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Best Practice in Disease, Pest and Weed Management The State of the Art

Edited by: D V Alford, F Feldmann, J Hasler and A von Tiedemann

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PREFACE

The production of plants for food or non-food uses requires complex strategies that must balance profitable and efficient farming with production quality (including environmental, product and process quality) and quantity concerns. Several production guidelines have been developed to benchmark existing processes and to give advice to producers on how to transform their farm operation into warrant economic, environmental and agronomic efficient plant production systems.

Plant disease, pest and weed management play a central role in plant production systems. The Symposium, therefore, will focus on management practices established in plant protection, reflecting the ongoing public and scientific debate about benchmarking existing practices within integrated plant protection and the development of alternative or innovative approaches.

Indicators for the environmental and economic effects of production systems need to be provided and evaluated. Can new technologies and biotechnologies help to meet the future societal demands for 'safe' or 'healthy' crop production?

This Symposium provides the stage for experts from various related fields (such as extension services, chemical or biotechnological companies, universities, research institutions and public authorities) to synthesize a critical evaluation of the state of management practices in crop protection, upon which a projection of future demands and developments should be derived.

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April 2007

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OPENING CEREMONY

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Opening lecture by: President and Professor Dr G F Backhaus Federal Research Centre for Agriculture and Forestry Braunschweig, Germany

Developments towards best management practices in plant protection

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INTRODUCTION

After decades, during which people did not find sufficient tools or means to control plant diseases, pests and weeds, discussion within the plant protection experts during the 1950s and 1960s mainly dealt with the recently developed chemical plant protection products (PPPs), in particular with their efficacy and phytotoxicity. Over the decades, critical views about the side effects of PPPs on the environment and the health of humans were also published (e.g. Richter, 1910), but the advantages were mainly considered to be on the side of solving practical plant protection problems by chemicals. Between 1950 and the 1980s chemical plant protection made great progress, owing to its positive effects on yields and economics of crops. However, at the latest when the book 'Silent Spring' (Carson, 1962) was published, the discussion about the effects of chemical plant protection on the health of humans and the environment became public, and ever since has never stopped. The discussions were accompanied by findings of herbicides and soil disinfectants in ground water, residues of PPPs in fruits and vegetables and other news which shocked the public or was used to shock the public. Because of ongoing controversial discussions, the evaluation of PPPs and their active ingredients was intensified, additional legal regulations were passed and new restrictions for users were introduced. However, some administrators had to realize one day that it might be impossible to control every single farmer in everything he does. Consequently, self-responsibility of farmers, horticulturists and foresters had to be addressed and guaranteed. For this purpose, in addition to the registration procedures, guidelines had to be developed about the minimum requirements for the use of PPPs and, of course, for the accompanying non-chemical measures of plant protection in the field. These guidelines were called 'good plant protection practice'. Very often they were embedded in guidelines of a broader sense, and called 'good agricultural practice', 'good horticultural practice' etc. In Germany, over the past 15 years, official authorities, extension services and grower associations developed their own guidelines (e.g. Reschke et al., 1987; Brinkjans & Scholz, 2003). There was great similarity between these guidelines with respect to the major items, although they differed considerably in their details.

BEST MANAGEMENT PRACTICE

Several efforts have been made to establish generally valid guidelines, e.g. by the EPPO standard PP 2/1(1) on 'Principles of good plant protection practice' which was first approved in 1993. In Germany, in addition to the important legal regulations based on the European guideline 91/414 (EWG), the German Plant Protection Acts of 1986 and, again, 1998 defined Good Plant Protection Practice as the most important basis for every operation in chemical plant protection. Good Plant Protection Practice on the one hand serves (in addition to the registration procedures for PPPs) for the maintenance of health and quality of plants and plant products, and on the other hand for the avoidance of dangers and risks which might arise for

the environment and for the health of humans and animals as a result of plant protection measures. The principles do not only concentrate on chemical plant protection but address all measures of plant protection. Good Plant Protection Practice requires the principles of Integrated Plant Protection (IPP) to be taken into consideration. These principles were described and published for the first time in 1998 (Burth & Freier, 1999) and the current version appeared in 2005 (Anon., 2005). Good Plant Protection Practice is the basic strategy and includes all measures a farmer can apply in accordance to the given rules and regulations. However, the farmer is obliged to keep in mind the principles of IPP:

- IPP requires a complex mode of action and represents a systemic approach.
- The concept of IPP includes the ecological relations of equilibrary with economic and social aspects, in order to secure sustainability.
- In IPP, preventive (prophylactic) measures should be preferred.
- IPP requires careful consideration of intending processes.
- IPP is a knowledge-based concept which places emphasis on the use of newest scientific knowledge and justifiable technological progress, and it makes high demands on the supply and transfer of location-oriented information.

This situation, however, might be merely a further mid-step on the way to finally approaching the model of future IPP. The aim for the near future is not only to define but also to internationally convert a certain minimum level of requirements for practical plant protection measures, beginning from selection, trade and transport of PPPs, all the way to the measures of application and waste management or disposal. In some discussions this development is called 'best management practice'. It is meant to deliver a standard for the behaviour of anyone who intends to use PPPs or to protect plants.

In the meantime, new methods of IPP management will have to be developed, to take account of new breeding efforts, precision farming, biological measures of plant protection, and innovative new active substances. In addition, we will have to improve application techniques. Furthermore PPPs are still not always used efficiently. Their potential for efficacy might, for example, be enhanced by use of more sophisticated application systems (which provide for close contact to the target area, such as plant leaves, and avoid contamination of non-target areas) and better application timing.

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PLENARY SESSIONS

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Qualitative and quantitative loss of pesticides during waste water treatment

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INTRODUCTION

In the past, investigations of possible pesticide contamination have concentrated on ground water. From 1985 to 1996, 450 groundwater sampling points were investigated in Rhineland Palatinate, and about 10% of the samples contained triazines (atrazine, simazine). Furthermore, bank filtrate of the river Rhine contained bentazone and dikegulac, deriving respectively from herbicide ascorbic acid production. Since dikegulac was not used in the area, this served as a tracer for the spread of bank filtrate into the ground water zone. Improved production processes stopped further emission. Other active ingredients were rarely found (single-source entries) and pesticide contamination of groundwater was less intensive than expected. In the following years, investigations concentrated on possible contamination of surface water.

METHODS

In 2003 the outlet water of six sewage plants within Rhineland-Palatinate was analysed for 43 different active ingredients and metabolites (29 herbicides, 12 fungicides, 2 insecticides). The plants meet all requirements of maximum mechanical, chemical and biological treatment processes. Mixed 14-day water outputs were continuously sampled automatically (Endress & Hausser) or by hand. The catchment area of Sewage Plants 1–3 was characterized by a large portion of specialized crops with a sampling period from March to October. Sewage Plants 4–6 are basically connected to arable land and samples were taken during March to June.

Following collection, samples were refrigerated and, subsequently, frozen until chemical analysis, which was done by LUFA in Speyer (according to acknowledged methods for active ingredients of pesticides (DFG – the German Research Foundation)). Especially for glyphosate, the laboratory developed an approved analytical method.

Financial support was given by the Ministry of Agriculture, Rhineland-Palatinate.

RESULTS

The average water flow, combined with the measured pesticide concentration, allowed the estimation of the pesticide quantity (a.i.) leaving the individual sewage plant. The amount of pesticide loss via sewage plants varied from 1 kg to 7.7 kg per sampling period. Losses were higher in catchment areas with mainly specialized crops, which need to be treated more often

(Table 1). According to their quantitative use, herbicide residues dominated the findings. This was especially obvious in areas with mainly arable crops (Sewage Plants 4–6). When specialized crops are present, fungicide losses gain in importance (Sewage Plants 1–3).

		Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
catchment area	(km ²)	58	52	50	57	21	92
arable land	(km ²)	3	19	36	31	11	45
specialized crop land	(km ²)	26	10	10	0	2	0,1
grassland	(km ²)	2	6	1	7	4	19
herbicides	(g a.i.)*	5,194	2,287	4,223	930	1,789	1,011
fungicides	(g a.i.)*	2,372	780	653	80	68	39
insecticidess	(g a.i.)*	199	1	591	0	3	1
Sum	(g a.i.)*	7,765	3,068	5,467	1,010	1,860	1,051

 Table 1. Specificities of six different sewage plants in Rhineland Palatinate sampled for pesticides in 2003.

* g a.i. \geq limit of quantitation LOQ.

DISCUSSION

Previous investigations pointed at sewage water as a major source for pesticides in surface water (Seel *et al.*, 1996; Augustin *et al.*, 2002). This was confirmed by these investigations. Pesticide concentrations of waste water in the course of the year indicate that they originate to a smaller scale from the actual application but more from the general handling of pesticides. Registration procedures for pesticides and enforced conditions of application aim to minimize environmental pollution. We need 'best pesticide management' to reach this aim and to prevent further environmental restrictions being placed upon pesticide use.

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Elaboration of a system for assessment of agricultural land bio-diversity in Siberia

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INTRODUCTION

System crisis in Russian agriculture has negatively affected the state of bio-diversity. In Siberia this is caused by the following: increased forest cutting and poaching, soil erosion, silting of water basins, loss of soil fertility, and degradation of pasture fields. The way out of this situation is seen in conducting agricultural activities on the basis of ecological principles. In addition to the improvement of the ecological situation in Russia this will make it possible to get maximum economic effect with low investments, which is extremely important in the present environmental crisis. In the last six years we have tried to develop a system for assessing agricultural land bio-diversity. Some groups of the most common soil and epigeous invertebrates have been selected for collecting the data concerning changes of agro-landscape bio-diversity caused by human activity.

METHODS

For the years 2000 to 2005 we have been studying the agricultural land bio-indicators in the south part of West Siberia. The territories under study are situated in the Tomsk and Kemerovo regions, which belong to a zone of so-called 'risky agriculture', especially for growing plants. The overall climate is continental, with long winters and warm, but short, summers. The frost-free period is 105–120 days a year. The standard annual precipitation level is 430–450 mm. According to some estimates only two out of every five years provide favorable weather for agriculture. The crops grown are spring wheat, winter rye, barley, oats, buckwheat and millet, as well as potatoes and other vegetables. Studied were done in fields of potato, cabbage and spring wheat. The methods of soil tests and transects of pitfall traps were used for collecting and monitoring soil and epigeous invertebrates (Vogel, 1983; Waage, 1985). The bio-diversity, density and the life form spectra of representatives of the most common group of arthropods: rove beetles (Coleoptera: Staphylinidae) have been used as indicators of agricultural land conditions.

RESULTS AND DISCUSSION

The maximal diversity of rove beetles populations (49 species) was found in cabbage fields. There were 36 species in potato fields and 34 species in wheat fields. The density of rove beetles in the studied fields (individuals/m²) were as follows: cabbage – c. 25; potato – 18; spring wheat – 19.5 (Table 1). Some species of beetles may serve as indicators of cabbage fields (*Philonthus addendus* and *Aleochara moerens*), potato fields (*Staphylinus sibiricus*) and wheat fields (*Tachyporus solutus*).

Agro-ecosystem	Number of species	Density (individuals/m ²)
Cabbage	49	24.8 ± 2.2
Potato	36	18.0 ± 2.0
Spring wheat	34	19.5 ± 1.4

Table 1. Bio-diversity and density	of rove beetles in West Siberian
agro-ecosystems.	

All main classes and groups of rove beetle adult life forms were found in agro-landscapes in the region of study. In all fields the most beetles were small epigeobionts (a significant number of *Philonthus*) and forest litter stratobionts (mainly forest litter Aleocharinae). The greatest variety of rove beetles life forms (4 classes and 10 groups) were found in cabbage fields. Approximately 75% of these were zoophagous, mostly 'epigeobios' and 'stratobios' (*Staphylinus, Ocypus, Quedius* and *Philonthus*). Relatively few rove beetles were mycetophous, but those that were (*Megarthrus* and *Gyrophaena*) were most numerous in the wet cabbage fields, and decreased simultaneously with increasing micro-climate severity.

Bio-diversity of rove beetles belonging to the classes 'geobios' (*Lathrobium* and *Meotica*) and (to some extent) 'psammocolymbetes' (*Astenus*) increases in the direction: wheat \rightarrow cabbage \rightarrow potato. Generally, an increase in the severity of micro-climatic conditions (in the direction: cabbage \rightarrow potato \rightarrow wheat) leads to a decrease in the diversity of adult rove beetles..

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Promotion of antagonistic mymarids of the grape leafhopper by planting dog roses along vineyards

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INTRODUCTION

Several mymarid species are known to be efficient egg parasitoids of the grape leafhopper (Empoasca vitis), a potential pest species (Böll & Herrmann 2004). However, overwintering mymarids depend on the eggs of other cicadellid species that predominantly occur in hedges. Dog roses (Rosa canina) are by far the most preferred hibernation sites of Anagrus atomus (Remund & Boller, 1996; Böll & Schwappach 2003); for two other mymarid species (Anagrus avalae and Stethynium triclavatum) the main overwintering sites are still unknown.

METHODS

As, during the past few decades, most shrubs have been cleared in intensely cultivated vineyards, it was examined in a 3-year study whether dog roses planted at the beginning and the end of vine rows established and promoted mymarid populations. With a dense net of yellow sticky traps in the vineyard and in an adjacent hedge (in the third year, also in the planted roses), the population dynamics of these mymarid species and the grape leafhopper were monitored throughout the growing season on a weekly basis. Hatching experiments with wild and planted rose shoots during the third year provided data on the number of overwintering mymarids/m shoot as well as of the number of hatching mymarids/m shoot during the vegetation period.

RESULTS AND DISCUSSION

In the third year, after most of the planted roses had reached a height of more than 2 m (similar to that of wild dog roses), results showed that:

- mymarids used the planted roses, both as overwintering sites and as a breeding habitat;
- the planted roses predominantly housed A. atomus (97%), whereas A. avalae (3%) and S. triclavatum (0%) could not be promoted;
- with few exceptions, only young rose shoots were used as egg laying sites by A. atomus and its cicadellid hosts;
- the planted dog roses were intensively used as overwintering sites, with an average of 24.4 cicadellid host eggs/m shoot and 14.6 A. atomus/m of shoot –corresponding to a winter parasitation rate of 59%;
- for the greater part of the vegetation period the planted roses were continuously used for

reproduction, with the tallest-grown roses housing similar numbers of A. atomus as wild dog roses in the adjacent hedge;

- with increasing biomass of the planted dog roses, densities of A. atomus over the study period significantly increased in adjacent wild dog roses but not in other shrub species;
- in the vineyard, grape leafhopper numbers were low, although almost equalled by the number of A. atomus over the season.

Similarly, studies in California have demonstrated that the egg parasitoid Anagrus epos of the Californian grape leafhopper (Erythroneura elegantula) can be enhanced and shows higher parasitation rates if prune (Prunus) trees are planted nearby as a refuge (Wilson et al. 1989; Murphy et al. 1996). Likewise, eggs of the host Edwardsiana prunicola serve as overwintering sites and are continuously used for reproduction over the growing season (Wilson et al. 1989). However, Rosenheim & Corbett (1996) found that the effect of prune refuges was limited to a few vine rows downwind and that A. epos exhibited a gradual decline with increasing distance from the refuge. In contrast, by planting dog roses within the vineyard along the vine rows, rather than in its vicinity, a more even distribution of the egg parasitoid was ensured in this study. Thus, establishing and promoting high-density populations of A. atomus could be an effective alternative to insecticide applications in areas with grape leafhopper problems.

In Franconia, the grape leafhopper seems to have been naturally controlled by mymarids for many years, and grape leafhopper numbers have continuously dropped. Furthermore, a close monitoring of five representative sites in the Franconian wine-growing area over the past 8 years has shown that irrespective of the number of immigrating grape leafhoppers the relationship of mymarids to grape leafhoppers at the hatching peak of the first generation stayed remarkably constant over the years, with one mymarid to 1–10 leafhoppers. In contrast to other German wine-growing areas, where two or three grape leafhopper generations per season occur, only one generation is observed in Franconia. The pattern of the population dynamics strongly indicates that mymarids effectively control the second generation of the grape leafhopper in Franconian vineyards.

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The Standardized Treatment Index as an indicator for pesticide use intensity on farms in North-East Germany

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INTRODUCTION

In 2004, the German Government issued a National Reduction Programme for the Use of Chemical Plant Protection Products, with the aim to reduce risks associated with their use. In order to monitor the progress of the programme a number of indicators were developed. Here, the pesticide use intensity will be analysed using the Standardized Treatment Index (STI). The STI counts the number of pesticide applications to a crop over one season. One application of a fungicide, herbicide, insecticide or growth regulator at the full permitted dosage over the whole area accounts for an index of 1. Reduced dosages and non-spraying of field parts decrease the index value. For monitoring or studying pesticide intensity, the index can be seen as a more accurate indicator than the amount of active ingredient(s) or amount of money spent. Owing to the standardized calculation procedure, it is possible to compare STI values for different crops and farms or even regions.

MATERIAL AND METHODS

In a study in Mecklenburg-Vorpommern (North-East Germany), on-farm data of pesticide use were collected to calculate the STI under practical field conditions. Two data-sets of pesticide use are analysed for a five-year period (2000 to 2004). The major focus was on crop rotations with oilseed rape and cereals. One data-set was collected by the State Plant Protection Service through a survey (data-set LPS). This comprised information on 36 single fields of different farms in the region and, over the five years, amounted to 80 records of winter wheat and 52 records of winter oilseed rape. The second data-set was acquired from the State Research Centre for Agriculture (data-set LFA), and originally collected for economic research. This data-set included information from all fields of seven farms in the region, 447 records of winter wheat and 227 records of oilseed rape. Together with the pesticide data, information was collected on cultivation practices such as cultivar choice, seeding time and tillage. Thus, analysis was possible on how far intensity of pesticide use is influenced by cropping practices. The effect of cultivation practices on STIs in winter wheat was examined by univariate or a multivariate ANOVA.

RESULTS

The variability of index values was high between years, but also between farms or individual fields. The yearly mean STI in winter wheat ranged from 4.3 to 5.6 in data-set LPS, and from 5.2 to 6.8 in data-set LFA. Comparable values for oilseed rape were 4.4–5.9 (LPS) and 4.4–6.9 (LFA). The means increased from year 2000 to 2004, due mainly to higher fungicide and herbicide intensity in wheat, and to greater insecticide use in oilseed rape.

The results of the univariate ANOVA show significant effects on fungicide and herbicide STI values by the following factors (P < 0.05): cropping region and year, cultivar susceptibility, amount of cereal crops in the rotation, tillage and seeding time. Cultivar susceptibility had the highest value of explained variability (eta²) for fungicide STI in data-set LPS. All fields of the data-set LPS were grown with cultivars of medium to low susceptibility; therefore, no ANOVA could be run on this factor. No effect appeared for winter wheat following winter wheat, for integration of a summer crop in the rotation or for the amount of nitrogen fertilization.

In the multivariate ANOVA, region and year were combined into one factor that represented the non-manipulable conditions of cropping. All cropping practices were combined into another factor, representing the susceptibility of the crop through management. The categories were developed with expert knowledge, particularly in relation to disease and weed pressure. The results indicate that the use of pesticides in the analysed data-sets is influenced mainly by environmental conditions (with about one third of variability explained by this factor). Only herbicides in data-set LFA seem to be less influenced by this factor. Moreover, data-set LFA shows that the combination of cultivation practices has a significant relationship to the intensity of fungicide and herbicide use. The factor 'crop susceptibility through management' could explain around 5 to 10% of STI variability. The effect could not be seen in data-set LPS, presumably owing to the smaller number of record sets.

CONCLUSIONS

Cultivation practices are a good means to significantly influence and reduce the intensity of pesticide use, as these measures of precautionary plant protection reduce the necessity for pesticide treatments.

ACKNOWLEDGEMENTS

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Reference farms for pesticide use and state of IPM implementation in arable farming

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INTRODUCTION

The German Action Plan for Reduction of Pesticide Use, starting in 2007, includes the establishment of a network of reference farms. These farms will provide reference data on the behaviour of farmers in relation to plant protection, and will deliver data on the intensity of pesticide use (as defined using the treatment frequency index (TFI)) and on the minimum need for pesticides in defined regions.

Two main aims of the action plan are described below.

• Annual collection of data on the intensity of pesticide use in major crops

The available TFI data demonstrate variable behaviour of farmers in different crops, years and regions. The statistical analysis of TFI data will be linked with data from the NEPTUN survey, which is conducted in a large number of farms every 3 or 4 years. Because of the large sample size, NEPTUN yields useful information on mean values, frequency distributions and corridors of the standard deviation of TFIs in the target regions. Because only a few reference farms can be established in each region, they provide typical examples but are not a statistically representative sample. However, the advantage of reference farms is that they permit data to be collected annually.

• Analysis of TFI data in connection with background information, especially on infestation per crop and year

The collected TFI data are analyzed by specialists from the advisory service in regard to minimum pesticide requirements. Reduction of pesticide use to the necessary minimum, in favour of cultural, natural and biological control methods, is a central demand of integrated pest management.

METHODS

The reference farm network is a collaborative project between the BBA and the plant protection services of German Länder. The BBA developed a concept that was discussed, together with the Länder, at a meeting in February 2007. This concept includes the following methodological approach:

- nomination of contact persons at the state and BBA level (Länder, BBA);
- annual collection of data on pesticide use in major crops (3 fields of each) of the reference farms and collection of other farm-related data;
- TFI calculation (BBA);
- monitoring and evaluation of field-specific infestations in the major crops (Länder);
- farm-specific evaluation of pesticide use in regard to minimum need requirements and reduction potentials (Länder);
- publication of crop-specific information summaries on pesticide use and background data for each reference farm (Länder, BBA), and public communication of results (BBA, Länder).

The number of reference farms selected, and criteria for their selection, will be based on the defined regions used in the NEPTUN surveys (Rossberg, 2002).

RESULTS AND DISCUSION

To date, 19 arable farming regions have been defined, covering all Länder except for the city states. Also, c. 60 reference farms have been earmarked, 41 of which have already been notified by the Länder. Most of these farms grow winter wheat, winter barley and winter rape, which are important crops for the analyses.

