural products to control insect pests in urban environments. An example of a natural product is diatomaceous earth (DE), which is composed of fossilized deposits of diatoms. Chemically, DE consists of more than 90% silica. In recent years there have been several popular articles, reviews, and research reports regarding the availability, efficacy and performance of various commercial DE products against insect pests. DEs have been most effective in conditions of low humidity because they induce mortality by causing desiccation; water is lost because DE removes the waxy layer of the cuticle of the exoskeleton by physisorption. Nano-structured DE acts like a sponge with lipophilic properties. Under wet or highly humid conditions (above 81 % relative humidity (r.h.)), non-modified DEs exhibit only reduced toxic action. New DE-formulations and synthetically produced silica are even effective under higher r.h.

Occupational exposure to crystalline silica dust is associated with an increased risk for pulmonary diseases such as silicosis and lung cancer. However, there are no studies to show amorphous silica to be carcinogenic in humans. The International Agency for Research on Cancer has listed amorphous silica as group III substance (= not classifiable as to its carcinogenicity to humans). Therefore, modified DEs and synthetic silica (both amorphous) products are regarded as non-poisonous alternatives, in contrast to synthetic chemical pesticides, for use in urban settings.

The purpose of the study described here is to analyze the toxic effects of different modified DE products against the mustard beetle (*Phaedon cochleariae*) on *Brassica chinensis* in open field and under different r.h. in laboratory experiments.

METHODS

Laboratory assessment: the DE products Fossil Shield® FS100, FS90, and FS90s were purchased from Bein GmbH (Eiterfeld, Germany). The products are made of marine diatoms with different degrees of hydrophobicity. FS100 is a natural non-modified DE, whereas FS90 is a medium hydrophobe and FS90s is a high hydrophobe DE product. To demonstrate the effect of hydrophobicity as major modification of DE we kept all materials for 48 h at three different r.h. to allow water saturation.

Experiments were conducted in climate chambers at 30, 60, and 90% r.h. For this, 0.05 mg cm^{-2} DE were weighted into petri dishes and 25 unsexed beetles per dish were added. Each experiment was replicated five times and untreated petri dishes served as controls.

Open field experiment: *Brassica chinensis* plants were grown in plant trays (60 cm × 90 cm). In June 2006, trays were kept for three weeks in an open field, surrounded by cloth material with the top open to keep *P. cochleariae* on the plants. Half of the plants were treated with FS90s, applied electrostatically, whereas, half were untreated. In each cage 50 adult beetles were released. Leaf damage was assessed three weeks after release by scanning all leaves and measuring leaf damage, using Sigma Scan-Pro image analysis software. Weather data were obtained from the meteorological station at Humboldt University Berlin.

RESULTS

In laboratory experiments at 30% r.h beetle mortality was above 30%, even in the controls. At higher r.h. beetle mortality in the controls was significantly ($P < 0.001$) lower at less than 4%. All DEs significantly increased beetle mortality over the control, except the unmodified FS100 at 90% r.h. Here, we assume that FS100 was nearly water-saturated and, therefore, the lipid binding capacity reduced. Highest mortality was achieved with FS90s (high hydrophobicity). Mortality rate was negatively correlated with r.h. and positive correlated with exposure time. FS90s achieved (at 90% r.h.) > 80% beetle mortality within 1 day of exposure and 98% mortality after 7 days of exposure.

During the three-week experiment in June 2006 when plants were outside we had an average r.h. of 73.5% and average daily rainfall of 18 ± 7 mm. Mean leaf damage was reduced by treatment with FS90s from 38.5% in the control to 2.5%. Treated plants showed no growth reduction or phytotoxic symptoms.