Vegetable (cabbage, carrot), fruit (apple), wine and hop-growing regions were identified, and the following numbers of reference farms were selected: 12 for each vegetable crop, 26 for apple, 14 for wine and 7 for hop.

The comments of specialists will show how objective factors, particularly occurrence of weeds, diseases and pests as well cost-benefit assessments by the users, modify the TFI. We also expect to gain information on subjective influences, such as user skills and risk behaviour. The findings from reference farms will help us to identify shortcomings in IPM, in terms of best practice. The information will contribute to the identification of pesticide reduction potentials, and will be very important for transparency and for communication of plant protection matters in Germany.

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Interaction between weed management, faunal diversity and plant growth of apple stands in the dry region of central Germany

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INTRODUCTION

A main objective of agri-environmantal schemes is a reduction in the risk of environmental contamination resulting from plant protection measures. This includes the banning of herbicide use in perennial crops, especially apple orchards and vineyards. In line with this, investigations were done to clarify the effects and interactions that occur within the agri-ecosystem under the dry conditions of central Germany. This deals especially with different methods of weed control or management, and their effects on arthropod populations within an apple stand.

MATERIALS AND METHOD

Investigations were done over three years, to specify the agri-environmental effects of different weed management procedures. The following variants were established: (1) control – without weed management; (2) weed management by herbicide use (glufosinate @ 5 litres/ha); (3) weed management by mechanical soil tillage. Within the treated areas, data were collected as follows: (1) characterization of apple tree development by measuring shoot growth; pitfall trapping to verify the population dynamics of epigeous arthropods (six traps per unit); (3) abundance assessment by leaf counts (30 leaves/tree, 10 trees per unit); (4) observations of aphid colony development and presence of antagonists (beneficials) (50 marked colonies with and without ant visitation).

RESULTS

The type of weed management influenced considerably both the growth of trees and the appearance of various arthropods (Table1). Growth was very different, when comparing the result of variant 1 with those of variants 2 and 3. This underlines the significant reduction of growth in variant 1 by water stress, resulting from higher transpiration by the dense weed cover on the soil. Differences in ther presence of arthropods were also evident; Table 1 shows selected examples. The number of recorded ants, aphids, mites (winter eggs) increased significantly as an effect of the intensity of weed management. On the other hand, the spider abundance dropped with increasing weed management. These findings confirm clearly the interactions between the type of weed management and the presence of arthropods in the apple stand. Using contingency table analysis (Dammer & Heyer, 1997), these findings could be quantified in selected arthropod communities (see Table 2). The calculation quantify the influence of the complete factor complex on the appearance of selected insect groups. The abundance of aphids, ants and beneficials is influenced most strongly by weather conditions and by vegetative growth stage. Nevertheless, other parameters also had significant impact on arthropod abundance. Concerning aphids, there is a clear dependence on weed managment. On the other hand, the appearance of beneficials in aphid colonies is considerably influenced by the presence or otherwise of ants.

	Control	Herbicide	Tillage
Mean length of 50 shoots (cm)	17.7	21.1	22.0
Total no. of ants (6 pitfall traps, 350 days)	4,850	6,990	6,800
Total no. of aphid colonies (300 branches)	155	300	370
Total no. of mites (winter eggs, 10×10 cm fruiting branches)	169	229	311
Total no. of spiders (300 leaf clusters)	57	28	6

Table 1. Shoot growth and arthropod presence (selected data, Manuel, 1999)

Table 2. Interaction of selected parameters, quantified by contingency table analysis.

Arthropods and impact parameters	Interaction selected	Coefficient of contingency
Aphids, period of vegetation (date), habitat (type of weed regulation)	total impact (dependency)	0.478
	date \times aphids	0.338
	habitat × aphids	0.278
Ants, beneficials*; year	total impact (dependency)	0.431
	year \times ants	0.201
	year \times beneficials	0.368
	ants \times beneficials	0.255

* Ladybirds (adults and larvae), hover flies (larvae), spiders and gall midges (larvae)

DISCUSSION

Various structural parameters determine the presence or absence of organisms in an ecosystem. Due to the water scarcity (c. 490 mm precipitation/year) the alternative to banning herbicide use does not lie in leaving the sub-vegetation but in the mechanical elimination of weeds by soil tillage. The structure of the apple stand is modified considerably with this and noticeably affects arthropod communities. In particular, aphid abundance is increased and the mutualism between ants and aphids will be enhanced. Therefore, under the specific conditions within the dry region of central Germany, the agri-environmental scheme of 'banning of herbicides' is inappropriate and does not make sense to apply.

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Survey on pesticide use in vegetable crops in Germany

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INTRODUCTION

Publicly available information on the real use of chemical plant protection agents in agricultural practice is urgently needed to address a series of scientific questions as well as for political argumentation. Therefore, in Germany, a survey on the application of chemical plant protection products on the most important crops has been carried out on a regular basis since the year 2000 (NEPTUN-Project). This project aims to increase the transparency regarding the intensity of chemical plant protection and to provide solid data for individual crops.

METHODS

An extensive survey on the use of plant protection products for a range of important vegetable crops was carried out for the first time in Germany in 2005. The grower organization 'Fachgruppe Gemüsebau im Bundesaussschuss Obst und Gemüse (BOG)' acted as the coordinator for data collection. Data were collected for the year 2005, and included all chemical and biological plant protection measures. The survey was based on a voluntary cooperation of selected farms in the main vegetable-growing regions and was, except for greenhouse crops, region specific. To obtain a realistic situation, all collected data were stored anonymously. For data analysis the application frequency and the application index were calculated. Application frequency denotes only the number of treatments, without considering the number and the amount of pesticides used at the same time. The application index specifies these factors, index rankings of active substances of different product groups (fungicides, herbicides, insecticides) were calculated.

RESULTS

For the vegetable survey in 2005, a total of 11,788 plant protection measures in 1,103 datasets were documented and analysed (Roßberg, 2006). Table 1 provides an overview on the application index of the different groups of plant protection products for selected vegetable crops. As expected, great differences between crops existed in the total number of plant protection measures, as well as in the range of the different groups of plant protection products. Salads and cucumbers were the crops with the highest intensity of pesticide use in outdoor and indoor production, respectively. The lowest use of pesticides occurred in spinach and basil production.

Crop	No. of data sets	All measures	Fungicide	Herbicide	Insecticide + acaricide
Field					
Salads	137	12.17	5.56	0.63	5.98
Carrot	160	6.91	2.67	2.30	1.93
Asparagus	258	6.66	4.29	1.40	0.97
Onion	147	9.52	5.53	2.73	1.27
Spinach	69	2.34	_	2.30	0.04
White cabbage	163	9.70	1.75	0.89	7.05
Greenhouse					
Basil	47	1.15	0.58	_	0.57
Cucumber	65	9.46	7.67	_	1.79
Tomato	57	4.36	2.72	_	1.24

Table 1. Application index for selected vegetable crops in 2005.

In the field the most used active ingredients of the fungicides were mancozeb (salads and onions) and difenoconazol (carrots, asparagus and white cabbage). In spinach no fungicides were applied. In the greenhouse propamocarb (basil), difenoconazol (cucumber) and fenhexamid (tomato) were most important. Concerning insecticides, cypermethrin (salads), lambda-cyhalothrin (carrot and asparagus), dimethoate (onions), *Bacillus thuringiensis* (spinach and tomato), methamidophos (white cabbage), soap (basil) and abamectin (cucumber) were the most frequently applied active ingredients. In only a few cases were significant differences observed regarding the intensity of plant protection in the different growing regions of Germany. In greenhouse cultivation, on average, 85–90 % of the measures against insects and mites were releases of beneficials.

DISCUSSION AND CONCLUSIONS

The data collected by the NEPTUN-Project are very important for stakeholders. One of the major benefits is transparency for the public regarding the use of plant protection measures. Growers and consulting services will get valuable information about the status quo. The survey should be done on a regularly basis, to obtain information on the development of plant protection measures in practice. Politicians can use these data for the implementation and observation of special programmes concerning the use of pesticides and the introduction of new techniques.

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CERCBET – a tool for the optimization of disease management in sugar beet

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INTRODUCTION

Cercospora leaf spot (*Cercospora beticola*) is the most serious fungal disease in German sugar beet growing. It may cause severe reduction in sugar beet yields and quality, and losses in sugar yield may vary from 5% to 50% depending on the severity of epidemics. Since the beginning of the 1990s, fungicide application has increased dramatically and, often, routine treatments were applied irrespective of disease development.

In order to reduce the number of superfluous fungicide applications, and to optimise fungicide use, a strategy based on action thresholds was elaborated. After certain improvements the strategy was introduced into practice by governmental crop protection services and sugar industry advisers. The action-threshold strategy required weekly assessments of 100 sugar beet leaves until the first treatment was applied; two weeks after the treatment assessments had to be continued. High labour input was the main reason for little acceptance by sugar beet growers. To enhance acceptance, the elaboration of forecasting models which should be able to predict the start of field assessments and the dates on which the action thresholds (disease incidences of 5, 15 and 45% during different periods from July to mid-September) are overridden was started. The main aim was to maximize the reduction of labour input for field assessments. If possible, the results of one or at maximum two assessments should be sufficient to serve as input for the models.

THE CERCBET MODELS

Development of the CERCBET models followed three main aims. The first two aims refer to the start of field assessments. Advisers from governmental crop protection services and from the sugar industry are the first to start monitoring activities. Their results are published via warning services (bulletins, internet). The first aim was to predict the start of these monitoring activities. Farmers commence their field inspections with a certain delay, so the second aim was to forecast the dates when farmers should start. For both aims a forecasting model was developed which predicts the date of first occurrence and the course of first occurrence in sugar beet fields, represented by a meteorological station. We named the model CERCBET 1. The third aim was to predict the dates when action thresholds (see above) will be exceeded and farmers should treat sugar beet crops with fungicides. To reach this aim a model (CERCBET 3) has been developed which simulates the progress of disease incidence for cercospora leaf spot. As the sugar beet growing season is quite long, and action thresholds may be overridden more than once, a module was needed within CERCBET 3 which models the effect of fungicides on the course of disease incidence.

As inputs, CERCBET 1 needs both meteorological and agronomical parameters. Temperature and relative humidity serve as meteorological input. Sugar beet prevalence, length of breaks between two successive sugar beet crops and an estimation of disease severities (in four classes) at the end of the previous season are the agronomic parameters and these represent a regional inoculum factor. CERCBET 1 calculates the share of sugar beet fields within a region infested by *C. beticola*. As soon as 5% of the sugar beet fields are infested (first occurrence) advisory officers should start regional monitoring activities. When about 50% of the fields are infested farmers should start observations in their own fields at the latest, because then the first action threshold for *C. beticola* control may be reached. Validation of CERCBET 1 was done with data sets from 1995 to 2003. In 12% of the cases CERCBET 1 predictions were too late and 21% of forecasts were too early, in regard to the date of regional first occurrence of *C. beticola*, considered a satisfying result. Far more important is a correct forecast of the date when 50% of the fields would be infested, because first fungicide treatments may already be required. In this case CERCBET 3 gave 89% correct forecasts. Just 7% of the predictions were too early and only 4% were too late.

CERCBET 1 is a model working on a regional scale whereas CERCBET 3 is plot-specific. Meteorological input parameters for CERCBET 3 are temperature, relative humidity, precipitation and wind speed. Agronomical input parameters are virtually the same as for CERCBET 1, but the parameter 'sugar beet prevalence' does not refer to the region in which the sugar beet field is located but to the close vicinity of the field. In addition, a factor representing irrigation is included in CERCBET 3. Epidemiology of *C. beticola* in CERCBET 3 is modelled by including three variables: incubation rate, infection rate and sporulation rate. These rates are combined multiplicatively to a daily infection pressure index from which disease incidence progress is calculated, using a logistic regression model. For modelling fungicide efficacy a module using temperature and precipitation and input parameters was included.

CERCBET 3 may be used to plan a fungicide strategy for a whole season, based on weather data, action threshold and one or two field assessments. CERCBET 3 in general gives good forecasts, in a range up to 50% disease incidence. Above 50% the model tends to underestimate disease incidences, which is not of practical relevance as the maximum threshold value is 45%. CERCBET 3 gave satisfactory results in 98 validation trials carried out from 2001 to 2003. The action threshold of 5% was correctly forecasted in 91% of cases, the 15% threshold in 83% and the 45% threshold in 81% of cases. Often, also, the 'too early' forecasts (9%, 13% and 10%, respectively) have to be considered as correct, owing to the very strong increase of the following epidemic. In order to improve CERCBET 3 forecasts, a module accounting for differences in cultivar susceptibility to *C. beticola* has recently been developed.

From 2003 to 2005, CERCBET 1 and 3 have been successfully introduced into agricultural practice on a national scale. The CERCBET models proved to be a valuable tool within an integrated crop protection system for sugar beet leaf disease control.

Models for beet rust (*Uromyces betae*), powdery mildew (*Erysiphe betae*) and ramularia leaf spot (*Ramularia beticola*) are currently under development.

Biosensors for field-based detection of plant pathogens and pesticide residue analysis: the state-of-the-art technology as a key tool in Integrated Plant Disease Management

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INTRODUCTION

In recent years there has been a rapid increase in the number of diagnostics applications in phytopathology and food chemistry based on biosensors, which can be defined as devices incorporating a biological sensing element connected to a transducer. The most advanced biosensor technology includes live, intact cells as the sensory units. At their current status, cell-based technologies could directly compete with immunoenzymic assays and other immunoanalytical systems. To these promising methods belongs the Bioelectric Recognition Assay (BERA) (Kintzios *et al.*, 2001). This method utilizes the natural response of cells to pathogens or other toxic factors. Alternatively, it can utilize so-called membrane-engineered cells, which are cells with tens of thousands of target-specific receptor molecules artificially inserted on the cell surface. In the following we describe, as a representative example, the application of BERA biosensors for the detection of (1) a plant virus (*Cucumber mosaic virus* – CMV) and (2) a pesticide (metamidophos) in plant tissues. Furthermore, we present the profile of a novel analytical lab, 3QLabs, which employs biosensors as an integral BMP tool for vegetable and fruit production at a country-wide scale.

METHODS

For CMV detection, BERA biosensors were based on membrane engineered Vero cells, created by electroinserting CMV polyclonal antibodies (Moschopoulou & Kintzios, 2006). For metamidophos detection, sensors were based on neuroblastoma cells. In both cases, each consumable sensor was connected to a working Ag/AgCl electrode and through this to the recording device, which comprised the PMD-1608FS A/D card (Measurement Computing, Middleboro, MA). Sensors were used for assaying CMV or metamidophos in homogenized plant extracts, derived from individual plants (n = 100). The total assay time was less than one minute. Result evaluation was assisted by a multi-net classifier system using Artificial Neural Networks (ANNs).

RESULTS

As demonstrated in Table 1, the BERA system was able to rapidly detect the presence of CMV or metamidiphos in plant extracts. Detection was very selective and each sensor type (membrane-engineered or neuroblastoma) responded only to its corresponding target (CMV or metamidophos, respectively). Further processing with the Artificial Neural Network has shown

that the biosensor system was able to detect negative samples or samples positive for either CMV or metamidophos with 100% or 98% specificity, respectively.

Table 1. Response of BERA sensors to either CMV (membrane-engineered sensors) or metamidophos (neuroblastoma sensors) (n = 100 replications for each sample). Biosensor response is expressed in mV.

Biosensor type	Control	CMV	metamidophos	metamidophos
Membrane-	27 ± 2	101 ± 11	27 ± 2	27 ± 2
engineered				
Neuroblastoma	-5	-5	17	22

Furthermore, we conducted a market analysis for the feasibility of adopting the BERA technology as a routine method for pathogen testing and pesticide residue analysis. A model company, 3QLabs (www.3QLabs.org) was designed for this purpose. The analysis has shown that the company could achieve a net profit of 1.7 million \in within five years of operations (Table 2).

Table 2. Financial assumptions and ratios for a model company employing biosensors as an integral BMP tool for vegetable and fruit production at a country-wide scale.

	Year 1	Year 2	Year 3	Year 4	Year 5
Sales ('000 €)	500	930	1,770	2,090	2,900
Cost of goods ('000 €)	116	210	334	400	470
Profit before tax and interest ('000 €)	_	100	640	1,000	1,700

DISCUSSION AND CONCLUSIONS

Biosensor systems for field-based pathogen and pesticide residue detection offer a number of significant advantages, such as high speed, reproducibility, accuracy, selectivity and sensitivity, as well as the ability to monitor at real-time conditions and retrieve as much information as possible during a single assay. As revealed by the financial analysis, providing novel solutions for food quality assurance can be a very profitable business, especially in view of the new EU regulations for minimal residue concentration in marketed food.

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Introduction of GIS in decision support systems for plant protection

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INTRODUCTION

During the last 40 years many weather-based forecasting models have been developed for the control of late blight (*Phytophora infestans*) (Kleinhenz & Jörg, 2000). SIMPHYT I and SIMPHYT III have been established to provide the best control of late blight in Germany (Gutsche, 1999; Gutsche *et al.*, 1999; Roßberg *et al.*, 2001; Hansen *et al.*, 2002). SIMPHYT I predicts the first appearance and SIMPHYT III calculates the infection pressure for the disease. A new model class for late blight is in practical use – SIMBLIGHT1 (Kleinhenz, 2007). In future a combination of the forecasting models with Geographic Information Systems (GIS) should help to get better forecasting results for local areas between two or more meteorological (met.) stations. With the use of GIS, daily spatial risk maps will be created in which the spatial and the temporal process of first appearance and regional development of late blight are documented. To reach this aim it is necessary to prepare meteorological, geomorphologic and plot-specific parameters of the forecasting models with a spatial index.

METHODS

The building of spatial risk maps is done in six steps: Step 1: import of hourly met. data from the weather database. Step 2: combination of met. data with the geographic information of the met. station. Step 3: preparation of geographical baseline data. Step 4: interpolation of the met. data. Step 5: calculation of the forecasting model, using the results of the interpolation. Step 6: display of the results as a risk map. The first three steps deal with data management. Step four is the main and the most difficult step. Different kinds of interpolation methods are necessary to identify or modify a method which gives the best results in interpolating met. data. Step five uses the interpolated met. data as input parameters to calculate the forecasting models. The final step is to connect the results to an internet application in which spatial information is displayed as a risk map of the first appearance of late blight and, later, of the daily infection risk.

RESULTS

The first calculations showed that deterministic interpolation methods were not suitable. We therefore concentrated on geostatistical interpolation methods. The following results show a comparison between Kriging (K) and Multiple Regression (MR) methods. Temperature and relative humidity were calculated for the years 2000 to 2005 for two German Bundesländer (Brandenburg and Rheinland-Pfalz). To compare the measured data with interpolated data some met. stations have been left out of the interpolation process. After calculation the interpolated values were compared with the measured values of the met. stations. Both

interpolation methods were able to calculate results with high accuracy. The coefficient of determination in all cases ranged from 96 to 99%. The results showed no significant differences between the two interpolation methods in either Bundesland. The differences between K values and measured values ranged from 0.5 to 2°C. Differences were less for MR (0.3 to 1°C). The interpolation of relative humidity (RH) show similar results compared with temperature interpolation. The coefficient of determination varied from 92 to 96% and mean differences in RH were 5 to 10% of recorded values.

After met. data, interpolation with MR data was made available to the forecasting models. The model predicted that infection of late blight would start early in the north-western part of the area and spread to southeast. Two monitoring points (P1 and P2) are displayed on a map, and field records from these monitoring points used to verify the calculation results. Infections at P1 (in the area at maximum risk) were recorded earlier than at P2 (in the low-risk area). The recorded time difference of the first occurrence of late blight at P1 and P2 was 14 days, which coincides well with our calculations. Absolute differences of forecasted and recorded dates for first occurrence of late blight were 3 days, which must be regarded as a highly accurate result.

CONCLUSION

The combination of forecasting models with analyses and methods from GIS is a milestone for advising farmers. GIS methods and analyses will help to obtain more detailed information, and results will have greater validity than before. It will be easier to understand and to interpret the results of forecasting models. Spatial maps will show hot spots of maximum risk. GIS and forecasting models lead to easier control of late blight. Thus, the aim of reducing the number of sprays can be achieved and this guarantees an environmentally friendly and economic crop-protection strategy. The clear vivid presentation methods of GIS make decision support system results easier to understand and lead to a higher acceptance of warning systems by the farmers.

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The forecaster ZWIPERO for downy mildew of onion: applying a disease warning system in diverse culture systems of vegetable crops

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INTRODUCTION

Downy mildew of onion, caused by *Peronospora destructor*, is the most disastrous disease on leaves of onion in all onion-growing regions with a humid climate. Therefore, forecasting systems based on weather data have been developed for spring-sown onions and adjusted to the regional conditions in several countries (Canada, France and Italy and the Netherlands).

In Germany, the forecaster ZWIPERO (Friedrich *et al.*, 2003) was introduced for spring-sown onions in 2005. Furthermore, depending on the climatic conditions, onions are grown as an over-wintering crop and as all-year-grown salad or bunching onions, with very high regional importance. In order to meet different market demands, spring-sown and salad onions vary widely in variety, the canopy density chosen and the irrigation intensity applied. While in bulb onion production severe downy mildew epidemics affect the yield and the grading achieved, in salad onions even low disease incidence, as well as pesticide residues, result in an unmarketable crop. Therefore, the forecaster ZWIPERO has been adjusted with regard to the diverse culture systems.

METHODS

The forecasting model ZWIPERO determines the risk of sporulation and infection of downy mildew, based on simulated microclimatic input data. Such data are provided by the subroutine AMBETI (Braden, 1995), the soil-plant-atmosphere model of the German weather service (DWD). Input data of the subroutine are actual standard weather data and hourly-predicted weather data, as well as data from local model fields (soil type, plant density, seeding date, canopy development and calculated irrigation time). Field trials were conducted in spring-sown onions (2000–2004) and salad and over-wintering onions (2005–2006) at the experimental farm in Queckbrunnerhof (DLR-Rheinpfalz) as well as at commercial farms. The trials included: (i) determination of canopy development (green leaf area index); (ii) variety tests; (iii) validation of fungicide strategies according to daily ZWIPERO output data; and (iv) monitoring of sporulation periods as an estimation of the regional inoculum available.