DISCUSSION AND CONCLUSIONS

The results of this study indicate that modified DE can effectively control *P. cochleariae* even under high r.h. (90%) within less than seven days. In contrast, unmodified DEs failed to control beetles at r.h. above 60%. *B. chinensis* plants treated with FS90s showed far less leaf damage than control plants. Efficacy of FS90s in field experiments was astonishing, considering that rainfall started in the first week. Further studies should to be conducted to test economic parameters of electrostatic silica treatments, as well as to test if plant growth parameters are reduced under sub-optimal conditions. It might be that silica dusts plaster the stomata and thereby reduce photosynthesis. We also can not exclude the possibility that DE alters the leaf epidermis of some plants.

Current agricultural plant health situation in Poland

F Walczak, A Tratwal, M Gałęzewski
Plant Protection Institute, Miczurina Street 20, 60-318 Poznań, Poland
Email: A.Tratwal@ior.poznan.pl

INTRODUCTION

In Poland organized monitoring of pests and diseases has been provided since 1957. The Department of Methods of Forecast and Pest Registration of the Plant Protection Institute in Poznań is engaged in the work, aimed at monitoring the occurrence and detrimental impact of the more important pests and diseases on agricultural plants. The data obtained during field observations are used for every decision support system, since data concerning the stage of pests and diseases, and their status, are as important as meteorological data and details of fertilization level and cultivar susceptibility. Such information allows the most suitable time for effective chemical treatment or alterative non-chemical control measures to be identified. Evaluation of pests and diseases harmfulness in particular regions is also very useful for long-term forecasting.

METHODS

Every year, during vegetative season, State Plant Health and Seed Inspection Service evaluate the impact of pests and diseases on agricultural sites (cereals, potatoes, sugar beets, oil seed rape, papilionaceous plants, vegetables and orchards). Field observations are carried out according to the methods worked out by the Plant Protection Institute. Data obtained are evaluated and published every year by the Plant Protection Institute, at the Department of Forecasting and Pests Monitoring, as the 'Phytosanitary State of Agricultural Plants in Poland and Prognosis for the Next Year'.

RESULTS

Information has been obtained and promulgated on various pests and diseases, including: (a) apple sawfly etc. (*Hoplocampa* spp.), black bean aphid (*Aphis fabae*), cabbage moth (*Mamestra brassicae*), carrot fly (*Psila rosae*), cereal leaf beetles (*Oulema* spp.), codling moth (*Cydia pomonella*), Colorado beetle (*Leptinotarsa decemlineata*), European cherry fruit fly (*Rhagoletis cerasi*), European corn borer (*Ostrinia nubilalis*), frit fly (*Oscinella frit*), grain aphid (*Sitobion avenae*), large white butterfly (*Pieris brassicae*), mangold fly (*Pegomya hyoscyami*), pea moth (*Cydia nigicana*), plum fruit moth (*Cydia funebrana*), pollen beetle (*Meligethes aeneus*) and saddle gall midge (*Haplodiplosis equestris*), and (b) angular leaf spot (*Pseudomonas lachrymans*), anthracnose fungus (*Colletotrichum gloeosporioides*), brown rot (*Monilinia laxa*), brown rust (*Puccinia recondita*), canker (*Phoma lingam*), cercosporella leaf spot (*Cercosporella beticola*), downy mildew (*Peronospora destructor*), downy mildew (*Pseudoperonospora cubensis*), eyespot (*Ramulispora* (= *Pseudocercosporella*) *herpotrichoides*), grey mould (*Botryotinia fuckeliana*), late blight (*Phytophthora infestans*), powdery mildew (*Blumeria graminis*), scab (*Venturia inaequalis*), septoria tritici leaf spot (*Septoria tritici*) and take-all (*Gaumannomyces graminis*). Further information is given in Tables 1 and 2.

Table 1. Occurrence of the more important pests of crops in Poland.

Pest/disease	Crops infested in 2006 (%)	Crops infested in 2005 (%)	Long-term average (%)	Regions of greatest occurrence
Aphis fabae	4.6	5.2	10.6	south-east, south-west
Leptinotarsa decemlineata	12.4	14.2	25.6	south-east
Meligethes aeneus	10.6	11.4	12.2	west
Oscinella frit on maize	2.6	-	4.6	north-east, central
Oulema spp on winter wheat	7.8	6.3	9.6	north, south, south-east
Pieris brassicae	5.6	3.9	13.9	south-east
Rhagoletis cerasi	3.2	-	5.6	central, locally south-west
Sitobion avenae on winter wheat	4.0	6.1	7.7	south

Table 2. Occurrence of the more important diseases of crops in Poland.