RESULTS

Canopy development differed strongly among the different culture systems and, to some extent, among different onion varieties. To take this into account the development of green leaf area was determined as a function of leaf stage (onions for bulb production) or canopy height (salad onions) and provided as an additional subroutine to AMBETI. The currently available varieties are all susceptible to downy mildew, with some varieties showing partial resistance. On average, two sprays fewer than in the grower routine were applied when using ZWIPERO to determine fungicide application in experimental field trials and on farm trials in spring-sown onions. The experimental trials also indicated an increasing fungicide efficacy when using the predicted infection risk values to determine fungicide application. Additionally, the sporulation risk values may be used as an efficient tool for assessing sporulation and disease incidence in commercial fields.

ZWIPERO is provided by ISIP via the internet (information system integrated **p**lant production: www.isip.de) in cooperation with the advisory services of the various Länder in Germany. The advisory services supply data from local model fields and communicate ZWIPERO information to the growers. Alternatively, growers have directly internet access to ZWIPERO forecast of their specific region.

DISCUSSION AND CONCLUSION

The forecaster ZWIPERO for downy mildew in onion was adapted for diverse culture systems, such as all-year-grown salad onions or spring-sown and over-wintering onion crops. ZWIPERO is a proven tool to increase fungicide efficacy by timed fungicide sprays according to predicted infection risk. It may also lead to a reduction in the number of fungicide sprays and, therefore, contributes to the national pesticide reduction programme. In 2005 and 2006, ZWIPERO was provided, for spring-sown onions, via the internet and was universally accepted by advisory services and growers. The implementation of ZWIPERO for salad onions in the information system ISIP will be continued in 2007.

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The phytosanitary strategies for control of plant-parasitic nematodes in the Ukraine

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INTRODUCTION

The introduction and spread of plant-parasitic nematodes depend to a great extent on the phytosanitary legislation employed. The Ukrainian General State Inspection on Plant Quarantine is issuing a number of new actions to improve statutory regulations, and the importance of plant-parasitic nematodes as a constraint to crop production in the Ukraine has recently been recognized (Movchan *et al.*, 2004). For the first time, a new national list of quarantine and regulated plant-parasitic nematodes has been prepared on the basis of technical justification and pest risk analysis. The latter revealed the necessity to collect and analyze information on the detection of phytonematodes in export and import commodities.

METHODS

The results of nematological diagnostics conducted in 24 quarantine laboratories of the Ukraine were submitted to one database, within which statistical data were grouped into different categories (nematodes: systematic order, feeding type; commodities: type, place of origin, import / export / local trading etc.). Statistical analysis of the data was made following these categories.

RESULTS

During the years 2004–2005, 146,730 nematological analyses were carried out in quarantine laboratories, of which 30,773 were conducted for commodities imported from 19 countries. In total, 60 nematode species were detected in a broad range of quarantine samples: 28 species were identified in potted plants, 13 in commercial turf, 9 in sawn coniferous timber, logs and wooden packaging materials, 7 in seedlings, 6 in bulbs and 5 in potato. The orders Araeolaimida, Dorylaimida, Enoplida, Monhysterida, Rhabditida and Tylenchida were represented by 1, 6, 4, 1, 24 and 24 species, respectively. No species rated as a 'quarantine pest not present in the Ukraine' were found.

A larger number of nematode species (78) were found in commodities specified for local trading or export from the Ukraine: of these, 8 species were identified in potted plants, 28 in soil samples, 34 in sawn coniferous timber, logs and wooden packaging materials, 9 in seedlings, 5 in bulbs and 17 in potato. The orders Araeolaimida, Dorylaimida, Enoplida, Rhabditida and Tylenchida were represented by 1, 2, 30 and 42 species, respectively.

DISCUSSION AND CONCLUSIONS

Nematological diagnostics conducted for the imported commodities proved at potted plants were the main pathway for plant-parasitic nematodes to enter the Ukraine. Further, identification of nematode species detected in the commodities specified for local trading or export from the Ukraine improved knowledge of nematode fauna associated with different environmental sites in the Ukraine. The latter revealed, for example, that sixteen species of *Heterodera* were present in the country (*H. avenae, H. cacti, H. carotae, H. cruciferae, H. estonica, H. galeopsidis, H. goettingiana, H. humuli, H. leptonepia, H. millefolii, H. paratrifolii, H. punctata, H. rumicis, H. schachtii, H. trifolii and H. urticae*). However, further studies are necessary to prove the identifications, that were based on morphological characteristics.

Detection of other nematode species, more or less common in Ukrainian agriculture, included those in the genera *Aphelenchoides*, *Ditylenchus* and *Meloidogyne*. Further, *Bursaphelenchus mucronatus* was detected several times in sawn coniferous timber and logs.

All this information was submitted to the pest (nematode) risk assessment programme, which finalized the preparation of a new Ukrainian national list of regulated plant-parasitic nematodes.

In contrast with the current list, which includes seven nematode species, the new one will include twelve: here, there will be an attempt to use official regulations not only for quarantine nematode species (*Bursaphelenchus xylophilus*, *Globodera pallida*, *G. rostochiensis*, *Heterodera glycines*, *Meloidogyne chitwoodi*, *M. fallax* and *Nacobbus aberrans*) but also regulated non-quarantine species which could be spread by mean of seeds, seedlings and other planting material (*Aphelenchoides besseyi*, *Ditylenchus destructor*, *D. dipsaci*, *Radopholus citrophilus* and *R. similis*).

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FOOTPRINT – functional tools for pesticide risk assessment and management

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INTRODUCTION

FOOTPRINT (www.eu-footprint.org) is a 3-year research project in the EU 6th Framework Programme (Project No. 022704). FOOTPRINT aims at developing a suite of three pesticide risk prediction and management tools, for use by three different end-user communities: farmers and extension advisors at the farm scale, water managers and local authorities at the catchment scale, and policy makers and registration authorities at the national/EU scale.

The tools will be based on state-of-the-art knowledge of processes, factors and landscape attributes influencing pesticide fate in the environment and will allow users:

- to identify the dominant pathways and sources of pesticide contamination in the landscape;
- to estimate levels of pesticide concentrations in local groundwater resources and surface water;
- to make assessments of how the implementation of mitigation strategies would reduce pesticide contamination of adjacent water resources.

The three FOOTPRINT tools will be complementary and tailored to the different needs of the different user groups. They share the same philosophy and underlying science (e.g. the development and subsequent modelling of a large number of scenarios representing agro-environmental conditions in the EU) and will provide a coherent and integrated solution to pesticide risk assessment and management in the EU. The predictive reliability and usability of the tools will be assessed through a substantial programme of piloting and evaluation studies at the field, farm, catchment and national scales. Beta-versions of the three tools will be publicly available for testing in September 2007; the final versions are due in November 2008.

THE FOOT-FS (FARM SCALE) TOOL

FOOT-FS is mainly targeted at farmers and extension advisors. It will be available both as a stand-alone application and as a web portal. The aims of the tool are:

- to identify the pathways and those areas that most contribute to contamination of water resources by pesticides at the scale of the farm;
- to provide site-specific recommendations to limit transfers of pesticides in the local agricultural landscape.

The classification of the agricultural land according to the pathways leading to contamination of water resources by pesticides will be based on a hybrid between the CORPEN and HOST methodologies. The estimation of pesticide concentrations in water resources due to leaching, drainage and surface runoff/erosion will rely on the deterministic models MACRO and PRZM, while simpler, more pragmatic approaches (e.g. drift calculation formulae according to FOCUS) will be used for assessing pesticide inputs via spray drift and point sources (storage places, farmyards). Predicted concentrations in edge-of-field surface water bodies will allow risk assessments to be performed for aquatic taxa as all three FOOTPRINT tools will include a database of ecotoxicological threshold values for fish, invertebrates, higher aquatic plants and algae.

THE FOOT-CRS (CATCHMENT AND REGIONAL SCALE) TOOL

FOOT-CRS is mainly targeted at local authorities, stewardship managers and water managers in charge of implementing the WFD and/or limiting the contamination of water resources by pesticides. However, it may also have applications with regulators or the crop protection industry, e.g. to investigate a region more closely when an application of the national and EUscale tool FOOT-NES has identified this region as a potential 'hot spot' of pesticide exposure. FOOT-CRS will be available as an ArcGIS extension. The main objectives of the FOOT-CRS tool are:

- to identify those areas in a catchment that most contribute to pollution of waters by pesticides;
- to define and/or optimise action plans (monitoring, mitigation, application restrictions etc.) at the scale of the catchment.

The classification of the agricultural land according to the dominant pathways leading to pesticide contamination of water resources will be based on remote sensing data (satellite imagery or aerial photos) and an adaptation of the HOST/CORPEN methodology used in the farm-scale tool FOOT-FS.

THE FOOT-NES (NATIONAL AND EU SCALE) TOOL

FOOT-NES is mainly targeted at decision and policy makers, but also has relevance to the registration context. The tool will have the potential to support the pesticide registration authorities and the crop protection industry for higher-tier modelling purposes. FOOT-NES will be available as an ArcGIS extension. The main objectives of the FOOT-NES tool are:

- to identify the areas or regions in the EU or a member state that are most at risk from pesticide contamination;
- to assess the probability of pesticide concentrations exceeding legal or ecotoxicologically-based thresholds.

Exposure/risk assessment in FOOT-NES is, thus, exclusively prospective. For risk assessment for the current situation, the user is referred to the two smaller-scale tools. In FOOT-NES, the classification of the European agricultural land according to the dominant transfer pathways will be undertaken using the innovative, data-parsimonious IDPR methodology.

ENDURE – a European network of excellence on pesticide reliance reduction

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INTRODUCTION

ENDURE is an initiative to reshape European research and development on pesticide use in crops, for the implementation of sustainable pest control strategies. It was selected for funding by the European Commission in response to call FP6, Food Quality and Safety, in the Area 'Safer and environmentally friendly production methods and technologies and healthier food stuffs' and Topic 'Reducing the use of plant protection products (NoE)'.

The consortium is made up of partners from 10 European countries:

- INRA (ENDURE Coordinator) France;
- Association de Coordination Technique Agricole (ACTA) France;
- CIRAD France;
- INRA Transfert (IT) France;
- International Biocontrol Manufacturers' Association IBMA International;
- Consiglio Nazionale delle Ricerche CNR (Italy);
- Scuola Superiore di Studi Universitari e di Perfezionamento Sant'Anna (SSSUP) (Italy)
- Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA) Germany;
- Rothamsted Research (RRES) UK;
- Aarhus University, Faculty of Agricultural Sciences (FAS) Denmark;
- Danish Agricultural Advisory Service (DAAS) Denmark;
- Agroscope Swiss Federal Research Station (AGROS) Switzerland;
- Plant Breeding and Acclimatization Institute (IHAR) Poland;
- Szent István University (SZIE) Hungary;
- Universitat de Lleida (UdL) Spain;
- Plant Research International (PRI) (also representing PPO and LEI of Wageningen UR) - the Netherlands.

Our objective is to reshape European research and development on pesticide use in crops, and to establish the network as a leader in the development and implementation of sustainable pest management strategies.

We will create a coordinated structure that takes advantage of alternative technologies, builds on advances in agricultural sciences, ecology, behaviour, genetics, economics and social sciences, and connects researchers to other stakeholders in extension, industry, policy-making and civil society. This multi-disciplinary and cross-sector approach is designed to foster the development and implementation of strategies rationalising and reducing pesticide inputs, as well as reducing risks.

Our operational goals are:

• to bring together research capacity and resources currently fragmented across Europe. We will share knowledge and people, and pool our facilities, biological resources and equipment through a joint crop protection research programme and the creation of a coordinated and geographically decentralised European resource facility – a 'virtual laboratory' – on pest control;

• to enhance the research-to-R&D innovation process by creating working relationships between researchers and practitioners in extension services and farming;

• to bring in industry, policy-makers and civil society to help define the research agenda;

• to pass on knowledge, know-how and resources through training, education and dissemination, targeting farmers, advisors, researchers, policy-makers and civil society – our European Pest Control Competence Centre is designed to become a source of knowledge and expertise, to support public policy-makers, regulatory bodies, extension services and other crop protection stakeholders;

• to endure, by building a sustainable, coherent and transnational institution made up of leading European crop protection research, R&D, extension, and industry organizations.

We will advance toward these goals in three ways:

• integrating activities that will help us identify priority research areas, link up with other relevant research and civil society groups, and plan our legal and financial sustainability;

• jointly executing research that will stimulate and develop a culture of collaboration in areas that are key to achieving progress in reducing reliance on pesticides;

• cross-fertilisation (or spreading) that will extend our activities and outputs to farmers, extension agents, students, policy-makers, consumers and society-at-large, as well as to elicit feedback and dialogue, ensuring that activities and outputs meet the needs of these stakeholders.

Our four-year programme started in January 2007. The initial 18-month period – with funding spread over a large number of participants and activities to foster interaction and sharing – will serve to review and collate research, and will lead to a focused research programme shaped by competitive bids in priority areas for collaborative projects submitted by at least three partners from three countries.

Potential and limits of biological control with beneficials in greenhouse ornamentals

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INTRODUCTION

Biological control with beneficials (biocontrol) is a long known successful story in vegetable production. In the last decade biocontrol in ornamentals became an interesting field of use in various ornamental crops such as poinsettia (Euphorbia pulcherrima) and bedding plants. Owing to the specific demands of ornamentals, biocontrol is also a field of research in manyt countries and is, therefore, discussed in international working groups (e.g. the International Organisation of Biological and Integrated Control, IOBC). The increasing importance of biocontrol in practice is due to different aspects: to a sophisticated view towards the side-effects of pesticides on the environment and to problems with pests resistant to insecticides; thirdly, to a lack of registered and recommended pesticides; and lastly, but not least, to developing concerns of growers towards their own health and safety.

ADVANTAGES AND DISADVANTAGES OF BIOCONTROL IN ORNAMENTALS

Biocontrol has several advantages over chemical control (e.g. beneficial organisms generally have no negative impact on the environment). This is true for native species, although not necessarily so for alien species. Certainly, beneficials do not leave chemical residues in food and there is no pre-harvest interval; nor do they hold any risks for the user. Instead, their implementation in cropping systems often offers a longer period of efficiency and a very low risk of resistance to the controlling agent. Nevertheless, there are some difficulties in handling beneficials particularly, where many different crops (which might be infested with different pests) are produced in varying production systems. In the beginning beneficials act slowly, the action threshold is pretty low and problems rise if additional pests or diseases occur. Consequently, biocontrol requires high advisory input and growers need up to 3 years patience until the biological system is established. Above all, biocontrol is slightly more expensive.

SPECIAL DEMANDS ON USING BIOCONROL IN ORNAMENTALS

Ornamental species and cultivars vary considerably – there are foliage and flowering plants, herbaceous and woody plants, and native as well as exotic species. Exotic species are often imported from other countries, which means from other climates and areas with a different, unknown and diverse spectra of pests and diseases. The wide range of pests and diseases with their corresponding beneficials makes biocontrol a complex system. Even the production systems vary immensely – for example, potted plants vs cut flowers; production in soil vs production in artificial media. In general, quality standards for ornamentals are extremely high. The produce must be completely free of insects, mites or any damage. For potted plants this means the whole plant must be free compared with greenhouse vegetables (such as cucumbers or tomatoes) where only the fruits are considered.

INTEGRATED PLANT PROTECTION MANAGEMENT

To cope with the high demands for quality of ornamental plants, there is often a need to integrate pesticides with biocontrol. For example, during the summer, adult thrips invade greenhouses and cause damage to flowers, often over a period of several weeks; they then leave the greenhouses. Generally, predatory mites can succesfully cope with a thrips outbreak in the crop, by feeding on the nymphs. However, the invading insects are adults. In springtime, some beneficials are insufficiently active, as it is either too cold or there is not enough light. This is the case for whiteflies and their parasitoid *Encarsia formosa*. If a grower wishes to adopt a biocontrol strategy, he should use selective pesticides for at least six months before commencing. This is necessary to decontaminate the crop. A heavy outbreak of a pest shortly before marketing can make a pesticide treatment necessary. Also, for some pest species no adequate beneficial organisms are available. Diseases are usually controlled by fungicides. Thus, there is an urgent need to know about the side-effects of pesticides (i.e. whether they are

harmful to beneficials and how persistent is their detrimental effect). This will help growers to select and time appropriate pesticide treatments. If a pesticide has a long-lasting detrimental effect on beneficials its use can lead to serious problems.

BIOLOGICAL/INTEGRATED CONTROL OF WHITEFLIES IN POINSETTIA

Controlling whiteflies (*Trialeurodes vaporariorum*) with *Encarsia formosa* in poinsettia has been a successful example for biocontrol programmes for many years. However, in recent years this strategy has been questioned because of its reduced efficacy. That is why the influence of different insecticides, particularly imidacloprid (Confidor WG 70), on searching and parasitation behaviour of the parasitoid was examined. In addition, tobacco whitefly (*Bemisia tabaci*) is on the increase in German horticulture, a species that chemical insecticides as well as beneficial organisms fail to control; one reason for the reduced efficacy of chemical protection is the fast development of resistance. Biocontrol could prove to be an alternative. Hence, the parasitic capacity of *E. formosa* was examined. The results showed that many insecticides have a repellent impact on *E. formosa*, so the wasps do not approach treated plants. In particular, imidacloprid (frequently used in stock plants) has a long-lasting repellent and lethal effect, lasting for 16 wk after spraying and for even longer after drenching. To control tobacco whitefly with *E. formosa* a minimum release of one wasp per plant is necessary and the mode and quantity of parasitoids released have to be adapted.

ECOMONIC EVALUATION OF BIOCONTROL

Implementing a biological control system requires a phase of reorganisation and adaption, with a high input of beneficials and monitoring. A recent economic evaluation of long-term benefits included consideration of the direct costs (over 6 years) for plant protection measures from two nurseries producing cut-flower roses. When the project began, costs in both nurseries were much higher than for conventional pest management (at, overall, $2.79 \notin/m2$ and $2.89 \notin/m2$). During the project, however, costs could be reduced significantly to $1.20 \notin/m2$ and $1.27 \notin/m2$, respectively, which is comparable to conventional production. Biological control systems have other important benefits that cannot be evaluated directly, such as growers' concerns towards their own health and safety, better plant quality, and the availability of alternatives if there is a lack of efficient pesticides or if pests become resistant. Hence, biological control has essential advantages in the long-term, as well as social and environmental benefits.

www.isip.de - online plant protection information in Germany

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INTRODUCTION

ISIP, the Information System for Integrated Plant production, is a Germany-wide online decision support system. It was initiated in 2001 by the German federal extension services as a common advisory portal, thus achieving synergies by pooling existing information. Despite the centralised character of the system, the regional identity of the co-operating services was to be preserved by a dispersed administration and data input. With the start of the system, the ISIP association was established; by 2007, this comprised eleven of the sixteen federal extension services in Germany. The office of the association, currently with four employees, is in Bad Kreuznach, Rhineland-Palatinate. Since information transfer is the primary task of extension services, the system is intended to make this work more efficient by using modern information technology. Therefore, a bi-directional data flow between the services and farmers was developed. By combining general with specific data, recommendations can be refined from regional to individual.

INFORMATION CONCEPTS

Three types of information can be distinguished in ISIP, each differing in scale. Decision support modules (DSMs) deliver the most specific results. They comprise results from a simulation model and/or monitored field observations, as well as a comment from the regional extension worker. This 'threefold decision support' gives a comprehensive overview for a defined pest or disease. More general information is provided in regional news. The members of ISIP can maintain their own starter pages in the system, where they can distribute topics ranging from contact data to legislative news. Furthermore, paper-based warning and information services are made available for download as PDF documents. The most general information is given in the encyclopaedia, where background information and standard recommendation for more than 20 crops and 200 pest and diseases are stored in a database.

Subsequently, a closer look will be drawn to the DSMs as implemented in the system. The different elements of the modules are represented in a defined colour scheme: the comment of the regional extension worker is marked in red; simulation results are shaded in orange and monitoring data shaded in green. This scheme and a limited set of icons provide a consistent interface for the user: e.g. a calculator symbol links to a form, where the user can input his information. With sending these to the server, the data are stored and the model is run, returning an individual result. The model is re-run whenever the weather data are updated, thus giving a new result every day. To release the user from having to check the system daily, an automatic warning service can be set up. When a module-specific threshold is reached, an SMS or e-mail is generated by the system and sent to the user. As of 2007, eight DSM are available, while another seven modules are currently under development.

TECHNICAL CONCEPTS

The software architecture of the system can be distinguished in three main tiers: the presentation, the application and the database. The presentation tier consists mainly of HTML pages, to be viewed in a standard web browser. The application tier comprises the system kernel, with prognosis models and other modules, such as import and export routines or scheduling functions. Finally, in the database tier, all necessary information – primarily weather data – for the model calculations is stored.

To facilitate the integration of new models, a 'master component' was developed. This component comprises an application programming interface (API) to both the presentation and the database tier. A model frame connecting the two APIs is ready to receive new simulation algorithms; thus, this master component can be used as a template for model development. Apart from JAVA programming knowledge, the model developer is relieved of technical details of the system framework, and can focus on the quantification of the functional relationships. The final outcome is a fully functional ISIP component, which can easily be implemented into the system. Model development with the ISIP master component is a three-step process, the first of which is development of the scientific model. To support this, a bare-bone ISIP system is installed on a local computer, comprising the application and the database tiers only. After the model has been evaluated, the integration into a non-public internet environment follows. Here, a number of technical tests are run. If the new model passes these tests, the final step is the release to the public production server.

DISCUSSION

The advantages of the ISIP system differ between the two target groups. On the one hand, the farmer gains most from the on-line calculation of prognosis models which deliver site-specific recommendations. Furthermore, the consistent user interface eases the acquisition of information. The automatic warning service by SMS or e-mail reduces online and response times, especially for time-critical decisions. On the other hand, extension workers benefit from the web-based input of monitored field data and advisory comments. This eliminates further processing, and ensures a fast and efficient transfer of information.

In the near future, new DSMs for plant protection will be included. Additionally, a special focus will be set to agronomic and horticultural model approaches. The encyclopaedia will also be extended on an even more comprehensive scale. On the technical side, the data exchange with farm management information system (FMIS), via the exchange language agroXML, will be enforced. A milestone will also be the upgrade of ISIP with a geographical information system (GIS). The added value of ISIP are its up-to-date site-specific DSS modules, complemented with the latest regional news and a large database of background information. The software framework of ISIP is built in an open and extensible architecture, which helps to speed up model development and ensures rapid transfer of knowledge. Hitherto, the information flow was more or less unidirectional, from the extension services to the farmer. With ISIP, an interactive network for information exchange between model developers, data providers, extension services, farmers and others is established. Using the internet as the linking platform, ISIP is a comprehensive tool for decision support in integrated plant production.

Development of new forms of biopreparation on the basis of biocontrol *Trichoderma* strains by using wooden residuals

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INTRODUCTION

Some of the most widely used biocontrol agents in the world belong to the fungal genus *Trichoderma* (Samuels, 1996). In particular, isolates of *T. harzianum*, *T. virens* and *T. hamatum* are used against diseases in a wide variety of economically important crops. However, standart strains in agriculture practice are giving inconsistent control between different nurseries and seasons, and seemed to be ineffective for reforestation in unfavourable years. Screening effective isolates within the aboriginal strains of *Trichoderma* may open new perspectives for biological control soil-borne pathogens (Gromovykh *et al.*, 2003). Wooden organic compounds, as byproducts of paper industries, have great potential as native suppressives of damping-off in forest seedlings. Multiplying *Trichoderma* spp. on such substrates could be beneficial for field application in forest nurseries.

METHODS AND RESULTS

All isolates of *Trichoderma* and *Fusarium* were obtained from forest nurseries soils of Central Siberia. *Trichoderma* spp. were tested against *Fusarium* isolates, using dual culture and antibiotic disk techniques (Egorov, 1985). Five organic substrates (pine bark, larch bark, the same substrate after CO_2 and also hydrolysis lignin) were evaluated for their ability to support the growth of different *Trichoderma* spp. After sterilization, the substrates were inoculated in a fermenter under aseptic conditions, with 1×10^6 spore/g. Deep solid fermentation was done for 8 days with aeration. The population of *Trichoderma* on the organic substrates was assessed by a serial dilution technique, using *Trichoderma* medium. Populations of *Fusarium* and *Trichoderma* were monitored in forest nurseries in two fields in 2002–2004 by the serial dilution technique. Disease severity was recorded at the bunch maturing phase, using a 1–5 scale. Trials were laid out in a randomized block design, each being conducted at least twice.

Collections of 197 selected isolates of Trichoderma (T. asperellum, T. viride, T. harzianum, T. koningii, T. virens) were analyzed with respect to their antagonistic activity against the main representatives of *Fusarium*. Strains providing the best control in the artificial light laboratory were then evaluated in small field plot tests. The screening has led to the selection of 15 aboriginal strains as a potential biocontrol agents. Monitoring of the single-spore clones of these 15 wild isolates has demonstrated high heterogenity with respect to culture-morphological properties, sporulation and the antibiotic activity of *Fusarium* species. Regarding these indexes, all isolates can be split into four distinct groups, with which

vegetative compatibility corresponds. These data were used as a basis for further selection within the given group for the development of biopreparations. For this purpose, solid biotechnology systems on different substrates (including pine bark, larch bark, the same substrate after CO_2 and ethanol extraction and also hydrolysis lignin) were investigated. Larch bark after CO_2 extraction was the best substrate to support the growth of *Trichoderma*, which quickly multiplied and covered the entire surface within 6 days (Table 1).

Substrate	Yield of spore, $(M \pm m)$, $*10^8 * r^{-1}$					
	ΜΓ/6	К-12	10 - 99/5	MO		
Hydrolysis lignin	0.65 ± 0.04	0.13 ± 0.03	0.85 ± 0.01	0.19 ± 0.03		
Spruce bark	2.34 ± 0.06	1.79 ± 0.01	2.29 ± 0.04	1.53 ± 0.06		
Spruce bark after CO ₂ extraction	3.57 ± 0.02	3.57 ± 0.02	2.40 ± 0.03	2.14 ± 0.03		
Larch bark	1.90 ± 0.01	1.21 ± 0.03	1.81 ± 0.03	1.33 ± 0.03		
Larch bark after CO ₂ extraction	3.90 ± 0.01	3.20 ± 0.06	2.67 ± 0.03	2.38 ± 0.06		

Table 1. Number of *Trichoderma* propaguls on different wooden residuals.

A different form of biopreparation was done for the evaluation in forest nurseries of *Picea* obovata seedlings: M Γ 97/6 *Trichoderma asperellum* on pine and larch bark after CO₂ extraction (containing 3 × 10⁸ spores/g). Complex biopreparation consisted of M Γ – 97/6 *T. asperellum*, M 99/5 *T. harzianum*, K-12 *T. asperellum* and MO *T. hamatum* (containing 2.5 × 10⁸ spores/g) on pine bark. The results showed that treatment of spruce seeds and seedlings could increase the number of healthy seedlings: biopreparation on larch bark by 4 times; biopreparation on spruce bark after CO₂ extraction by 3.4 times. The maximum percentage of healthy seedlings (8.5 times greater than the control) was achieved with a complex of biopreparations.

DISCUSSION AND CONCLUSION

The success of biological control on crop plants depends not only on effective antagonists but also on the costs involved and the method of application. Complex biopreparation using larch bark were cost effective, had a long shelf-life, supported high propagules density, were easy to formulate and achieved effective disease control.

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Enhancement of biopreparation activity for plant protection

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INTRODUCTION

Biological preparations (biopesticides) based on natural biocontrol agents are a good alternative to synthetic chemicals in modern plant protection, especially in organic crop production. The merits of biological formulations of microorganisms and its metabolites are well known. Nevertheless, the use of biopesticides for protection of agricultural crops is not as widespread as desirable. Some explanations of this situation include narrow spectrum of host pest, more variable efficacy and field stability than chemicals. The aim of this presentation is to overview our previous and latest research and to show the possibilities of the enhancement of biopreparations activity for crop protection in some examples.

The experiments were carried out under laboratory and field conditions. Biopesticides of different origin were tested against insects of several orders and against phytopathogenic fungi. Some common methods for evaluation of efficacy of biological formulations for plant protection were described earlier (Shternshis et al., 2002; 2006).

RESULTS

In order to activate penetration of biological agents, such as baculovirus or Bacillus thuringiensis (Bt) into host targets, microbial chitinase (0.5 mU ml-1) was used as an additive to microbial insecticides. The enhancement in activity of baculoviruses, including the Cydia pomonella granulovirus (GV) and Mamestra brassicae nucleopolyhedrovirus (NPV), caused by chitinase was shown to be greater than the enhancement in Bt activity under the influence of the same enzyme. This fact allows us to use virus preparation containing 10-fold less biocontrol agent in the presence of chitinase against C. pomonella (Lepidoptera: Torticidae) in the Novosibirsk and Krasnodar regions of Russia. The results obtained in both regions showed the same efficacy of the traditional GV formulation $(3 \times 109 \text{ granule per ml})$ and the new one $(3 \times 108 \text{ granule per ml})$. To overcome the narrow spectrum of activity of some bioinsecticides, especially viral ones, mixture with other biological agents is useful. Taking into account the previous results concerning synergistic effect of Bt and M. brassicae NPV used together for cabbage protection, we developed the triple mixture consisting of Bt, M. brassicae NPV and chitinase (Shternshis et al., 2002). Such triple mixture provided complete protection of cabbage against all lepidopteran insects. In some cases, formulations based on natural microbial metabolites could replace both synthetic chemicals and microbial insecticides based on propagules. The application of such formulations allows to avoid some negative environmental factors and to achieve quick effect concerning plant protection. Therefore, we applied bioinsecticide based on natural Streptomyces avermitilis metabolite for vegetable and soft fruit protection in both field and greenhouse. The results showed that this formulation provided a good crop protection against several insects, such as beet webworm Pyrausta sticticalis (Lepidoptera: Pyralidae), raspberry cane midge Resseliella theobaldi (Diptera: Cecidomyiidae) some species of aphid, and against two-spotted spider mite (Tetranychus urticae) (Acari: Tetranychidae). In addition, this commercial formulation appeared to be a dual function biopesticide. Namely, in laboratory and field testing, the formulation recommended so far for insect control suppressed the growth of the phytopathogenic fungus Didymella applanata that causes raspberry spur blight. The efficacy of this S. avermitilis metabolite and synthetic chemical traditionally used against raspberry spur blight was shown to be similar:.

Treatment	Spur blight surface severity (%)*				
	2001	2002			
S. avermitilis metabolite 0.2%	19	13.8			
S. avermitilis metabolite 0.1%	21	11.4			
Chemical standard 0.1%	11	9.8			
Control	46	21.4			
LSD $(P = 0.05)$	6.6	6.6			

Table 1. Effect of S. avermitilis metabolite on raspberry spur blight severity (2001–2002).

* Spur blight surface severity means the damage to epidermis, parenchyma and periderm.

DISCUSSION AND CONCLUSIONS

There is no doubt that crops grown all over the world require ecologically safe pest control. Particularly, it concerns vegetable and berry crops, to avoid chemical residues in fresh fruits. The use of natural agents for pest control promotes the biodiversity of other natural enemies useful for insect and plant disease control. Although, in some cases, biological formulations were started for crop protection several decades ago, application is still in its infancy. Some observed disadvantages in the use of biopreparations could be reduced by enhancing their potency with one or more additives. Mixtures combining biocontrol agents with low concentrations of ecologically friendly components are more preferable to enhance biocontrol activity. Disadvantage in the practice of baculovirus-based formulation concerning its narrow spectrum of host could be reduced by addition of Bt-formulation based on synergistic strain. In some cases, the microbial metabolite formulations have some advantages over living organismbased preparations. Metabolites are less susceptible to environmental factors such as temperature, humidity and UV-radiation. Metabolite preparations also appear to have a wider spectrum and quicker action. Also, metabolite-based pesticides are environmentally safe and are not subject to accumulation in fruits as compared with synthetic chemicals. In addition, dual properties of these products concerning both insect and disease control observed in some cases, are rather valuable for plant protection.

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FRIS – best practice in viticultural disease and pest management in the Franconian winegrowing region

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INTRODUCTION

Database information systems have become fundamental for economic decisions in agriculture and horticulture. Also, in viticulture, such an information system can be helpful. To support local winegrowers the so-called FRIS (<u>FR</u>anconian <u>Information Service</u> for plant protection in viticulture) has been established since 1996 in the Franconian wine-growing area, located along the river Main. The focus of FRIS is to provide information for making individual decisions on pest management, involving a sound handling of resources and sustainable development in viticulture. Therefore, the system should provide highly up-to-date information and, at the same time, be adaptable to different microclimates and soil conditions in Franconia. All persons involved in viticultural advisory services in Franconia are involved and cooperate in FRIS. Thus, different or inconsistent recommendations (as sometimes happened in the past) are avoided.

STRUCTURE OF FRIS

FRIS is in structured into three parts: data collection, data processing and transfer of information.

Collecting data

Information concerning phenology of the vines and weather, as well as the occurrence of pests and diseases, is collected from four different sources: monitoring fields, reports of vineyard custodians, own field trials, and a network of 16 meteorological stations spread all over Franconia. At the heart of FRIS are five selected vineyards, representing the different soil and micro-climate conditions typical for the Franconian wine-growing region. These are monitored regularly (i.e. once a week within the growing season) by a qualified viticultural technician, for the presence of about 20 diseases, pests and important beneficial insects. In addition, we utilize the results of field trials conducted by the scientists of the Bavarian State Research Institute for Viticulture and Horticulture. Furthermore, vineyard 'custodians', located in almost every village of Franconia, report weekly about disease and pest development in their vines. These custodians are winegrowers, who act as representatives from all Franconian wine-growing villages, and are recommended for appointment by local winegrowers' associations. They act as mediators between research, winegrowers and advisory services. Being trained regularly by scientists of the Bavarian State Institute for Viticulture and Horticulture they provide helpful information for FRIS. Finally, the 16 meteorological stations record crucial data such as temperature, precipitation, humidity and leaf wetness. This ensures the high quality of information and, subsequently, of recommendations based upon FRIS.

Processing data

Data processing is done at the Bavarian State Research Institute for Viticulture and Horticulture. Information is compressed and transmitted to charts and graphics. Epidemiologic forecast models are then used as available. Results obtained by the different sources are also discussed and interpreted.

Transferring information

All information is transferred to winegrowers by all existing media. The 'Viticulture Fax Franconia' is issued twice a week by fax and e-mail and placed on the internet. It provides the latest information for winegrowers, e.g. timely information on specific pest development or a recommendation to use a certain method of pest control.

In addition, there is a Newsletter that provides up-to-date information on viticulture in general. Published monthly, all members of the Franconian Viticultural Association (c. 4,200 winegrowers from Franconia, including all cooperatives), get information about winegrowing, pest management, oenology and administrative regulations.

The annually published 'Guideline of Grapevine Pest Management' gives background information about pests and diseases. It considers all the climatic and geographical characteristics of the Franconian wine-growing region. Moreover, this free booklet contains a list of pesticides recommended for sustainable viticulture. Selected by the scientists of the Bavarian State Research Institute, and based on results of field trials, only those pesticides that do not harm beneficial insects are listed. The annual edition (with a circulation of more than 4,000 booklets) ensures that every interested winegrower can obtain information on environment-friendly methods of grape production. Besides the printed product, an internet version can be downloaded from the world wide web (http://www.lwg.bayern.de/wein-bau/rebschutz_lebensraum_weinberg/16334/).

RESULTS AND CONCLUSIONS

For more than 10 years, at the same sites, weather, plant growth, and epidemiology of diseases, pests and their antagonists have been monitored. This systematic and continuous sampling of uniform data has led to the establishment of a long-term data pool. Thus, a very helpful source of information has been established for new management practices and prognosis models. It is also a useful indicator for newly appearing diseases and pests.

FRIS is accepted very well by local winegrowers. In the meantime it is almost unthinkable to produce grapes in Franconia without information from FRIS. Not only do winegrowers and producers rely on its recommendations, but agricultural traders and representatives of agrochemical companies also use the information provided by FRIS and, thus, improve their sales. When thinking about best management practices in viticulture, FRIS represents the state of the art, at least within Germany.

Documentation of pesticide applications in arable farming – a study on German farmers' experiences and approaches

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INTRODUCTION

In Germany, recording of pesticide applications came into farmers' practice following the last official revision of the guidelines for good agricultural practice (GAP). These guidelines demand detailed documentation of pesticide measures taken. The recent plant protection act does not make documentation mandatory, but there is a demand for consideration of GAP. In fact, EU legislations 178/2002, 852/2004 and 183/2005 require documentation of the complete agricultural process chain, including plant protection. Additionally, in many cases, documentation of pesticide use and application data is already required by traders, millers, process labels and contract partners. Thus, farmers are forced in several ways to fulfil proper documentation. Nevertheless, critics of pesticide use argue that such documentation is insufficient and that misuse may still take place. Data are lacking, however, to evaluate the current state of agricultural practice in this area.

MATERIAL AND METHODS

An inquiry was carried out to obtain information on the implementation of documentation practices in arable farms. Accordingly, about 1,600 professional farmers in central Germany were contacted via a postal survey, which included questions about their documentation practices. This survey took place during June and mid-July 2006, before crop harvest. Participants were recipients of a plant protection and crop husbandry newsletter, issued by the official extension service in Lower Saxony (Niedersachsen). Questions asked related to technical issues and attitudes to statements. Responses were received from 36% of participants, which is quite a satisfactory proportion from a methodological viewpoint for socio-economic studies. The mean farm size of the respondents was 160 ha, which was above the average size (c. 50 ha) for farms in Germany.

RESULTS

All of the participants declared being involved in pesticide documentation. This is not surprising since those who refuse documentation would probably also have refused answering! Thus, the study cannot account for total share of documentation, but it can describe farmers'

approaches. Pencil-written documentation (e.g. calendar books and field records) was still commonplace, and used by 45% of the farmers. Computer-based systems (e.g. PC-based field records, 'palm') were adopted by 55% of the sample. On average, farmers stated an annual expense of 582 \in to maintain documentation equipment. Items documented are listed in Table 1. Consideration of items recommended by the German code of 'good agricultural practice in plant protection' is relatively high. However, the items 'name' and 'pest' may be considered infrequently since many farmers regard them as obvious. Optional data are considered less frequently in farmers' documentation.

Item of documentation	Proportion of farmers (%)
Name *	37
Date *	98
Field identity, location *	94
Crop *	92
Pest *	24
Plant protection product *	97
Amount per ha *	94
Buffer zones etc. **	54
Crop growth stage **	53
Spraying technique **	38
Weather **	30
Treatment index **	9

Table 1. Items of pesticide documentation considered by German farmers (n = 581).

* Recommended by code of 'good agricultural practice'. ** Optional items.

A cluster analysis of farmers' socio-economic statements identified four attitude groups with respect to mandatory documentation: 'opponents' (20%), 'those being afraid of farm checks' (20%), 'proponents on the farm level' (23%) and 'general supporters' (37%). The last-mentioned group sees documentation as an instrument for gaining acceptance by retailers and the public.

DISCUSSION

On-farm pesticide documentation by farmers is widely adopted and is carried out in a professional way. In Germany this is due to other reasons than national pesticide law. For far too long, mandatory documentation has been discussed at a political level and, in the public view, this could be seen as reservation by lobbyism. Farmers are recommended to be open and straightforward, to underpin their achievements, as is already stated by 'general supporters'.

Habitat and resistance management in renewable energy crops and set-aside land

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INTRODUCTION

In the last two decades the character of farming in the EU has changed from subsidized food and feed production into sustainable management of the farmland. Set-aside schemes or fallow periods were implemented as a control mechanism to reduce over-production and to stabilize prices for the crops and, more, recently as a regeneration strategy for the soil. Plant protection measures have always played a major economic role, to bring fallow land back into culture, to increase yields or as insurance of the harvest. Following several International Conventions the use of biomass for energy production or rising energy costs make farming of renewable energy crops more economical and necessary. In 2005 the world produced c. 40 million tonnes of bioethanol and bio-diesel. The main source is from processed plant oil and sugar. Escalating demand of energy will require biomass of complete plants to be converted to bio crude oil and methanol.

In Germany, grassland, maize, wheat and oilseed rape are used increasingly beyond their original destination for food production. Habitat and resistance management in renewable energy crops is an optimization tool of plant production techniques, and seriously needs to be taken into account when political or economical reasons ask for it.

HABITAT MANAGEMENT IN RENEWABLE ENERGY CROPS AND SET-ASIDE

The agro-ecosystem is a multi-zonal network of biotopes. Various levels of cultivation are directed by the farmer and he creates different habitats. Set-aside or fallow land can develop 30 to 80 different plant species within the first few years. Therefore, it is of considerable ecological importance, and can be used in the rotation (Knauer, 1993). Arable crops such assugar-beet, cereals, oilseed rape and maize can be used as alternative sources for renewable energy. However, the habitat or field must be managed similarly to conventional methods of arable farming to achieve highest yields. More and more silaged pasture grass is used in biogas plants as a substrate for co-fermentation, and fermentation is most effective when the C/N ratio is optimal. Highest yields of methane are achieved with silaged grass harvested from intensively managed pasture (Lemmer & Oechsner, 2003).

However, in terms of high floral species diversity of a landscape, it is acknowledged that fallow land, grassland, range and pasture are the closest to natural vegetation. Soil conditions and climate have determined floral distribution, plant community, frequency, status and level of establishment. Cultivation or melioration measures have produced habitats with a different proximity from nature. Moreover, increasing effects of biological globalization become evident in a shift of the floral composition, to the advantage of many exotic species (Hoffmann, 2005). Invasive alien species represent one of the primary threats to biodiversity.

For economical reasons the pasture destined as a source for renewable energy requires a culture of monocotyledonous grass species. Additionally, habitat management needs to avoid neophytes or invasive species, toxic or allergy-inducing weeds such as giant hogweed (*Heracleum mantegazzianum*), ragweed (*Ambrosia artemisiifolia*), ragwort (*Senecio jacobaea*) and japanese knotweed/bistot (*Reynoutria japonica* and *Fallopia sachalinensis*), to reach unacceptable levels of abundance on the fallow land ready for re-cultivation. Traditional pasture management tools of cutting or grazing may not be successful, especially when perennial weeds such as creeping buttercup (*Ranunculus repens*), broad-leaved dock (*Rumex obtusifolius*), creeping thistle (*Cirsium arvensis*), common nettle (*Urtica dioica*) are dominant and succeed the grass species. Specific active ingredients with herbicidal mode of action (Table 1) can control tricky weed species.