Pest/disease	Crops infected in 2006 (%)	Crops infected in 2005 (%)	Long-term average (%)	Regions of greatest occurrence
Blumeria graminis o winter wheat	22.3	22.6	27.1	north, locally south-east
Botryotinia fuckelian	3.7	3.9	8.4	north-west, south-east
Cercosporella betico	6.0	6.8	12.8	south-east
Peronospora destructor	5.3	7.0	15.6	south-east
Phoma lingam	2.5	3.4	4.4	north, north-east
Pseudomonas lachrymans	9.7	5.4	14.9	north-west, central
Phytophthora infestans	17.6	27	48.9	south-east
Phytophthora infesta on tomato	7.9	9.4	18.0	central
Puccinia recondita	8.4	5.6	11.4	south, south-east
Septoria tritici	6.5	8.2	14.4	south, south-east
Venturia inaequalis	5.6	6.7	14.8	south
Monilinia laxa	4.0	11.4	6.2	central

Regional forecasts and warning system for pests and diseases in agricultural crops in Poland

F Walczak, A Tratwal, M Gałęzewski
Plant Protection Institute, Miczurina Street 20, 60-318 Poznań, Poland
Email: A.Tratwal@ior.poznan.pl

INTRODUCTION

Nowadays, one of the most crucial elements of plant protection is the efficient monitoring of pests and diseases that occur on agricultural crops. In providing correct forecasts and advice, one has to remember that, in terms of 'first appearance' or in terms of 'developmental stages' of pests and diseases, significant differences (sometimes 3 weeks) may be observed between different regions of the country. Within voivodeships these differences can reach 2 weeks, and about 1 week within a county. Moreover, at the same place (one village – different plantations) a few days difference in the appearance of pests or diseases can be observed. The main purpose of regional forecasts is to determine the optimal time of chemical control on the specific field or plantation, which gives the opportunity to reduce the costs, the number of chemical treatments and, subsequently, the risk of environmental pollution.

METHODS

Taking into consideration the demand of producers for accurate information regarding pests and diseases, optimal chemical treatments and information on the necessity to apply treatments, the Plant Protection Institute has been undertaking regional pest and diseases monitoring since 2005. The results are published on the Institutes' website (www.ior.poznan.pl) under "Sygnalizacja Agrofagów" (Pests/diseases signalization). Except for information on first appearances and next developmental stages, the above-mentioned website also provides information on the biology of pests and diseases. Such information helps producers to estimate their individual requirements in the field.

RESULTS

During the vegetative season (in 2005 and 2006), observations were provide in four voivodeships – Wielkopolskia region (three places – Winna Góra, Słupia Wielka and Baborówko) – the western part of Poland, Śląsk region (Sośnicowice) – south Poland, Kujawsko-Pomorskie region (Więcławice) – north Poland and Mazowieckie region (Chylice) – central part of Poland. Observation were concentrated on: (a) aphids, cereal leaf beetles (*Oulema* spp.), saddle gall midge (*Haplodiplosis equestris*), brown rust (*Puccinia recondita*) and powdery mildew (*Blumeria graminis*) in cereals, (b) Colorado beetle (*Leptinotarsa decemlineata*) and late blight (*Phytophthora infestans*) in potatoes, and (c) aphids, cutworms, mangold fly (*Pegomya hyoscyami*) and cercospora leaf spot (*Cercosporella beticola*) in sugar beet.

Some information regarding regional infections of powdery mildew (*Blumeria graminis*) and infestations of cereal leaf beetles (*Oulema* spp.) are shown in Tables 1 and 2, respectively.

Table 1. Powdery mildew (*Blumeria graminis*) on winter wheat in Poland in 2005.

Date of observatio	Developmental stage	Winna Góra (Wielkopolska)	Słupia Wielka (Wielkopolska)	Remarks
29 April	shooting	no symptoms	5% of the infected are	
5 May	shooting	< 10% of the infected area	< 10% of the infected area	
9 May	shooting	> 10% of the infected area	< 10% of the infected area	Winna Góra – treatment recommended bu no opportunity (heavy rain)
12 May	shooting		> 10% of the infected area	Słupia Wielka – treatment recommended
13 June	shooting	> 10% of the infected area		Winna Góra – treatment recommended

Table 2. Cereal leaf beetles (*Oulema* spp.) on winter wheat in Poland in 2005.

Date of observation	Developmental stage	Winna Góra (Wielkopolska)	Sośnicowice (Śląsk))	Remarks
14 April	adult	5 items/m^2		
20 April	adult		> 10 items/m^2	
29 April	laying eggs	4 eggs/m^2		
6 May	laying eggs		5 eggs/m^2	
24 May	eggs hatching		1 larvae/10 stems	
30 May	eggs hatching	larvae hatching		mass of laying eggs
7 June	mass of eggs hatching	21 larvae/100 stem		Winna Góra – number of larvae 4 mm long below threshold – treatment not recommended
10 June	mass of eggs hatched			Sośnicowice – see above

Behavioural response of the predatory mite Phytoseiulus persimilis **in inert materials of application**

D Wendorf, H Sermann, C Büttner
Humboldt University Berlin, Lentzeallee 55, D-14195 Berlin, Germany
Email: helga.sermann@agrar.hu-berlin.de

P Katz
Katz-Biotech AG, Industriestraße 38, D-73642 Welzheim, Germany

INTRODUCTION

A large-scale application of the predatory mite *Phytoseiulus persimilis* for use in the biological control of spider mites, for instance in cucumber fields, involves specific problems. Owing to the need to mechanize this customary manual method of application, an inert material for transport and distribution of the predatory mites must be provided. This material has to hold the mites for the duration of the application and must be suitable for use in a mechanical procedure. *Therefore, the behaviour of P. persimilis was tested in chosen materials. Special interest was given to the distribution of the animals in the material, the time of remaining in the material and certain factors that might have an impact on the time of their remaining in the material.*

MATERIALS AND METHODS

The materials used in the laboratory studies were buckwheat husks, millet husks, wood shavings (0.8–2.0 mm), spelt husks (Germany wheat) and vermiculite (1–3 mm). To examine the effect of abiotic influences on the migratory behaviour and the time of remaining in the material, both the dampness of the materials was varied (0%, 5%, 10% and 20%) and the temperature (6°, 8° and 10°C), as well as the duration of exposure to the different temperatures (2, 4 and 16 h), were varied. The animals used in the studies were mass-reared predatory mites (*P. persimilis*).

To study the influence of dampness, 10 ml of the material with the chosen dampness (0%, 5%, 10% and 20%) were put into a petri dish. Then, 50 mites were positioned centrally on this material and immediately covered with additional 10 ml of the same material. The effect on the emigration of the mites was observed after 5, 10, 15, 30 and 45 minutes by controlling the number of individuals that vacated the material.

To study the effect of temperature, small transparent pipes (ø = 3.7 cm, ht = 12 cm) made of synthetic material were used. Again, 10 ml of the material (10% dampness) were put into the container; 50 mites were then positioned centrally on this material and immediately covered with an additional 40 ml of the same material. The container was closed with a lid made out of gauze and stored for 2 h, 4 h and 16 h at 10°C, 8°C and 6°C in cooling chamber. Effects of temperature and time of exposure to the corresponding temperature were examined. The time until resumption of movement and the moment of emigration after the cooling was recorded quantitatively.

RESULTS

Dampness

Emigration from all dry materials was completed 15 minutes at the latest after the beginning of the test. Emigration from buckwheat husks and spelt husks was especially fast. After 5 minutes, 88% and 92% of the mites, respectively, were already found outside the material. No effect on mortality or disturbance of mobility could be detected.