Herbicide	Ambrosia	Cirsium	Heracleum	Ranunculus	Rumex	Senecio	Urtica
aminopyralid	98%	96%	_	93%	94%	99%	_
clopyralid	_	90%	_	—	_	_	_
triclopyr	—	I	100%	87%	70%		100%

RESISTANCE MANAGEMENT IN RENEWABLE ENERGY CROPS

Oilseed rape has been discovered as a major source for bio-fuel and is grown on c. 500,000 ha of set-aside land in Germany. Traditionally, in NW Europe, oilseed rape is part of a crop rotation with winter wheat and winter barley. Blackgrass (*Alopecurus myosuroides*) can be present in all three crops and, in some areas, may have already may have developed resistance to herbicides. The occurrence of graminicide resistance and cross resistance is of major significance to both current and future weed control programmes. Herbicide and insecticide resistance jeopardize expectations of high yield in oilseed rape. Non-specific-acting herbicides may preserve the efficacy of specific-acting herbicides against blackgrass, and suitable active substances, e.g. glyphosate, propyzamide and trifluralin, allow long-term, consistent 98% control as part of a herbicide resistance-management strategy in a narrow crop rotation. In 2006 some areas of Germany experienced total yield loss as result of attacks of pollen beetle (*Meligethes aeneus*). Predictable yield expectations for oilseed rape will require resistance-breaking insecticides (e.g. chlorpyrifos), since some pyrethroides already exhibit limited levels of control. Trials in Germany with chlorpyrifos have shown high levels of control of pollen beetle

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IPM in a developing country: Turkey's experience

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INTRODUCTION

Agriculture plays vital role in Turkey's economy and social life, over one third of the population living in rural areas and being employed in the agricultural sector. The area under cultivation in Turkey is 27 million ha, which represents 35% of the total land area. Turkey's geographical, climatic and agro-ecological diversity reflect her crop pattern. Wheat is grown throughout the country, but tea plantations occur only in northern Turkey (which is a humid area and where temperatures are mild). Crops produced in Turkey range from subtropical crops (such as banana, kiwifruit and tea) to winter cereals, the foremost crops being wheat, barley, corn, pulses, cotton, sugar beet, potato, tobacco, sunflower, vegetables, pome and stone fruits, nuts, citrus fruits, grapes and olives. Differences in cropping patterns, geography and climate result in varying pest and disease patterns in the different areas. For instance, the key diseases in vineyards are downy mildew (*Plasmopara viticola*) and powdery mildew (*Uncinula necator*); the key pests in grain crops are shield bugs (*Eurygaster* spp.) (Scutelleridae) or wheat bugs (*Aelia* spp.) (Pentatomidae), depending on the growing region.

Turkish agriculture is different from that of under-developed and developed countries. More than 65% of agricultural enterprises are of 5 ha or less. The use of tractors is increasing and man/animal power is decreasing. Currently, there are over one million tractors and ploughs but fewer than 100,000 animal-powered tools for ploughing. Hand-hoeing in cotton production is reduced to once per season and replaced by inter-row tillage with tractor-powered machinery. Pesticide use in Turkey, however, has been increasing (from c. 8,000 t in 1979 to > 13,000 t in 2004). However, the amount of pesticide used varies from region to region. Although pest resistance to pesticides has not been well documented, some cases of resistance have been reported and studied in Turkey. Alternative methods, such as biological control and systems such as ecological agriculture and integrated pest management (IPM), have been implemented.

IPM IN TURKEY

Biological control of pests started in Turkey in the early 1900s, and the first IPM research project began (in cotton) in 1970. This project was followed in 1972 by others, to establish IPM on apple and hazelnut. The results were applied in the field soon afterwards, and spraying against insects in cotton fields, for example, dropped from 10–11 applications to 4–5. Forecasting and warning systems were established for codling moth (*Cydia pomonella*) and scab (*Venturia inaequalis*) in the early 1980s. A cornerstone of IPM in Turkey is that, in 1990, projects were implemented by the Ministry of Agriculture in 10 main crops: apple, cherry,

cotton, hazelnut, maize, olive, pistachio, potato, sunflower and protected crops (vegetables and ornamentals). These projects mostly focused on collating earlier data and producing new data, to establish IPM programmes. They were conducted by researchers in the main production areas of each given crop. Extension agents were trained as well as researchers. Projects covered not only insect pests but also diseases, physiological disorders and weeds. Results of these projects and the future of IPM were discussed at a meeting held in 1994. The meeting was considered one of the most important steps in IPM in Turkey, because new attitudes to IPM were established. Goals, objectives, policy and strategies, that had been determined in 1988, were revised. The name of the umbrella project was changed (from the 'National research, development and training project for IPM' to the 'National research, implementation and training project for IPM'), which resulted in active and greater involvement by extension agents and producers. Following the meeting, IPM in Turkey became applied in the field instead of merely remaining a theory within research institutions. The number of crops under IPM was increased. Apricot, chickpea, citrus, lentil, peach, grapevines and wheat were added; the protected crops project was restricted to vegetables; and the sunflower project was cancelled. IPM was added to the national pest management programme book in 1997, and 'Directives for IPM Projects', which covers responsibilities of all stakeholders and project implementation methods, was published in 1999. After 2000, IPM activities were mainly implemented by extension agents, although researchers and research institutions kept their involvement as trainers and regional coordinators. New crops, such as rice, bean, pear and walnuts, were added. Some crops, such as soybean and sunflower, were added but then cancelled. Some universities conducted their own IPM projects independently, but they produced only data for research fields belonging to participating farmers and some training material. Currently, over 2,000 ha area is under IPM; although an additional 6,000 ha is ready for developing IPM for potato mildew (a very small percentage of both the chemically sprayed area and the total arable area). IPM in fruit crops covers 127,500 trees (including apple, cherry, peach, apricot, pear, sour cherry, walnut, pistachio, hazelnut, citrus and olive). The forecasting project for apple covers over 12 million trees (out of 42 million), and that for vineyards is implemented on 130,000 out of 560,000 ha. However, the area/tree where IPM has been implemented has not increased for a decade.

The projects resulted in pesticides in Turkey being classified according to their toxicity and impact on the environment. IPM guidelines have been prepared for citrus, apple, grapevines, cotton, chickpea, potato, peach, olive, cherry and protected vegetables (cited here in the order of the publishing year), and these are available in hard copy and on the internet.

Regrettably, IPM in Turkey has been adopted in only limited areas, in spite of farmers and extension agents having been trained. After 15 years of intensive IPM projects, pesticide use continues to spread, and implementation of IPM is not recognized by administrators; also, vast numbers of farmers are not aware of IPM. There are many bottlenecks, but the main one is the lack of awareness of environmental issues. Additionally, lack of consumer education and market-related activities are among the most important weak points of the projects. These projects are only one step back from integrated crop management; however, new data on different subjects (such as pesticide resistance, pest/environment relations, novel methods, and thresholds) should be produced to improve IPM. Turkey's experience can be used by many countries, as well as by herself.

On the occurrence and monitoring of wheat blossom midges (Diptera: Cecidomyiidae) in Central Germany

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INTRODUCTION

Lemon wheat blossom midge (*Contarinia tritici*) and orange wheat blossom midge (*Sitodiplosis mosellana*) belong to the most prominent insect pests in winter wheat (Holland *et al.*, 1996). However, no practical method exists to predict or monitor the impact of these insect pests. There is also a lack of recent research on the issue, particularly for Central Germany (older studies include: Lübke & Wetzel, 1984; Volkmar & Wetzel, 1989). Consequently, this study attempts to provide new data on the occurrence, monitoring and crop damage of wheat blossom midges. It also focuses on the impact of changing agricultural conditions, such as wheat-to-wheat crop rotation, reduced soil tillage or different crop cultivars.

METHODS

A systematic survey of midge occurrence and crop damage in a wheat-to-wheat crop rotation was carried out at a research field in 2005 and at a conventional winter wheat field in 2006. Orange wheat blossom midges were monitored by means of pheromone traps. In 2006, white traps were tested as an alternative method, to collect data on both midge species.

Flight activity of adult midges was monitored on 13 different dates (BBCH scale 45-85). Crop damage was evaluated by line assessment on eight dates (BBCH scale 65-87). For this assessment, a line with five control points was drawn at 20-m depth into the field. A total of 50 spikes per crop cultivar and date were randomly selected for microscopical examination. The analysis included parameters such as number of midge larvae per infested seed, number of grain thrips (*Limothrips cerealium*) (nymphs and adults) per spike or number of infested seeds per spike.

RESULTS

In 2005, the activity of adult orange wheat blossom midges reached its peak at the phenological growth stage of full flowering (BBCH 65-69). The activity density was higher in cv. Elvis with 260 midges per trap (monitoring until mid-flowering), compared with cv. Altos with 89 midges per trap (monitoring until the end of flowering period). In 2006, the activity of adult orange wheat blossom midges reached its peak at BBCH 51 (beginning of heading), with 246 midges per trap (cv. Tommi). The alternative monitoring of midges by white traps did not produce accurate results. Even at the peak of midge activity during BBCH 55 the traps contained an average of only 5 individuals.

In 2005 the greatest abundance of midge larvae per ear was established for BBCH 75, with averages of 2.1 (cv. Altos) and 1.6 (cv. Elvis). In 2006, larval numbers were significantly

higher (Table 1). In cv. Tommi, an average of 14.3 midge larvae per ear was reached during BBCH 70-73. Midge-damaged kernels in 2005 at BBCH 80-85 reached 6.3% (cv. Altos) and 4.4 % (cv. Elvis), whereas in 2006 the extent of damage reached 23.5% (cv. Tommi) (Table 1).

Date*	BBCH	Midges		Thr	Midge/thrips	
	Code	Larvae per ear	Infested kernels	Nymphs per ear	Adults per ear	Damaged kernels
21 June	65	4.7	4.1	6.0	1.6	1.5 %
23 June	65-69	8.8	7.0	7.7	1.9	2.8 %
28 June	69-70	12.8	8.9	8.4	4.4	15.2 %
1 July	70-73	14.9	10.7	11.0	6.3	22.7 %
5 July	73-75	13.6	9.4	18.2	7.7	20.0 %
9 July	80-85	4.9	4.1	22.9	11.1	23.5 %
11 July	85	5.6	4.5	16.4	13.0	19.7 %
16 July	87	5.8	4.9	11.5	15.1	23.1 %

Table 1. Occurrence and crop damage of wheat blossom midges and grain thrips on a winter wheat field in Halle (Saale), 2006.

* Sample n = 50 ears per date.

DISCUSSION AND CONCLUSIONS

The results suggest that the intensity of crop damage depends on the correlation of two factors. The closer the activity peak of midges correlates with the critical wheat growth stage of heading (BBCH 50-59), the greater the crop damage. This is highlighted by the results of 2006. In 2005, on the other hand, orange wheat blossom midge reached its activity peak much later (during BBCH 65-69) and the ensuing crop damage was significantly less.

In conclusion, the results of this survey in Central Germany stress the influence of changing agricultural conditions and regional cultivation concepts. They also suggest that further studies covering several years in open habitats would be worth while.

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POSTER PRESENTATIONS

A sequential testing programme to evaluate the efficacy of seed-treatment insecticides on cotton flea beetles as indicators of early-season pests in Sudan

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INTRODUCTION

Seed treatments promote seedling establishment, help ensure yield and reduce quality losses due to many pests and diseases. Protecting cotton plant from the attack of early-season insect pests and diseases is of prime importance to ensure a healthy and strong establishment of this strategic crop. The present study tried to measure the susceptibility of cotton flea beetles (*Podagrica* spp.) to the neonicotinoid imidacloprid as a single seed treatment or in a mixture with two antimicrobial pesticides.

MATERIALS AND METHODS

The efficacy of some single pesticides or mixtures at different dosage rates on cotton flea beetles was measured using three different kinds of experiment: visual counts in the field, no-choice semi-field laboratory tests, and no-choice laboratory tests. Flea beetle damage was assessed by counting shot-holes resulting from adult feeding. The data were subjected to appropriate transformation (square root for counts). Statgraf software was used for data analysis (ANOVA).

RESULTS AND DISCUSSION

Using the antimicrobial bronopol alone did not prevent flea beetle damage (Table 1). Treatments containing imidacloprid significantly reduced damage in the three experiments, but not 10 weeks after sowing in field experiments. Wilde *et al.* (2004), evaluating the efficacy of various seed treatments, reported that imidacloprid was effective in reducing populations of flea beetle and other pests. Since the side-effects of imidacloprid on natural enemies is minimal (Albajes *et al.*, 2003), this insecticide can be used successfully in integrated pest management programmes to combat early-season pests.

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	Dose (g	Laboratory experiment (4 WAS) – holes/3	Semi-field laboratory Experiment (4WAS) – holes/5		isual counts blants/subplot
Treatments	product/ kg seed)	adults/leaf (72 h exposure)	adults/leaf (72 h exposure)	(5 WAS)	(10 WAS)
Bronopol	3	5.3 (3.7) b	10.8 (117.3) f	3.84 (16.3) c	10.43 (111.0) ab
Bronopol	5	5.5 (29.3) b	9.6 (93.3) ef	3.73 (13.7) c	11.74 (140.3) b
Bronopol	7	2.9 (10.7) a	8.7 (75.7) def	3.24 (14.0) bc	12.89 (168.7) b
Imidacloprid	5	1.7 (3.0) a	3.4 (11.7) a	1.46 (1.7) a	10.48 (112.0) ab
Imidacloprid	7	1.5 (1.7) a	5.1 (33.7) abc	1.95 (3.3) ab	10.80 (117.7) ab
Bronopol + imidacloprid	3 + 5	2.0 (3.7) a	4.6 (24.3) ab	1.85 (3.3) ab	9.89 (99.3) ab
Bronopol + imidacloprid + tebuconazole Bronopol +	3+5+2	1.6 (2.7) a	6.6 (46.0) abcde	1.56 (2.0) a	9.38 (88.7) ab
imidacloprid + tebuconazole	5+5+2	1.3 (1.7) a	6.5 (42.7) abcde	1.82 (3.0) ab	9.16 (89.7) ab
Bronopol + imidacloprid + tebuconazole	7 + 5 + 2	1.7 (3.0) a	6.0 (41.7) abcde	1.56 (2.0) ab	10.75 (121.3) ab
Bronopol + imidacloprid + tebuconazole	3 + 7 + 2	2.6 (6.3) a	4.2 (20.3) ab	1.74 (2.7) ab	6.96 (52.3) a
Bronopol + imidacloprid + tebuconazolel	5 + 7 + 2	1.9 (4.0) a	7.7 (59.3) bcdef	1.90 (3.3) ab	9.48 (111.0) ab
Bronopol + imidacloprid + tebuconazole	7 + 7 + 2	0.7 (0.0) a	8.3 (69.3) cdef	1.97 (3.7) ab	8.76 (80.7) ab
Gaucho + tebuconazole	7 + 2	1.0 (0.7) a	6.0 (44.3) abcd	1.35 (1.7) a	6.74 (48.3) a
Control					
(= untreated)		7.2 (58.0) b	10.3 (107.3) f	3.66 (15.7) c	11.89 (145.0) b
S	Е	0.81	1.2	0.62	1.46
CV%	/o	84.63	42.70	55.94	27.00

Table 1. Susceptibility of cotton flea beetles (*Podagrica* spp.) to various treatments during the cotton-growing season 2004/2005 in Gezira (Sudan). (Mean number of holes $\sqrt{x} + 0.5$).

WAS = weeks after sowing. Figures followed by the same letter within a column were not significantly different at 5% Multiple Range Test (Statgraf Software); figures in parentheses are actual values.

Dynamics of the parasitoid complex of the summer fruit tortrix moth (*Adoxophyes orana*) in the first year of conversion of apple trees to ecological production in north-eastern Romania

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INTRODUCTION

The apple is the most important cultivated fruit tree in Romania. The intensive crop systems, with a high number of phytosanitary treatments, led to the adaptation of certain secondary pests, which became main pests. One of these species is the summer fruit tortrix moth (SFTM), *Adoxophyes orana* (Lepidoptera: Tortricidae) (Diaconu *et al.*, 2006).

METHODS

The dynamics of SFTM parasitoids, especially of the larval stage, were analyzed in an experimental 10 ha intensive orchard plot at the Fruit Growing Research Station Fălticeni (north-eastern Romania), in ecological production since 2006. A conventional plot was established as a control. Samples of pupae and the final two larval instars of SFTM were collected weekly. For the 1st generation, 3 samples were collected (from 10 to 26 May) and for the 2nd generation 4 samples (from 19 July to 10 August 10), from both plots. Each sample contained at least 50 individuals, with a total of 985 individuals. The collected material was reared in laboratory until the emergence of either SFTM adults or parasitoids.

RESULTS

The main parasitoid species of the SFTM, common to both experimental plots, were two species of Hymenoptera: *Teleutaea striata* (Ichneumonidae) (a solitary oligophagous larval endoparasitoid) and *Colpoclypeus florus* (Eulophidae) (a gregarious polyphagous larval ectoparasitoid) (Table 1). In the ecological plot the following parasitoid wasps were also reared

from host larvae: *Phytodietus polyzonias*, *Scambus* sp. (Ichneumonidae), *Cotesia xanthostigma* (Braconidae), *Sympiesis* sp. (Eulophidae) and *Pteromalus chrysos* (Pteromalidae). From the same plot *Itoplectis* sp. (Ichneumonidae) and *Brachymeria intermedia* (Chalcididae) were obtained from pupae.

		Average parasitism (%)					
Plot	Generation	Teleutaea striata	Colpoclypeus florus	Other species	TOTAL		
Conventiona	al I	7.5	0.0	0.0	7.5		
	II	0.0	33.9	0.0	33.9		
Ecological	Ι	13.3	0.4	1.1	14.6		
-	II	20.8	18.2	2.3	41.3		

Table 1. Parasitism of Adoxophyes orana larvae during the vegetative season of 2006.

DISCUSSION AND CONCLUSIONS

T. striata established a higher rate of parasitism in the first generation in the ecological plot compared with the conventional one because the former is surrounded by other untreated vegetation on three of its sides, while the latter is surrounded only by chemically treated plots. The presence of *T. striata* in the first generation in the conventional plot was due to the fact that the parasitism occurs in autumn, the species hibernates inside the immature host larvae and its biological cycle is closely correlated with that of the host (Evenhuis & Vlug, 1983). The absence of the parasitism activity in the second generation in the conventional plot was due to the insecticide treatments. As for *C. florus*, it is known that this species is a limiting factor for SFTM in the second part of the vegetative season (Carl, 1974; Diaconu *et al.*, 2006), a situation also confirmed in both experimental plots. The higher rate of parasitism in the conventional plot is due to several factors, e.g. the small number of other competitors and the use of only four insecticide treatments before the last sampling.

After the 1st year of ecological production, the parasitoids associated with SFTM increased both in diversity (9 species in the ecological plot; 2 in the conventional one) and also in their efficiency (55.9% global rate of parasitism in the ecological plot; 41.4% in the conventional).

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New strains of *Streptomyces* as producers of biofungicides and biological stimulators for protection of the shoots and seedlings of Tiang-Shang spruce fir (*Picea schrenkiana*)

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INTRODUCTION

The Tiang-Shang spruce fir or the spruce fir of Schrenkiana (*Picea schrenkiana*) is the main forest-forming species of winter green forests of Kyrgyzstan. The major factors limiting the germination and survival of shoots of the spruce fir in the mountain climatic conditions are diseases caused by phytopatogenic fungi. The antibiotic substances of *Streptomyces* bacteria have a great significance in phytopathology, particularly in protecting coniferous species (Novikova *et al.*, 2002). This is because coniferous plants are more sensitive to fungicides than the majority of other agricultural and woody-shrubby plants (Stakman & Harrar, 1957).

METHODS

In order to identify the spread of fungal diseases and their degree of disutility, we carried out phytopathologic inspections of seedlings of the spruce fir in nurseries and forest areas of Northern Kyrgyzstan. Registration sites were arranged at the size of 1×1 m on the diagonal of the site with an interval of 1.5 to 15 m (on average from 4 up to 10 registration platforms). The reasons of diseases were determined by a mycological analysis of 50–100 samples taken from each site. The determinants (Cheremisinov *et al.*, 1970; Zhuravlev *et al.*, 1979; Barnet & Hunter, 2003) were used to determine the taxonomic classification of the moulds. The biological activity of *Streptomyces* metabolites was determined as raising the resistance of seedlings to fungal diseases. The seeds were processed in a water suspension of *Streptomyces*, dried until friable and then immersed in a water suspension of phytopathogenic fungi for 6 hours. Seeds processed in a solution of 0.03% KMnO₄ served as indicators.

RESULTS

During this study, we detected 5 types of disease agents harming seedlings and saplings. Our researches determined that the drowning of seedlings in the above-mentioned forest sites was caused by fungi of genera *Fusarium* and *Alternaria*. Further, the red rust damaged not only adult trees but also the seedlings of all ages, except shoots. The disease agent of grey mildew of needles was *Hypodermella sulsigena* (a conidial stage is *Hendersonia acicola*). The disease was detected mainly on 15- to 20-year-old trees. The disease agent causing crown rot of shoots was *Sclerotinia graminearum*, and the first symptoms of the disease were detected immediately

after melting of snow. This work used 22 natural strains of *Streptomyces* derived from various soil biotopes of Kyrgyzstan. As our data show, 6 out of the 18 tested *Streptomyces* strains have the most expressed antifungal influence on the test objects – phytopathogenic fungi. The widest spectrum of antibiotic influence belongs to *S. griseogromogenes* 24-8 strain which suppresses the growth and development of all tested disease activators of Tian Shan spruce fir. Then, S. *rubrogriseus* TK2-5 strain that demonstrates an antagonistic effect on all phytopathogenic fungi, except *Sclerotinia graminearum* (Table 1). A narrow spectrum of antifungal effect belonged to *S. bambergiensis* K1-3 strain which demonstrated antagonism only to one species of phytopathogenic *Alternaria*.

Streptomyces Strains	Phytopathogenic fungi					
	Alternaria	Fusarium	Sclerotinia graminearum	Hypodermella sulsigena		
S. rubrogriseus TK2-5	+	+	-	+		
S. bambergiensis K1-3	+	-	-	-		
S. noursei 24-10	+	-	-	+		
S. griseogromogenes 24-8	+	+	+	+		
S. heliomycini 24-7	+	+	-	-		
S. viridobrunneus 3K-2	-	+	-	+		
S. fumanus TM2-2	-	+	-	-		
S. wistariopsis CП3-13	-	+	-	+		
S. albadancus AП3-6	-	+	-	+		
S. afghaniensis 1K-6	-	-	-	+		

Table1. The spectrum of antibiotic effect of *Streptomyces* strains on phytopathogenic fungi of Tian Shan spruce fir.