The increase of dampness had an obvious effect on the duration of time mites remained in the material. This could be due to the 'comfort' of the mites in the material. Depending on the material, emigration was completed after 30 to 45 minutes. In this respect the materials millet husks and wood shavings showed the most favourable effect at 10% dampness.

Temperature

Depending on temperature and duration of cooling, an obvious delay in the resumption of movement and the moment of emigration from the material after the cooling was observed. Decreasing temperatures and increasing times of exposure prolonged the time of remaining in the material and, therefore, show a differentiated and repressive effect on both migration and emigration. Overall, however, brief (2 h) and moderate cooling (10°C) was not very effective.

The strongest effect on delaying the resumption of movement was recorded after cooling for 16 h at a temperature of 6°C, and the beginning of emigration was delayed by up to 40–50 minutes.

Under these circumstances, the emigration of 50% of the inserted mites was accomplished in 68 minutes (vermiculite) to 176 minutes (wood shavings). At room temperature, however, the mites had already emigrated from each material after c. 45 minutes (10% dampness). In addition, for each material, a comparable effect on the migratory behaviour was recorded for the following combinations: 10°C for 16 h, 8°C for 4 h and 6°C for 2 h. In respect to the materials, the strongest effect of cooling was detected for millet husks and wood shavings. Cooling of vermiculite showed only a moderate additional effect on delaying the migratory activity of the mites.

CONCLUSIONS

The time of remaining in the dry materials proofed to be comparatively short. By increasing the dampness of the materials to 10%, in combination with the effect of cooling the time can be prolonged considerably. The effect of slowing down the mobility of the mites in the material has to be judged positively in respect of mechanised application. However, it depends on the chosen form of application technology whether this effect is sufficient for the mechanised application process. Further factors that might have an impact on the mobility and distribution in the materials are still being examined.

High multi-drug resistance to chemically unrelated oomycete fungicides in *Phytophthora infestans* and *P. nicotianae*

B N Ziogas, A N Markoglou
Agricultural University of Athens, 75 Iera Odos, Votanikos, 118 55 Athens, Greece
Email: ziv@aua.gr

INTRODUCTION

Fungicide research has produced a diverse range of new oomycete fungicides, such as morpholines, amidocarbamates, QoIs, QiIs and benzamides, which are expected to have a significant impact on the control of downy mildews. Biochemical studies on the mode of action showed that their fungitoxicity is based on different mechanisms of action from those of phenylamides and acetamides, which interfere with nucleic acid (RNA and DNA, respectively) biosynthesis (Ziogas & Davidse, 1987). To our knowledge very limited information is available concerning the risk for resistance development to the above novel fungicides. The objective of the present study was to explore the genetical potential of *Phytophora infestans* and *P. nicotianae* for resistance to new oomycete fungicides and to access the risk related to the build-up of field resistance. The research project was co-funded by the European Social Fund and National Resources – EPEAEK II.

METHODS

The strains CBS 430.90 of *P. infestans* and BPI 1384 of *P. nicotianae* with wild-ype sensitivity to metalaxyl-m, were used to obtain mutant isolates resistant to amidocarbamates. Mutant isolates were obtained after ultraviolet irradiation and selection on fungicide-amended medium.