DISCUSSION AND CONCLUSIONS

The test results indicate that the preliminary processing of the spruce fir seeds in the suspensions of *Streptomyces* provides the safety of the seedlings which was higher (up to 72-77%) at the end of the vegetative period than in the control variants, where the survival of the shoots reached only 32% of those that emerged. Here, *S. wistariopsis* (CII3-13), unlike other biological preparations, raised ground germination of the seeds by up to 103% in comparison with the control variant.

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Fungicide resistance and aflatoxin production: the effect of resistance mutations to triazoles, phenylpyrroles and anilinopyrimidine fungicides on aflatoxigenic ability of *Aspergillus parasiticus*

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INTRODUCTION

Aflatoxins are highly toxic secondary metabolites, predominantly produced by Aspergillus *flavus* and *A. parasiticus*. The contamination of food and feed by mycotoxigenic fungi is a serious worldwide health hazard to both human and livestock (Chu, 2002). An approach for the control of mycotoxigenic fungal species should be the use of appropriate antifungal agents (Buchanan *et al.*, 1987; Badii & Moss, 1988; D'Mello *et al.*, 1998). However, like other many organisms, these fungal species may become resistant to fungicides. In this case, an important consideration is the influence of fungicide resistance mutations on the mycotoxigenic ability of mutant strains. To our knowledge no information is available concerning the risk for resistance development to fungicides in *A. parasiticus* and the impact of these mutation(s) on mycotoxin production. The research reported here was co-funded by the European Social Fund and National Resources – EPEAEK II.

MATERIALS AND METHODS

The aflatoxigenic wild-type strain ATCC 15517 of *A. parasiticus* was used to obtain mutants resistant to phenylpyrroles, triazoles or anilinopyrimidines. Resistant isolates were obtained after UV-mutagenesis and selection on fungicide-amended medium. The aflatoxin (B₁, B₂, G₁ and G₂) production by the wild-type and mutant strains was determined by thin layer chromatography (TLC), enzyme linked immunoassay technique (ELISA), and high performance liquid chromatography/mass spectrometry using a fluorescence detector and electron spray ionization-mass spectrometry (LC/ESI-MS).

RESULTS AND DISCUSSION

Mutants of *A. parasiticus* resistant to triazoles, phenylpyrroles or anilinopyrimidines were isolated at frequencies of 3×10^{-7} , 3.3×10^{-5} and 1.3×10^{-5} , respectively. Studies on the effect of mutation(s) on the aflatoxin production showed that all cyprodinil, and most fludioxonil-resistant isolates, produced aflatoxins at similar or even higher concentration than the wild-type parent strain. Contrary to the above, a loss of the aflatoxigenic ability was observed in most of flusilazole-resistant strains (Table 1). Study of fitness parameters showed that the mutation(s) for resistance to triazoles or to phenylpyrroles may or may not affect mycelial growth, sporulation and conidial germination. However, in the case of cyprodinil-resistant isolates, the resistance mutation(s) do not significantly affect the saprophytic fitness-determining characteristics. Cross-resistance studies with other fungicides showed that the mutation(s) for resistance to fludioxonil or to cyprodinil affect the sensitivity of mutant strains only to the aromatic hydrocarbon and dicarboximide fungicides (AHDs) and to anilinopyrimidines, respectively. The aflatoxigenic flusilazole-resistant mutants of

A. parasiticus showed a reduced sensitivity only to the demethylase inhibiting fungicides (DMIs). However, in non-aflatoxigenic flusilazole-resistant mutants, the mutated gene(s) also reduced the sensitivity to chemically unrelated fungicides, such as benzimidazoles, anilinopyrimidines and phenylpyridinamines, but not to the QoIs or to the non-site-specific fungicides chlorothalinil and maneb.

		Aflatoxins J	production ^b	Fitness Parameters		
Strains	$\mathbf{R}\mathbf{f}^{a}$					
		AFB_1	Total	Mycelial growth ^c	Sporulation ^d	
wt (ATCC 15517)		100	100	100	100	
Flusilazole-resistant						
Ap/FLZ-11	21.3	371.4	289.7	68.1	156.4	
Ap/FLZ-4	12.3	0.38	0.28	51.8	4.5	
Ap/FLZ-5	10.7	0.38	0.28	56.2	2.2	
Ap/FLZ-25	11.4	0.38	0.28	57	8.1	
Fludioxonil-resistant						
Ap/FLD-19	>700	247.8	146.2	80.5	83.8	
Ap/FLD-43	>700	265.8	169.9	60.9	1.1	
Ap/FLD-46	>700	183.7	92.8	70.4	32.1	
Ap/FLD-29	>700	0.05	0.08	68.6	211	
Cyprodinil-resistant						
Ap/CPR-6	2660	103	99.3	118.5	97.4	
Ap/CPR-33	3900	106.4	88.5	108.9	81.6	
Ap/CPR-37	2300	105	99.3	108.2	52.6	
Ap/CPR-42	2650	136	118.2	124.4	76.3	

Table 1. Comparison of *Aspergillus parasiticus* isolates resistant to fungicides with their parental wild-type strain with respect to aflatoxins production and some saprophytic fitness characteristics.

^{*a*} Resistance factor based on EC_{50.}

^b Aflatoxin production as % of wild-type; measurements made after 10 d of incubation (n = 3).

^c Mycelial growth as % of wild-type. Measurements made after 8 d of incubation (n = 3).

^{*d*} Conidial production as % of wild-type. Measurements made after 10 d of incubation (n = 3).

There is a risk of the appearance and predominance in agricultural environments of highly aflatoxigenic mutant strains resistant to site-specific fungicides. Also, the application of antifungal agents requires careful implementation of appropriate anti-resistance strategies to preserve their effectiveness, followed by monitoring to detect aflatoxigenic mutant strains.

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Managing fungal diseases of tomato and wheat by potential biocontrol agents in salinated soils of Uzbekistan

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INTRODUCTION

Up to 30% of crop plants are lost before harvesting in Uzbekistan, mainly due to fungal diseases caused by pathogenic fungi. The ecological balance in naturally suppressive soils favourable to crop plants is now considered to be a concerted action of several microorganisms, with their own mode of action against pathogens, often in conjunction with the host plant (Lemanceau & Alabouwette, 1993). A variety of rhizobacteria with biological control activities has been described, and these bacteria use diverse mechanisms to protect crops (Lugtenberg et al., 2002). Potential biocontrol agents include Pseudomonas and Bacillus (Weller, 1988). Although numerous commercial biocontrol strains are already being marketed, there is much interest in the development of new biocontrol agents, to extent the area of application and to target pathogens. In the present work we screened and developed salt-tolerant biological control organisms against tomato foot and root rot and wheat root disease in salinated, extreme conditions of Uzbekistan.

Methods

Bacterial strains were examined for its ability to suppress the wheat (*Triticum aestivum*) root rot caused by *Fusarium culmorum* 556 and tomato (*Lycopersicon esculentum*) root rot *Fusarium oxysporum* f. sp. *radicis-lycopersici* in salinated soil. The experiments were performed with a completely randomized design, with twelve replications and three different sets. The first set contained seed inoculated with bacterial strains and sown in soil mixed with fungal pathogen. The second set contained non-inoculated seeds sown in soil mixed with fungal pathogen, and the third set contained non inoculated seeds sown in soil without fungal pathogen (control). The number of diseased plants was determined when a substantial part of the plants in the untreated control was diseased, usually 21 days after sowing. Plants were removed from the soil, washed, and the plant roots were examined for crown and root rot (indicated by browning and lesions). Roots without any disease symptoms were classified as healthy. Data were analyzed for significance after arcsine square root transformations using analysis of variance, followed by Fisher's least significant difference test ($\alpha = 0.05$), using SAS- software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

In pot experiments, 41% of healthy wheat plants were diseased after treatment with the pathogen *F. culmorum* 556. The number of plants showing disease symptoms in pathogen-mixed soil was reduced to about 25%, a result of seed bacterization with *Bacillus*

subtilis NCAM. The bacterial strain also stimulated shoot, root dry matter and nutrient uptake of wheat, which was statistically significant compared with diseased plants (Table 1).

Bacterial strains	Dry w	eight]	N	I	D		K
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	100	100	100	100	100	100	100	100
	$(0.39)^1$	$(0.27)^1$	$(0.01)^1$	$(0.004)^1$	$(0.002)^1$	$(0.001)^1$	$(0.02)^1$	$(0.005)^1$
Pseudomonas sp.	116*	125*	144*	124*	138*	151*	147*	141*
NCAM								
B. subtilis NCAM	115*	119*	140*	146*	127*	143*	117*	149*
Bacillus sp. NCAM	143*	137*	155*	157*	134*	136*	148*	147*
LSD $\alpha < 0.05$	10	15	12	21	11	18	11	19

Table 1. The effect of inoculating wheat seedlings with bacteria.	Table 1. The effect	of inoculating	wheat seedlings	with bacteria.
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¹ mg/plant.

To test the biocontrol ability of Bacillus subtilis NCAM, tomato seedling were inoculated with the bacterial strain and grown in pots containing F. oxysporum f. sp. radicis-lycopersici spores. After 21 days the plants were analyzed for disease symptoms and statistical analysis was performed. The presence of the pathogenic fungus F. oxysporum f. sp. radicis-lycopersici caused disease symptoms in 45% of the plants. The plants inoculated with bacterial strain Bacillus cereus 80 reduced the percentage of sick plants to 28%.

After application of biological control organisms (*Bacillus subtilis* NCAM), sick wheat and tomato plants was reduced to about 25–28%. Mahaffee & Kloepper (1994) have shown that biological control by endophytic bacteria is possible and can involve induced resistance to soil-borne pathogens. Most crops in Uzbekistan are cultivated on agricultural land that is salinated. However, the salt-tolerant and temperature-resistant biological control organisms can easily withstand the local salt stress and will help improve cropping methods, plant health and crop productivity. Through this sustainable practice, soil quality is also expected to improve.

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Mycorrhizal fungi as biological IPM components in vegetable production: BIOMYC – an international co-operation as basis for preventive consumer protection

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THE BIOMYC PROJECT

The BIOMYC project was initiated by the German Ministry of Consumer Protection, Food and Agriculture and the Chinese Ministry of Agriculture. The project is a collaboration between partners of the Chinese Agricultural University, Beijing, and the Xinjiang Academy of Agricultural Sciences, Urumqi, China, with the German Federal Biological Research Centre for Agriculture and Forestry (BBA) and the German company INOQ GmbH. BIOMYC (a) introduced new mycorrhizal technology for an integrated plant protection strategy to Chinese horticulture, (b) expanded the basic knowledge of Chinese scientists on the population dynamics of pests and pathogens on vegetables under greenhouse conditions, (c) promoted the development of new soil improvers/biofertilizer products in Germany and China, and (d) demonstrated sustainable, consumer-oriented methods for horticulture to Chinese students, scientific professors and supervisors of plant producers. Accordingly, future developments of Chinese plant protection strategies will have the chance to match compliance criteria of farm assurance systems which are now important in food quality control. Furthermore, the cooperation of BBA with Chinese partners increases the expertise of German scientists in the use of biological plant protection factors under biotic stresses in greenhouses, and enhances knowledge of Chinese horticultural and agricultural plant production systems.

MYCORRHIZAL SYMBIONTS AS A FACTOR IN PLANT PRODUCTION

Most vascular plants live in a symbiotic association with soil fungi, the mycorrhiza. In this symbiosis, the fungus takes up nutrient salts and water from the soil and makes them accessible for the plant partner, while the plant supplies the fungus with essential carbohydrates produced in photosynthesis. As the fine fungal hyphae can penetrate and exploit the soil to a much greater extent than the plant's own root hairs, mycorrhizal symbiosis increases both the ecological and the physiological fitness of the plant. This has a huge impact on agriculture and forestry by increasing plant growth, health and crop yield. The absence of effective symbiotic fungi in native soils can lead to reduced growth or even to failure of plants when they are

introduced. The most relevant areas for practical implementation of mycorrhizas (especially arbuscular mycorrhizal fungi –AMF) include plant production in horticulture and landscaping, land restoration, erosion control, phyto-remediation and vegetable production. Benefits obtainable from optimal use of AMF can include: enhanced tolerance against soil-borne diseases, pests and nematodes; increased drought tolerance and reduced water consumption; more plant material of higher quality classes; faster and better growth (including root growth); higher and earlier marketable yield; earlier ripening of fruits; advantages for plants that are cold-stored during the winter, efficient use of fertilizers, leading to more environmentally friendly production.

MYCORRHIZAL PRODUCTS FOR THE HORTICULTURAL MARKET

In recent years various AMF products have been introduced into the European and Chinese market for a range of purposes. Although the achieved progress in commercialization of this biotechnological supplement in the last five years is impressive, experiences obtained so far have shown that the quality of the product and, thus, quality control of production is really a bottleneck for general establishment in the marketplace. As AMF are obligate biotrophic organisms they have to be propagated commercially on living plant roots, e.g. in greenhouses. There are various conventional and modern molecular biological tests that can be applied to the quality control of AMF inoculants, based (since 1997) on voluntary agreements of the German Committee of Mycorrhiza Application (CMAG). The use of AMF inoculum is recently facing a highly diverse host plant spectrum and diverse substrates for specific uses at the front-end in the market. Mycorrhizal technology, therefore, has to overcome specificity of symbiontal interactions and has to adapt the application procedure (by hand or machine, integration into common procedures or use of specific technological developments) to mycorrhizal inoculum demands. The quality declaration allows choice of the proper product for a particular application, which will fulfil the expectations of the buyer.

FUTURE OF THE BIOMYC PROJECT

Since 2002, in demonstration projects under practical conditions, the following steps have been realized: mycorrhizal technology at XAAS (Urumqi, China) was established, and biological control of biotic stressors (e.g. nematodes, fungal pathogens, insects) on tomato, bell (green) pepper and cucumber (by beneficials and mycorrhizal fungi under greenhouse conditions) was studied. Also under greenhouse conditions, eco-physiological studies on mycorrhizal functioning in nematode-infested soils (e.g. influence of light, nutrition, population biology) have shown the strongly opposed influence of mycorrhiza/nematode interactions on vegetable yields. It is probable that protocols can be developed in 2007 for mycorrhizal use in nematode-infested soils (e.g. in German and Chinese organic horticulture), especially for cucumber production. The standards for quality control of mycorrhizal inoculants have recently been discussed at a European level in the COST Action 870. Hopefully, these will be the basis for Quality Assurance and Certification in business and industry in Europe, and in China, to assure best inoculum quality and (at the same time) to avoid spreading unwanted organisms with inocula. Therefore, all partners agree that the BIOMYC project had an important starter function for consumer-oriented research on, and the environmentally friendly application of, mycorrhizal technology in their countries.

Use of microorganisms for overcoming the pollution of soil by herbicides

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INTRODUCTION

Using herbicides during the cultivation of pine (*Pinus*) seedlings in forest nurseries results in the formation two phenotypes of teratomorphic seedlings – conditionally normal (= conditional) and abnormal. The first is characterized by disruption of the correlation between stem and needles, the second by the development of a number of additional shoots. Creation of forest cultures from teratomorphic seedlings leads to low survival. It is known that herbicides and their metabolic products can remain in soil for many years. Thus, it is impossible to rely only on the natural auto-purification of soil.

Herbicide residues in soil are typically removed by microbiological decomposition. However, the use of pure cultures of microorganisms is difficult. Now, data on the benefits of microbiological transformation of herbicides, that relate to the cooperative action of microorganisms, are gathered. This method for ridding soils of herbicide residues is preferable, because the meliorative organic substance involved occur sloe to the forest nurseries – a forest litter which is enriched with microorganisms. The forest litter is occupied by various microorganisms which, together, possess a wide range of enzymes capable of transforming organic substance (Vedrova, 1997), and will promote the decomposition of herbicides. This process is connected and controlled by hydrothermal conditions, by weight of the decaying vegetative matter and by other factors.

METHODS

An earlier experiment, created in a forest nursery where herbicides were applied, gave a positive result. An application (at a rate of 10 kg/m²) to forest litter derived from pine (*Pinus*) and birch (Betula) resulted in 23% of normal phenotype seedlings, i.e. almost a quarter of plants in the experiment. The further research, reported here, was carried out in a forest nursery with a soddy-podsolic loamy soil; density of the arable horizon of soil was 1.03 g/m^3 , pH of a salt extract 4.9, the humus content was 4.23%, available potassium and phosphorus of 1.6 and 5.2 mg/100 g of soil, respectively, i.e. the level of soil fertility would not prevent cultivation of standard pine seedlings. However, as shown by the development of a large number of seedlings with teratomorphic phenotypes, the soil was polluted because herbicides (2,4-D, glyphosate and others) had been used for more than 20 years in the forest nursery. The purpose of the experiments was to examine the influence on the morphology of pine seedlings of entering a mixed pine-birch forest litter, incorporated into soil before the sowing in the spring at rates of 10 and 20 kg/m². The effects were assessed by examining the morphology of seedlings (number of pine seedlings with normal, conditionally normal and abnormal phenotypes), the intensity of emission CO_2 by soil and the activity of a catalyst, which correlates with the number of soil microorganisms (Kurbatov, 1962) and is a non-specific indicator of pollution (Kovalenko & Babushkina, 2003). Experiments were done in field conditions. Seeds were sown and soil prepared in the ways typically used in forest nurseries. Seedlings were raised for two years. In the second year, in mid-September, they were dug up and sorted according to earlier-developed criteria for identifying phenotypes (Freiberg *et al.*, 2004).

RESULTS

The results of these experiments show that a dose rate of 20 kg/m^2 was most effective (Table 1). The number of seedlings with a normal phenotype, following cultivation in forest litter, improved from 32% to 40%. Data on the intensity of CO₂ emission by soil and data on the activity of a catalyst confirm the considerable contribution part played by microorganisms. Our data indicate that colonization of soil by microorganisms in the first year of planting pine seedlings in forest litter is to be recommended.

Forest litter (kg/m ²)	Emission of CO ₂ (mg/100 g soil)		Activity of a catalyst* (ml O ₂ /g soil) during 1 day	Distribution of seedlings			No. of seedlings/m
	year 1	year 2	uurring r uur	normal	conditional	abnormal	
Experime	nt 1 (year	· 2000)					
10	2.25	1.59	1.0	15.1%	65.7%	19.2%	97
20	4.75	1.73	1.3	40.4%	38.8%	20.8%	103
Control	0.88	0.67	1.1	0.3%	88.7%	11.0%	97
Experime	nt 2 (year	· 2003)					
10	1.74	1.08	1.5	22.2%	72.7%	5.1%	138
20	3.31	1.56	1.9	32.9%	60.7%	6.4%	109
Control	0.10	0.76	0.7	1.0%	89.3%	9.7%	109

Table 1. Distribution of 2-year-old pine seedlings of various phenotypes, and biological activity of soil.

*In the second year of seedling growth.

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Root weevils (Coleoptera, Curculionidae) and their control in nurseries in Serbia

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INTRODUCTION

Forest and ornamental nurseries in Serbia are grown on the area about 718 ha, involving 274 growers. Nurseries in Serbia are either government-owned (50.3%) or under private ownership (49.7%). An average nursery area is 4.24 ha, but private nurseries are smaller (averaging 2.5 ha). The most frequent grown plants are: *Acer* spp., *Betula* spp., *Buxus sempervirens*, *Cedrus atlantica*, *Chamaecyparis lawsoniana* cv., *Corylus* spp., *Cotoneaster* spp., *Ginkgo biloba*, *Lonicera* spp., *Thuja occidentalis* (various cultivars), *T. orientalis* (various cultivars), *Juniperus* spp., *Magnolia* spp., *Pinus* spp., *Prunus laurocerasus*, *Picea omorika*, *Pseudotsuga mensiessi*, *Salix* spp., *Spiraea* spp. and *Taxus baccata* (various cultivars).

Insect pests and mites in nurseries in Serbia were studied. Altogether, 56 species of insects and 8 species of mites occur frequently and cause the damage to leaves, roots, twigs, shoots and flowers (Glavendekić & Mihajlović, 2006). In nurseries, attacks lead to stagnation of plant growth, reduction of aesthetical and economic values of cultivated plants or the death of seedlings and plants. Root weevils are among the most important pests in nurseries in Serbia. Both as adults and larvae they cause serious damage to seedlings, container-grown seedlings and other nursery stock. The most important are the strawberry root weevil (*Otiorhynchus ovatus*) and vine weevil (*Otiorhynchus sulcatus*) (Coleoptera, Curculionidae). Owing to their broad distribution (Europe, North America, some parts of Australia, New Zealand and Japan) they are the most ubiquitous and damaging species in nurseries and young plantations (Nielsen, 1989).

METHODS

The biology of root weevils was studied in several nurseries in Serbia. Paper cups dug into the soil were used for monitoring adults. These pitfall traps were checked twice a week. Feeding behaviour of the weevils was also observed. Chemical control measures were applied against adults. Biological efficacy of insecticides based on following active substances was tested: acetamiprid, cypermethrin, dimethoate, pirimyphos-methyl and thiamethoxam. Larval development of vine weevil was studied in detail.

RESULTS

Investigations on the biology and control of strawberry root weevil were done in a forest nursery in central Serbia, where mainly coniferous trees are, e.g. *Picea*, *Abies*, *Pseudotsuga*, *Chamaecyparis*, *Larix* and *Pinus*. Adults were observed from June. They fed on and notched

leaf margins. Severe damage and losses occurred amongst *Picea pungens* and *Abies alba* seedlings.

Active ingredient	Dosage/100 m ²	Efficacy (%)	
aetamiprid	2.5 g	78–89	
acetamiprid	5.0 g	81–93	
cypermetrin + nu-film	3 ml + 10 ml	67-80	
cypermetrin + nu-film	6 ml + 10 ml	70-85	
dimethoate	20 ml	75–90	
dimethoate	40 ml	100	
pirimyphos-methyl	20 ml	100	
thiamethoxam	8.0 g	80–98	
thiamethoxam	4.0 g	75–92	

Table 1. Efficacy of chemicals against adult strawberry root weevils.

Evaluation of efficacy of various chemicals (Table 1) showed good efficacy of dimethoate, pirimyphos-methyl and neonicotinoids. Against larvae, however, such treatments were less effective.

Vine weevil was observed in urban green areas, where a great lost (90% of plants) was observed in a new plantation in April. Surveys in nurseries showed considerable infestations on *Prunus laurocerasus*. Autumn damage, causing c. 40% dieback of plants, also occurred.

DISCUSSION AND CONCLUSIONS

Adults of strawberry root weevil could be sufficient controlled with chemicals. Among others, neonicotinoids should be recomended. Our results correspond to those of Labanowska *et al.* (2004). Efficacy against larvae was limited. Vine weevil is not common in the Balkan region. The first data on this pest originate from Slovenia (Maček, 1968) and Croatia (Kovačević, 1977). It is recorded in nurseries and on ornamentals in Serbia for the first time.

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The use of active strains of *Trichoderma* and *Streptomyces* in biological monitoring of coniferous seedlings

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INTRODUCTION

At present, around 30% of all forest seedlings in Siberia are destroyed by plant pathogens. Pesticides and organic compounds are widely used to control plant pathogens in many countries. However, the degradation of such compounds is very difficult and the concentration and/or accumulation of them are leading to higher toxicity levels. Trichoderma species have been investigated for over 80 years. These fungi are well studied and have shown efficience on biocontrol of different phytopathogens, including some (such as Fusarium and Alternaria) from the phyloplane. They have been used recently as biocontrol agents, and their isolates have recently become available commercially. Nowadays, biofungicides formulated with Trichoderma, are used to control several soil-borne pathogens which cause damping-off and root rot diseases. This development is largely the result of a change in public attitude towards the use of chemical pesticides (including fumigates, such as methyl bromide, which had been widely used in forest nurseries to control soil-borne pathogens) (Prochzkova et al., 1997). The other prospective organisms recommended for biologocal control are actinomycetes. However, these are few in world reforestation practice (Dumroese et al., 1998). This paper highlights factors that have influenced the acceptance and use of biological control of forest seedling production systems used in reforestation in Siberia.

METHODS

The following active strains of *Trichoderma* and *Streptomyces* were obtained from forest nursery soils in Central Siberia: *T. asperellum* (MG-97), *T. harzianum* (M-99/5) and *S. lateritius* (19/97-M) (Gromovykh *et al.*, 2003). *Trichoderma* spp. were tested against *Fusarium* isolates, using dual culture common agar-well diffusion assay (Egorov, 2004). Living preparations were made for testing in forest nurseries by deep-fluid and solid fermentation. The influence of the strains *T. asperellum* MI-97 and *Trichoderma harzianum* 'Universal' on phytopathogens was studied, following their introduction to soil of the forest spruce crop *Picea obovata*. Efficiency of the introduced antagonist was measured, using the following parameters: symptom levels in seedlings; population of strains; fungal numbers and species composition; microbes using organic and mineral forms of nitrogen.

RESULTS AND DISCUSSION

Both strains of *Trichoderma* had a period of maximum abundance: *T. asperellum* up to 60 days and *T. harzianum* up to 30 days after inoculation. Introduction of antagonistic *Trichoderma* strains into soil resulted in a decreased distribution of symptoms, modification of

the fungal populations and loss of plant pathogens from the genera *Fusarium* and *Alternaria*. The populations of *Trichoderma* isolates decreased in soil after 21 days, but remained at moderate levels for 60 days after introduction. Results indicated that the level of control of disease was consistent and satisfactory over two years (Table 1).

Treatment	Yield of healthy seedlings (numbers/100 m)			
	2004	2005		
untreated control	4,130	4,740		
trichodermin-m Trichoderma asperellum	6,730	8,540		
trichodermin-c Trichoderma harzianum	9,580	7,570		
laterin <i>Streptomyces lateritius</i> trichodermin-c +	9,650	6,280		
laterin Streptomyces lateritius	10,320	7,350		
trichodermin-m + trichodermin-c	_	12,600		

Introduction of antagonistic *Trichoderma* strains resulted in a decreased distribution of symptoms, and the loss of plant pathogens from the genera *Fusarium* and *Alternaria*. Maximal effect was achieved by using laterin and two trichodermins (streptomycete plant growth promoters and antibioses of *Trichoderma*). The largest number of healthy seedlings was achieved by using combined preparations, as a result of the double action of streptomycete's metabolites: growth promotion and antibiotic action of *Trichoderma* with respect to phytopathogenic fungi that have infected the seedlings.

Our results maximized the opportunity for the biocontrol agent to be incorporated into mainstream reforestation practices, reducing the number of pesticide applications and safeguarding against the risk of 'failure' under high disease pressure.

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Analysis of pesticide use in reference farms with regard to necessary minimum

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INTRODUCTION

With the passage of the German Action Plan for Reduction of Pesticide Use in 2004, the treatment frequency index (TFI) was introduced in Germany as an instrument to measure the intensity of use of plant protection products. One advantage of the TFI is that it makes it possible to compare different units such as kilograms, litres and grams. Furthermore, it makes it possible to determine whether any actual reductions in pesticide use have been achieved. It was decided that the intensity of plant protection product usage at reference farms should be analysed over a period of 8 years, in order to determine the potentials for reduction to the necessary minimum. Potential correlations between the intensity of pesticide use and relevant agricultural factors such as the time of sowing, preceding crops and the type of tillage were further questions for analysis. The relationship between conservation tillage and the use of glyphosate herbicides was of particular interest.

METHODS

To answer the aforementioned questions, from 1998 to 2005, our group conducted an analysis of pesticide use in various crops, especially winter wheat and winter rape, at five German reference farms with different climate and soil characteristic. TFIs were calculated for each farm, year and crop, as described previously (Kudsk, 1989). The TFI data were then used to calculate the mean and annual intensities of pesticide use for all the main crops. Potential correlations between the TFI and the time of sowing, preceding crops and kind of tillage were investigated by defining factors of influence and calculating Spearman's correlation coefficient.

RESULTS

Our findings show that in none of the investigated crops (winter wheat, winter rape, barley and sugar beet) had a decrease in pesticide use been achieved within the last 8 years. In fact, the intensity of pesticide use in winter rape and winter wheat even increased in two of the five farms studied. The mean intensity of pesticide use at the five farms was generally higher in winter wheat and winter rape than in sugar beet and barley. The variation between the years was less pronounced in herbicides and more pronounced in insecticides than in other plant protection products. The highest TFIs were found in sugar beet herbicides, winter wheat fungicides and winter rape insecticides. In the investigated crops, TFIs below a mean of 1.0

were achieved in growth regulators, in fungicides (except in winter rape) and in insecticides. In winter wheat, there was a correlation between the date of sowing and the intensity of fungicide use in two out of five farms, and between the date of sowing and the intensity of herbicide use in two of the farms. Furthermore, the intensity of growth regulator use correlated with the date of sowing in one farm, and with variety properties in another. farm. During the investigated period (1998 to 2005), for this crop, there was a measurable increase in growth regulator use at two farms, in herbicide use at two farms and in fungicide use at three farms.

Farm	Crop	Pesticide type	TFI
Klützer Winkel	sugar beet	herbicides	3.73
Klützer Winkel	winter wheat	fungicides	2.90
Magdeburg	sugar beet	herbicides	2.60
Halle	winter oilseed rape	insecticides	2.31
Halle	winter wheat	fungicides	2.24
Halle	sugar beet	herbicides	2.21
Halle	sugar beet	insecticides	0.10
Macham	barley	insecticides	0.09
Macham	sugar beet	fungicides	0.00
Klützer Winkel	sugar beet	fungicides	0.00
Klützer Winkel	barley	insecticides	0.00

Table 1. Intensity of pesticide use at different reference farms in Germany.

DISCUSSION AND CONCLUSIONS

The TFI is more suitable for representing the intensity of pesticide use than the quantity of plant protection products. As expected, the intensity of pesticide use varied between fields, farms and years. Early sowing, conservation tillage and unfavourable preceding crops led to higher herbicide indices in winter wheat within the investigated period, owing to intensive use of glyphosate herbicides and herbicides for control of *Bromus* weeds. Significant increases in TFIs for growth regulators were observed in years with early sowing of winter wheat. Fungicide TFIs rose over the years in half of the investigated farms. Early sowing was shown to be associated with higher TFI values.

At the present state of research, it still is not possible to define local necessary minimum application values for plant protection products based on TFI values. The question of whether soil protection or reduction of pesticide use should be given more attention must still be decided, depending on local conditions for soil erosion.

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Indication and evaluation of plant protection measures on a farm level within the REPRO concept

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INTRODUCTION

In the context of the common agricultural policy of the EU, environmentally compatible agricultural production and higher requirements of health and consumer protection are gaining increased attention. This is reflected in the development of environmental and quality management systems. These support the farmer in decision making and farm management. They also form an important tool for implementing good agricultural practice. By recording the operational farm activities, external inputs and product chains are documented at one and the same time. Thus, the internal farm matter cycle (e.g. biomass, nutrients, energy) can be analysed retrospectively, and compliance with predefined standard or target values for agricultural production and environment protection is provable.

THE REPRO CONCEPT

REPRO is a computer-based tool for farm and environmental management. The software allows a virtual farm (including farm site, farm structure and the farmer activities) to be established. This information forms the basis for data analysis, allowing economic and environmental evaluation. Key parameters managed within the system include: (a) farm site (weather and basic soil data); (b) farm structure (fields, cropping patterns, crop rotation, livestock categories, livestock performance); (c) cropping (technology, fertilization, yields, products); (d) yield (main and byproduct) and product quality; (e) storage (product in- and output); (f) costs (gross margins, total costs). These data are completed using comprehensive data master files. These contain product information (fertilizer, pesticides), results of long-term experiments (e.g. humus formation) and various other standard data and coefficients (e.g. soil characterization, machinery). These data allow farm processes to be analysed, and enable the impact of farm operations on environmental goods to be evaluated. In addition to plant protection data, analyses also consider, for example, on-farm matter cycle (N, P, K, C), N-turnover, humus and energy balancing, erosion risks and biodiversity.

PLANT PROTECTION INDICATORS

Plant protection forms an important part of the whole-farm evaluation. Data input is done with the support of comprehensive master files. This allows the correct recording of applied products, whether using the product name or the official registration number. In addition, the date of application, product quantity per ha, extent of treatment (complete field or field parts), the application method (spray or seed treatment) and costs (product and process) are also

elevated. REPRO involves the plant protection indicators shown in Table 1 (after Heyer *et al.*, 2005).

	Reference unit / level	Content and aim of indicator application
Indicators used in		
documentation		
Product quantity (litres/ha,	Field and sub-field;	Quantitative indicators, used with the aim of
kg/ha)	crop groups and crop;	farm management.
Costs (€/ha)	crop rotation;	
Number of applications	arable land,	The share of non-treated area is important in
Treated area (ha or %)	grassland;	case of ecological evaluation.
Non-treated area (ha or %)	farm	
Indicators used in		
evaluation		
Farm application index		Indicator with aggregated information about
(without dimension)	10 main field crops	frequency, amount and area of treatment.
REPRO valuation index	10 main field crops;	Adaptation of the 'Farm application index'
(without dimension)	farm level	to the REPRO concept.
		Purpose of comparability to other REPRO
		indicators.
Fossil energy use (MJ/ha)	See above	Basic information for energy balancing.

Table 1. Plant protection indicators within the REPRO concept.

RESULTS AND CONCLUSIONS

Analysis of agricultural enterprises (n = 25) on the basis of 'treatment index' and 'REPRO assessment number' showed an agriculturally acceptable use of pesticides. Nevertheless, between the enterprises, differences in plant protection intensity could be demonstrated, which could not be explained by subtly different crop patterns or by farm site. This finding indicates that the plant protection management of compared enterprises was handled very differently and that there are possibilities to optimise plant protection activities. It illustrates either that more intensive plant protection measures often did not result in higher yields or that N efficiency was reduced following adoption of sub-optimal plant protection measures.

The REPRO plant protection indicators were suitable for both decision making within the farm management process and assessment of the environmental impact of plant-protection intensity. Considering the different evaluation levels (sub-field, field, crop, crop rotation or the complete farm), the analyses were comprehensive and ways to improve plant protection strategies could often be recommended. Further qualification of the REPRO results requires improved means for complex data analysis of factors such as the interactions between plant protection, fertilization and energy gain. This work is currently underway.

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Diabrotica virgifera virgifera in confrontation mood: simultaneous geographical and host spectrum expansion in southeastern Slovenia

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INTRODUCTION

The western corn rootworm (WCR) (Diabrotica virgifera virgifera) (Coleoptera: Chrysomelidae), is an alien invader from the New World to Europe. WCR arrived in Europe from North America on at least 3 separate occasions (Miller et al., 2005) and expanded its territory with amazing effectiveness and speed. Within 15 years after first introduction to Belgrade airport, the entire area of southeastern and central Europe (except Germany and Denmark) is now considered infested. Several areas, including Hungary, Romania, Serbia and Croatia, are already suffering economic losses in maize, so far the only known host plant in Europe. This is in contrast to the Americas where WCR also attacks members of the plant family Cucurbitaceae (Rhodes et al., 1980) and, very recently, soybeans (Spencer et al., 2005). For the last 7 years, we have been on constant watch for possible expansion of WCR's host range in Europe. In August 2006 we finally succeeded and obtained evidence of WCR not only greatly expanding its geographical range in Slovenia, but also accepting a new host - the pest visiting the yellow blossoms of oil pumpkin (*Cucurbita pepo*) in fields south of Gaberje near Lendava, Eastern Slovenia. Oil pumpkin has regional economic importance as a source of valuable vegetable oil and seeds for health food. This is the first report documenting, in the Old World, of WCR on this crop.

METHODS

Sticky cup traps of the Metcalf type (for details see Hummel *et al.*, 2005 and related papers) were baited with MCA (4-methoxy-cinnamaldehyde) kairomone or female sex pheromone and established with a minimal distance of 20 m in maize at 1-1.5 m, in oil pumpkin at 0.5 m, above ground. Traps were monitored daily. In oil pumpkin the blossom itself serves as a natural 'trap' site where WCR adults like to feed and can be easily observed.

RESULTS & DISCUSSION

In blossoms of a field of oil pumpkin (0.28 ha), 4 WCR were counted at a field south of the village of Gaberje, Slovenia, on 19 August 2006, followed by 2 WCR adults on 22 August. The incidence of detection in the crop is still quite low (about 0.1 %) but reflects increased WCR activity during late July and throughout August. In daily counts, 6,209 blossoms were

systematically sampled from 31 July to 29 August 2006. Females not only fly to the flowers, attracted by their yellow colour, but are also attracted to colourless MCA kairomone-baited traps. On 28 August 2006, 5 female WCR were caught on such traps within 24 hours. Four sex pheromone-baited traps each caught 1 male, whereas two unbaited sticky traps serving as controls caught no beetles at all. In maize, from 31 July to 29 August 2006, we detected 3,780 WCR adults on 40 traps equally distributed over an area of 4.01 ha; at first mainly males were seen but, as time progressed, an increasing number of females were caught. In a parallel study conducted throughout Eastern Slovenia, Modic *et al.* (2006) reported geometrically increasing WCR numbers from 2003 to 2005. In 2005 alone, the WCR population advanced 40 km westward. In total, 1,349 WCRs were found at 120 locations whereas, in 2003, only 19 were found at 14 locations.

We take this newest finding in Europe as an additional indicator for the successful and irreversible establishment of WCR in the Old World, and as a sign of its active and aggressive colonization strategy, not only for new territory but now also for oil pumpkin as a newly emerging host plant. The discovery has direct consequences for the future effectiveness of crop rotation as one of the few readily available, inexpensive and so far quite successful IPM approaches. The more hosts WCR will colonize, the less effective crop rotation will become, a hard lesson learned in Illinois and parts of Indiana, USA, where "the crop rotation resistant" WCR ecotype has been well studied by Spencer *et al.* (2005). Independently, Kiss *et al.* (2005) conclude that although "rotation of maize with other crops is a primary control method for WCR populations, there are still major questions concerning the long-term management of WCR". We are probably now witnessing the first steps of WCR in its search for new hosts in Europe. What is our collective answer?

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Determination of water extractable deltamethrin metabolites in different kinds of tea and non-extractable residues in tea

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INTRODUCTION

Synthetic pyrethroids are a class of widely used insecticides that have relatively low mammalian toxicities and reasonably short lifetimes in the field. Therefore, the European authority allows countries to use synthetic pyrethroids in tea crops cultivated for the European market (Anonymous, 2004; 2005). However, so far, there have been no studies of the metabolic fate of deltamethrin in tea plants. The aim of this study was to find out which metabolites of deltamethrin are present in tea infusions.

METHODS

With a metabolism study of 14C-deltamethrin we examined its major pathways in tea plants grown under greenhouse conditions. After optimizing application, the treated tea plants were grown-on for three months. Sampling took place every second week following the application. The degradation of deltamethrin in this study was prolonged by a 'waiting period' of 26 days, owing to greenhouse conditions. The most important process in the deltamethrin degradation is the cleavage of the ester group in the middle of the pyrethroid molecule. The most important of metabolites are 3-PBA, 3Br2CA, their mono- and diglucosidic conjugates and 3-PBAld.

Another main point of this study was the investigation of the migration of water-soluble metabolites, and conjugated and free residues into the tea infusion. Different kinds of tea were produced from the treated plant materials. These teas and their infusions were analyzed for deltamethrin residues and metabolites. After the concentration of these conjugates and free metabolites with SPE, we isolated fractions of free and conjugated metabolites. Conjugated metabolites could be broken with the use of ß-glucosidase. We measure amounts of these aglucons and free metabolites with GC-MS/MS after methylation with diazomethan. In this part of work we could show differences in the distribution of the metabolites in different kinds of tea.

RESULTS

Besides traces of deltamethrin, the metabolites 3-PBA, 3-PBAlc, Br2CA and their conjugates with glucose were identified in infusions of all kinds of tea. Their distribution, however, varied. In green tea infusions they were mostly present in conjugated or glucosidic form. The metabolites of black tea infusions, however, were present in their agluconic or free form. In the case of half-fermented (or oolong) tea infusions we observed a degree of conjugation with glucose of the formed metabolites between black and green teas. The degree of fermentation during tea processing is responsible for this phenomenon. <A[extracted|gaiThese data supplied the information on the dependence of the release of deltamethrin metabolites on the degree of fermentation of the tea.

Apart from the determination of the formed extractable residues, another special aspect of this study was the determination of the bound or non-extractable residues. After the chemical cleavage of the plant cell wall, we were able to measure the radioactivity in each hydrolyzed fraction.

For this investigation five different cell wall fractions were isolated. The fractions contained lightly polymerized polyphenols and proteins, pectin, cellulose, hemicellulose and lignin. The largest part of the non-extractable radioactivity (about 30%) was found in the lignin fraction. Another part of the deltamethrin (without the radio labelling) was present, in the form of Br2CA, in the hemicellulosic fraction. After 82 days the value of non-extractable radioactivity amounted to 25% of the total applied value.

We measured the main metabolites from deltamethrin 3-PBA, 3-PBAlc and Br2CA in free form in black tea infusions and their β -glucosidic conjugates in the green tea solutions. One part of the polar metabolites from deltamethrin (12%–14%) is water soluble, and this will be extracted from the tea infusion.

The same metabolites were found in infusions of the different kinds of tea. However, their distribution varied. In green tea they were present mostly in their conjugated or glucosidic form. By contrast, the main part of black tea metabolites are aglucons in their free form. Because of the low water solubility of pyrethroids, just a small amount of deltamethrin (< 1% of the total residues) was found in both kinds of tea infusions.

ACKNOWLEMENT

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

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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.

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Approaches to cultivating prospective strains of mycoherbicide producers in liquid media

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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 $24 \pm 3^{\circ}$ 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°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 2^{nd} and 3^{rd} day of the growth) on all of the studied media; thereafter, a smooth increase in pH up to 9.5 (by the 10^{th} day of the growth) occurred. Subsequently, up to the 15^{th} 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 \pm 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.

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Competitive tests of herbicides on spring wheat in western Siberia

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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 litres/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

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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.

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

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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 & Павлющин, 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.

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	$7,5 \pm 2$	12.5 ± 5	12.5 ± 5	20 ± 7

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)

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

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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.

Tre	eatment 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.	<i>B.t.t</i> @ 5 litres/ha	92 €/ha
5.	neem @ 2.5 litres/ha + pyrethrum/rape oil @ 8 litres/ha (2 days apart)	233 €/ha
6.	<i>B.t.t</i> @ 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

Table 1. Treatments for control of Colorado beetle

* 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 ϵ /ha) * $\alpha = 0.05$ Dunnett

Treatment	Defoliation (%) 22 days after first pesticide	Efficiency factor (%)	Yield in dt/ha	Additional profit (€/ha)
	treatment			
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.

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

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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 20^{th} 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.

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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.

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

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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 125^{th} 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 m²).

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 156th 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.

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Integrated management of small-holder fruit gardens in the Soconusco, Chiapas, Mexico

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INTRODUCTION

In the Soconusco, fruit growing extends actually over 60,000 ha and is characterized by highdiversity home gardens on the one hand, and by export orientated planting of mangoes (*Mangifera indica*), bananas (*Musa acuminata, M. paradisiaca*), papaya (*Carica papaya*) and rambutan (*Nephelium lappaceum*) on the other (Pérez Romero & Pohlan, 2004; Vanderlinden *et al.*, 2004; Pohlan *et al.*, 1997). Both types of fruit cropping systems are characterized by intensive mechanical and chemical cultivation of soil and by indiscriminate use of chemical products for weed and insect control. The interesting and very important question under these conditions is to find new strategies for soil conservation and soil fertility, for balancing and diversifying both the natural insect fauna and the weed flora, and for integrating cash and trap crops into the fruit areas (Pohlan, 2002; Gamboa & Pohlan, 1997).