RESULTS AND DISCUSSION

Mutants of *P. infestans* and *P. nicotianae* highly resistant to amidocarbamates were isolated at a low frequency of 1×10^{-7} and 6×10^{-10}, respectively. Fungitoxicity tests on the response of *P. infestans* mutant strains to other oomycete fungicides showed that the mutated gene(s) also reduced the sensitivity of mutant isolates to metalaxyl-m, cymoxanil, dimethomorph, zoxamide, QoIs azoxystrobin, kresoxim-methyl, pyraclostrobin, trifloxystrobin, famoxadone and fenamidone, to QiI cyazofamid and to chlorothalonil (Table 1). A reduction of the sensitivity was not apparent in the case of propineb, maneb and fluazinam. In the case of *P. nicotianae* a similar cross-resistance pattern, with the exception of chlorothalonil and zoxamide, was also observed in most mutant strains. Study of fitness-determining characteristics in the wild-type strains and mutant isolates showed that the mutation(s) leading to multi-drug resistance, in both fungal species, affect the ecological fitness of most mutant isolates. However, in few of them fitness characteristics such as mycelial growth rate, sporulation, sporiangial germination and differentiation into zoospores (*P. infestans*), clamydospores production (*P. parasitica*) and pathogenicity on tomato seedlings were practically unaffected. Our data are the first clearly indicating the existence of a genetical and biochemical potential for the development of high-level multi-drug resistance to chemically unrelated fungicides in *Phytophtora*. Previous studies have shown cross resistance among phenylamides (Gisi *et al.*, 1997; Sedegui *et al.*, 1999; Mitani *et al.*, 2001; Shattock *et al.*, 2002).

Table 1. Fungicide sensitivity of wild-type and representative mutant isolates of *Phytophthora infestans* and *P. nicotianae* selected by amidocarbamates amended medium.

Fungicide	Wild-type EC_{90} (μg ml^{-1})		Relative growth at 10 μg ml^{-1}					
			P. infestans				*P. nicotianae*	
	P. inf.	*P. nic.*	IPV-1[c]	IPV-2[c]	BVC-3[c]	BVC-5[c]	PNB-2[c]	PNB-3[c]
iprovalicarb	0.25	1	97	100	100	95	96	65
benthiavalicarb	0.075	0.1	96	100	94	95	98	63
cyazofamid	0.005	nt[b]	81	100	100	94	nt	nt
zoxamide	0.15	0.75	100	100	100	100	98	0
dimethomorph	0.5	2.5	100	100	89	100	100	92
cymoxanil	2.5	30	100	93	100	94	96	80
metalaxyl-m	0.75	0.75	97	100	100	98	98	87
chlorothalonil	0.075	>100	93	100	94	80	0	0
azoxystrobin	0.75	>50	64	49	60	57	nt	nt
kresoxim-me	5	nt	53	64	60	50	nt	nt
pyraclostrobin	1	>50	36	54	62	58	nt	nt
fenamidone	0.35	nt	nt	57	60	58	nt	nt
fluazinam	7.5	50	0	23	29	20	34	23
propineb	50	50	0	56	53	50	33	46

[a] Effective concentration causing 90% reduction in growth rate of wild-type. [b] nt: not tested.
[c] IPV mutant strains selected by iprovalicarb; BVC mutant strains selected by benthiavalicarb.

A target site change does not provide a reasonable explanation for the multi-drug resistance found in the present work. The reduction of sensitivity to inhibitors affecting different sites of cellular processes indicates that a mechanism other than target-site modification is the underlying biochemical mechanism of resistance.

REFERENCES

Gisi U; Hermann D; Ohl L; Steden C (1997). Sensitivity profile of *Mycospaerella graminicola* and *Phytophthora infestans* populations to different class of fungicides. *Pesticide Science* **51**, 290-298.

Mitani S; Araki S; Yamaguchi T; Takii Y; Ohshima T; Matsuo N (2001). Biological properties of the novel fungicide cyazofamid against Phytophthora infestans on tomato and Pseudoperonospora cubensis on cucumber. Pest Management Science 58, 139-145.

Sedegui M; Carroll R B; Morehart A L; Hamlen R A; Power R J (1999). Comparison of assay for measuring sensitivity of Phytophthora infestans isolates to fungicides. Plant Disease 83, 1167-1169.

Shattock R C (2002). Phytophthora infestans: populations, pathogenicity and phenylamides. Pest Management Science 58: 944-950.

Ziogas B N; Davidse L C (1987). Studies on the mechanism of action of cymoxanil in Phytophthora infestans. Pesticide Biochemistry and Physiology 29: 89-96.