METHODS

The investigations were begun in the first rainy season in 2005, in two typical fruit orchards in the Soconusco, Chiapas, Mexico. The experiments are located in a mango orchard in Cintalapa (15° 19' 431" N, 92° 37' 369" W, altitude 184 m a.s.l) and in a rambutan plantation in El Triunfo (15° 21' 147" N, 92° 33' 176" W, altitude 335 m a.s.l). The experimental design consisted of a split-plot system with a total area of 9,408 m². Each experiment (168 × 56 m) included eight intercropped treatments, arranged as a strip design with six replicates in which each subplot measured 14 × 14 m. Each treatment is divided in six sub-plots or replicates. In each replicate, one evaluation point for weed and crop measurement was fixed. Weed determination included abundance, biomass, diversity and insect incidence. Results of the 2° cycle of annual crops and their effects on fruit yield in intercropped mango and rambutan are reported here.

RESULTS

Weed biomass production is influenced by different site conditions, intercropped treatments and changes during the cropping cycles from dominant monocotyledonous to dicotyledonous species. The biomass of *Cajanus cajan* and *Phaseolus acutifolius* (genotype Frijol Escumite) and their incorporation to the ecosystem produced positive effects on fruit number and yield of mango and rambutan.

DISCUSSION AND CONCLUSIONS

Intercropping of either mango or rambutan with *Cajanus cajan* or *Phaseolus vulgaris* influences insect diversity in these systems (Florez *et al.*, 2006). This study demonstrates the high potential of *Cajanus cajan* and *Phaseolus acutifolius* (genotype Frijol Escumite) for improving sustainability of fruit orchards and for re-establishing entomophylous pollination potential. These legumes are an alternative for stable economic income, soil fertility, ecological diversity and low management costs, as well as labour opportunities during the whole year, and offer additional a self-consumption diet for farmer families.

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The effect of the EU review of active substances on plant protection in Poland

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INTRODUCTION

After accession to the European Union, Poland implemented a number of EU law regulations concerning among others agriculture and plant protection. The main EU Directive setting principles regarding plant protection is the Council Directive 91/414/EEC. The main deed implementing the requirements of the Directive 91/414 to Polish law is the Plant Protection Act voted in on 18 December 2003. The Plant Protection Act was accompanied by numerous executive regulations. One of consequences of accessing EU was the immense effort of Polish legislative authorities because it involved a hugh workload, which is still ongoing. A measure of the legislative effort is, surely, the number of legal acts issued in Poland: from the year 2001 to the end of April 2006 over 150 legal acts concerning plant protection were issued in Poland, more than in the whole of the 20th Century (Matyjaszczyk, 2006). To put the requirements of Directive 91/414 into effect the European Commission started to review the active ingredients of all plant protection products (PPPs), to check if they are safe for people, animals and the environment. The review is performed on the EU level and its results are binding for member states, and also for Poland.

RESULTS

Table 1 shows the influence of the membership in EU on the number of PPPs placed on the Polish market during two years after accession. The period considered is from 1 May 2004 (the date of accession EU) to the end of April 2006. From the date of accession, the products withdrawn outnumbered the new registered PPPs (Table 1). The decline of PPPs placed on the market is noticeable, especially in case of herbicides – during the two years of Polish membership in the EU the number of herbicides placed on the Polish market decreased by 52. In the case of zoocides (e.g. insecticides, nematicides, molluscicides, rodenticides and acaricides) there was decline of 19 PPPs. Decisions about the withdrawal of PPPs from the market (Table 1) do not concern results of the withdrawal of active substances following the lack of support in the fourth round of review. It is already known that 91 out of 225 active ingredients from the fourth round of the review are to be withdrawn from the EU market, as their producers are not supporting them through the review process. Because of this, 63 PPPs are expected to be withdrawn from the Polish market, among them 14 products approved for use in ecological farming (www.ior.poznan.pl).

To illustrate better the significance of the number withdrawn and new registered PPPs, data regarding total number of PPPs registered in Poland are necessary. According to data of the Ministry of Agriculture and Rural Development, 949 PPPs were placed on the Polish market in July 2006 (www.bip.minrol.gov.pl/DesktopDefault.aspx?TabOrgId=647&LangId=0). The most numerous group were herbicides (355 products), followed by fungicides (268 products). In the group of zoocides 196 products were registered. The most complex and the least

numerous (130 PPPs) is the last group 'other products'; this includes adjuvants, which are registered in Poland as PPPs.

Table 1. Changes in number of plant protection products placed on the Polish market two years after accession to the EU (1 May 2004 to 30 April 2006).

Products	Fungicides	Herbicides	Zoocides	Others	Total
New registrations	26	17	9	16	68
Re-registrations	3	5	4	8	20
Withdrawals	25	69	28	9	131

Source: Personal elaboration of data received from the Polish Ministry of Agriculture.

DISCUSSION AND CONCLUSIONS

Withdrawals of PPPs from the Polish market during the first two years after EU accession caused a decline of 6% in the number of all PPPs placed on the market: herbicides decreased by > 12% and zoocides by > 8%; fungicides increased by c. 1% and 'other products' by > 5%. The decline is accompanied, however, by another problem: the reduction in the contents of labels. According to the estimations of the Department of Expertises and Opinions about Plant Protection Products (the unit responsible for authorising PPP labels) > 70% of re-registered PPPs has fewer approved uses than before. It means that the number of products available to protect numerous crops is declining more significantly than the number of PPPs placed on the market. As a result problems with protection of some crops have arisen, especially for 'minor crops'.

EU accession has affected the Polish market of PPPs, the most noticeable for Polish farmers seem to be:

- withdrawals of PPPs from the market due to EU review of active ingredients;
- withdrawals of some approved uses from labels during re-registration;
- problems with protection of 'minor crops'.

It is difficult to sum up the changes on the Polish market of PPPs resulting from implementing EU regulations to Polish law unambiguously. The changes in legal requirements following proecological requirements of Directive 91/414 can, in future, contribute to improvements in the condition of the environment. This would be good not only for farmers but also for all Polish residents. However, because of the review of active ingredients, significant numbers of PPPs will be withdrawn from use in Poland purely for financial reasons. The list of PPPs available for farmers will be reduced and problems with protection of some crops, or with control some pests, will occur. This can influence the competitiveness of Polish agriculture.

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Biochemical methods for control of cereal crop resistance to biotic and abiotic factors

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INTRODUCTION

One of factors of achieving productivity of new cereal crop cultivars is their resistance to biotic and abiotic factors in the environment. To develop resistant cultivars it is important to know the mechanisms of plant resistance, and to have effective and definitive methods for the prediction and selection of resistant genotypes of such cereal crops. It is thought that glycoproteins (such as lectins and proteinase inhibitors) may have an important role in plant resistance mechanisms (Iljinskaja, 1991). It is known that these proteins protect a plant from attack by insects and pathogens, and that their contents in the grain are genetically determined. Further, a certain role in the regulation of plant responses to different natural stress factors belongs to salicylic acid (Raskin, 1992). Salicylic acid may be a trigger that activates plant protection mechanisms in response to diseases and abiotic stresses.

The purpose of the present investigation is to study the activity of lectin and trypsin inhibitors in the grain and seedlings of cereal cultures, to discover their roles in the formation of plant-protective reactions towards pathogenic and abiotic factors.

METHODS

Lectin activity was studied by using the haemagglutination response of white rats to trypsinised erythrocytes (Lutsik *et al.*, 1981). The concentration of the trypsin inhibitor was measured using the substrate N-benzoyl-arginine-4-nitroanilide (Levitsky, 1979). The pathogens *Fusarium graminearum* and *Fusarium culmorum* were used as the agents of infection.

This research was made on various cultivars of winter wheat and spring barley that differ in their resistance to *Fusarium* moulds (i.e. to fusariosis) and heat, using four methods of germination: (a) in pure water, (b) in the presence of 2 mM salicylic acid, (c) in presence of *Fusarium* spp., and (d) at a temperature of 100° C.

RESULTS

It has been established that the diverse changes in the trypsin inhibitor and lectin activity depends on the resistance of the winter wheat and spring barley genotypes to *Fusarium* spp. and heat, in the seed and in seedlings of infected plants, in plants treated with salicylic acid and in seeds which have been heated at high temperatures. Based on the results obtained, a new express method for the selection of *Fusarium*-resistant and heat-resistant genotypes of cereal crops (using biochemical parameters) has been developed. Three Ukrainian patents for these

methods have been taken out (Patent No 12639; Declarative patent No 43280 A; Declarative patent No 69859).

DISCUSSION AND CONCLUSIONS

It is supposed that, on the basis of differential reactions of cereal crop genotypes to the influence of fusariosis, heat and salicylic acid, there are not only cultivar differences in the specificity of the accumulation of trypsin and lectin inhibitors, and their redistribution, but also genetic differences. It is concluded that the studied physiological and biochemical indicators play a part in forming the mechanisms of resistance to *Fusarium* spp. and to heat and that salicylic acid is the activator of the resistance properties of both wheat and barley plants. Biochemical estimation methods for *Fusarium*-resistant and heat-resistant genotypes of cereal crops enable a plant breeder to assess, in a very short time, a considerable number of genotypes at the early stages of breeding. The methods can be used at the plant breeding centres and by grain storage companies to produce non-polluting consignments of grain.

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Comparison of the entomofauna on cabbage plants in Montenegro

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INTRODUCTION

In Montenegro, from 1998 to 2002, the average area covered by cabbage and cauliflower was 1,952.6 ha, yielding 13.13 t/ha. Following the increase in production, and during further development, it is important not to forget the main postulate of sustainable agriculture: to fulfil our needs and to find the optimum way of development, while not endangering the ability of future generations to fulfil their needs for food production and food security. In this sense, the main goal of the present paper is to portray relations between cabbage plants and insects in the agro biotope, by showing similarity of entomofauna in the different localities.

METHODS

Fieldwork was done in various geographic and agrarian regions of Montenegro: Coastal Region (locality Prčanj); Zeta-Bjelopavlići Region (Grbe, Vranjske Njive, Sadine, Grbavci, Balabani and Trešnjica) and Region of High Mountains and Deep Valleys (locality Smailagića Polje). Collection of insects, and all fieldwork, was done during the growing period of 2000 and 2001 on (a) heading cabbage, (b) cale or collards - species endemic to the entire south-eastern Mediterranean region (Pavlek, 1978; Pajović, 2005); (c) Brussel sprouts; and (d) broccoli; and (e) cauliflower and kohlrabi. Southwood (1977) stated that it is practically impossible to count all invertebrates in a locality, so that the most cost-efficient method is to collect them by traps. Malaise traps, Barber soil traps, yellow dishes and a light trap (Sivčev, 1981) were used. Insects were also collected manually from the plants. Collected material was separated in a laboratory and identified to species, genus and family. Jaccard's similarity coefficient was calculated for 'total' number of insects captured in all localities (unequal number of samples at different sampling sites all year round) and for the 'average' sample (same number of samples at each site taken at same intervals during June–October). The Shannon's diversity index (SDI) and Shannon's evenness index (SEI) were also used. Insects were grouped according to their relationship to cabbage plants or to other insects.

RESULTS AND DISCUSSION

By the Jaccard's similarity coefficient (per cent), the relations between faunas in the year 2000 were the same in both cases (total and average). The localities of Grbavci and Prčanj were the most similar (67.9% and 68.2%, respectively); they were followed by Grbe 67% and 67.6%, Vranjske Njive (66.3% in both cases) and, finally, Sadine (65.7%). For 2001, there was a

difference in the results between the calculation for the total number of captured insects and for the 'average' sample. Regarding the calculation for the total number of captured insects, there was the separation between two most similar localities, Sadine and Prčanj (70.2%) (i.e. the first group), and Grbe and Kolašin (68.9%) (i.e. the second group). These two groups were mutually similar at 68.6%, followed by Trešnjica (68.3%), Balabani (67.2%) and, finally, Vranjske Njive (57.1%). Comparison of the values for the 'average' sample was different. The two most similar groups were Sadine and Prčanj (69.2%) and Grbe and Kolašin (68.1%), but Trešnjica was the more similar to the first group (at 67.8%) than to the second. Furthermore, these five biotopes were similar (67.4%), followed by Balabani (67.3%) and, again, Vranjske Njive (58.2%). The highest SDIs were recorded in 2001 in Kolašin (2.876) and Sadine (2.818). Moderate diversity was observed at Balabani in 2001 (2.761), Vranjske Njive in 2001 (2.717), Prčanj in 2000 (2.657) and Grbavci in 2000 (2.615). Lower indexes were recorded at Grbe (2.453) in 2000 and Prčanj (2.338), Sadine (2.322), Grbe (2.241) and Vranjske Njive (2.158) in 2001. Trešnjica, in 2001, had the lowest diversity index (1.458). SEI showed even distribution of specimens in taxons. The highest index was in the locality of Vranjske Njive for both years (0.747). Very similar are localities of Prčanj in 2000 (0.676); Kolašin in 2001 (0.67); Balabani in 2001 (0.654); Grbavci in 2000 (0.636); Grbe in 2000 (0.618) and Sadine in both years (0.61 and 0.608). They are followed by Prčanj in 2001 (0.543); Grbe in 2001 (0.526) and, finally, Trešnjica in 2001, with 0.369.

The total of 49,929 insects was subdivided, according to the relations between insects & plants, insects & insects and, finally, the role of the taxa in agro biotopes and agro-ecosystems. Pest insects included 26 taxa, the most important being: *Pieris* spp. (Lepidoptera: Pieridae); *Brevicoryne brassicae* (Hemiptera: Aphididae) *Aleyrodes proletella* (Hemiptera: Aleyrodidae); *Phyllotreta* spp. (Coleoptera: Chrysomelidae, Halticinae); all sampled weevils (Coleoptera: Curculionidae); and *Eurydema ventrale* (Hemiptera: Pentatomidae). Beneficial insects included 23 taxa, dominated by Coleoptera: Carabidae and Coccinellidae; Neuroptera: Chrysopidae; Diptera: Syrphidae; and Hymenoptera: Ichneumonidae, Trichogrammatidae and Vespidae. 'Beneficials' also included Dermaptera (Forficulidae); Hymenoptera (Apidae) and protected species of Papilionidae (Lepidoptera). In the agro biotope of cabbage plants and all agro-ecosystems on the cabbage fields, huge numbers of 'neutral' species occurred. Of the 49 taxa found, the most numerous were Collembola; Heteroptera (Pentatomidae); Hemiptera (Cercopidae); Coleoptera (Chrysomelidae); Diptera (Stratiomyidae and Muscidae) and Hymenoptera (Formicidae).

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Invasion pathway of peanut flower by green fluorescence protein

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INTRODUCTION

Colonization of peanut (*Arachis hypogaea*) seed by *Aspergillus flavus* and subsequent aflatoxin contamination is a serious worldwide problem. Although colonization of peanut pods by *A. flavus* can occur either before or after harvest (Sander *et al.*, 1985; Dorner *et al.*, 1989) and this fungus can infect pods in the soil during fruit development or earlier during the flower or aerial peg stage (Mehan *et al.*, 1991). Little is known of the nature and mechanisms by which this fungus infects peanut flowers. The development of an *A. flavus* strain that produce green fluorescence protein (GFP) offers the opportunity to track pathways of infection which have not been clearly identified.

METHODS

Three peanut genotypes (511CC, 419CC and Tainan 9) were grown in a hydroponic system to determine flower and aerial peg infection by *A. flavus*. Peanut flowers were marked with coloured thread and inoculated with 0.5 ml of GFP *A. flavus* spore suspension. By 24 and 48 hr after inoculation, inoculated flowers were separated into stigma, style, hypanthium and ovary for observation of fungal invasion and colonization, by using a UV-illuminated microscope. At 10 days after inoculation, pegs were evaluated for the incidence of fungal colonization by being plated on M3S1B medium (Griffin & Garren, 1974).

RESULTS

Observation with an UV-illuminated microscope showed conidia of GFP *A. flavus* germinated within 24 hr and extensively colonized stigma and style, especially near the pollen grains. By 48 hr after inoculation, fungal hyphae grew down the style, eventually reaching the top of the ovary conidiophores, and conidia had formed over the peanut flowers. However, the visible fungal colonization in the ovules was sparse. The highest incidence of peg infection was found in Tainan 9 genotype.

DISCUSSION AND CONCLUSIONS

This experiment provides compelling evidence that seed infection by *A. flavus* may occur directly through floral infection. Initial infections may take place from: (i) *A. flavus* spore attached to pollen grain, (ii) spore germinated on the stigma surface and penetrated through the stigma follow the pollen tube, and (iii) spore germinated on the hypanthium and penetrated transversely through the style and ovary wall. Thus, knowledge of the floral infection could be a key to optimizing control of pre-harvest *A. flavus* infection and subsequent aflatoxin contamination, and then research would be warranted to identify irrigation, row orientation and other factors that would prevent the movement of conidia from the soil surface to the flowers.

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Variability of aliphatic glucosinolates in *Arabidopsis* and their influence on insect resistance

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INTRODUCTION

The glucosinolate (GS) myrosinase system of Brassicaceae comprises a characteristic defense especially against generalist herbivores (Renwick, 2002). GSs are usually found in members of the order Brassicales, which includes the Brassicaceae family with crops of economic and nutritional importance and the molecular reference plant *Arabidopsis thaliana* (Rodman *et al.*, 1996). Based on their side chain structure GSs are grouped into aliphatic, aromatic, and indolyl GSs. Indolyl GSs are widely distributed in *A. thaliana* ecotypes and in the Brassicaceae family, but the presence of aliphatic GSs is very variable and under strong genetic control (Raybould & Moyes, 2001). While GSs by themselves are unpalatable to a number of insect herbivores, after tissue damage the GSs come in contact with myrosinase, releasing additional biologically active compounds such as isothiocyanates and nitriles (Wittstock *et al.*, 2003). Few studies pay attention to the impact of certain GSs on insect resistance. Therefore, we have investigated the plant resistance of *A. thaliana* ecotypes with variable aliphatic GS profiles against two lepidopteran insect pests with different feeding specializations.

METHODS

For the study we used 19 different *A. thaliana* ecotypes, which were divided into three classes after chemical analysis with HPLC: containing methylsulfinyl, 3-hydroxypropyl, and 2-propenyl GS. The corresponding hydrolysis products of GSs in the different ecotypes were determined with GC-MS. Larvae of the generalist *Spodoptera exigua* (Lepidoptera: Noctuidae) and the crucifer specialist *Pieris brassicae* (Lepidoptera: Pieridae) were selected for the feeding studies. The bioassays with insects were done with 10 replications per ecotype and with 30-day-old *A. thaliana* plants. Tests were performed in transparent and gauze-covered insect cages at 21 ± 1 °C, a 10.5 h photo period, and 200 µmol m⁻²s⁻¹ light intensity. Initial larval weight was determined and the final weight was documented after 48 h feeding on ecotypes.

RESULTS

Eight ecotypes contained methylsulfinyl or 3-hydroxypropyl GS as the main compound, respectively, and three ecotypes had 2-propenyl GS. The bioassays revealed significant differences in host plant suitability of ecotype groups for the generalist as well as for the specialist herbivore. Larval percentage weight gain on *A. thaliana* plants containing

3-hydroxypropyl GS and 2-propenyl GS was significantly higher in both insects compared with methylsulfinyl GS containing ecotypes. Additionally, the constitutive GS contents were correlated to the percentage weight gain of larvae, to examine a possible relationship between a certain chemical profile and the weight gain of larvae. The percentage weight gain of *S. exigua* and *P. brassicae* on 3-hydroxypropyl GS containing ecotypes was higher than on methylsulfinyl GS producing ecotypes at comparable GS levels, indicating a better host plant suitability. Weight gain of *S. exigua* was negatively related to constitutive GS levels in methylsulfinyl GS-containing ecotypes only (ANOVA: $P \le 0.05$). Also, a low negative relation to constitutive GS levels was found for *P. brassicae* in methylsulfinyl GS containing ecotypes. Furthermore, the correlation of hydrolysis products to the bioassay data revealed that ecotypes with 3-hydroxypropyl GS at similar concentrations. However, this was independent of the type of hydrolysis produced: the ratio of isothiocyanates vs. nitriles.

DISCUSSION AND CONCLUSIONS

GS levels as well as different types of hydrolysis products influence plant resistance, especially against generalist herbivores (Wittstock *et al.*, 2003). In our study we found negative effects of increasing GS levels in *A. thaliana* on both insects, the generalist *S. exigua* and the specialist *P. brassicae*. Negative effects of GSs and their hydrolysis products on another cruciferous specialist (*Pieris rapae*) are also reported (Agrawal & Kurashige, 2003). The side chain of aliphatic GS obviously influenced insect performance, but the effect of the type of hydrolysis product was transient. Surprisingly, 3-hydroxypropyl and 2-propenyl GS containing ecotypes were less suitable for consumption for the generalist and the specialist than methylsulfinyl GS producing ecotypes. A reason for a better host plant suitability of ecotypes with 3-hydroxypropyl GS might be the different chemical structure and reactivity of this GS compared with methylsulfinyl GS.

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Chances of uptake and fate of the explosives TNT and RDX in conifers

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INTRODUCTION

Former military sites (ammunition plants and military training areas) represent 2.8% (9,997 km²) of the entire German territory (Schröder et al., 2003). Many of these areas are contaminated with residues of explosive specific compounds. Main contaminants are TNT (2,4,6-trinitrotoluene) and RDX (Royal Demolition eXplosive, hexahydro-1,3,5-trinitro-1,3,5-triazine). The hazardous potential, mammalian toxicity, mutagenic and carcinogenic features of explosives are reviewed by Talmage et al. (1999). Most of German former military sites are covered by woodlands dominated by conifers. This causes our hypothesis, if conifer trees may contribute to natural decontamination processes in explosive-polluted soils. Besides tolerance features of conifers to explosives, uptake of explosives by coniferous species are the focus of our investigations.

METHODS

Three-years-old plants of Scots pine (*Pinus sylvestris*) and of a dwarf mutant of Canadian white spruce (*Picea glauca* 'Conica') were first cultivated in 8-cm pots in field soil. After one year of growth in field soil, the trees were transplanted into quartz sand. The 8-cm pots were supplied with glass fibre wicks and placed on 500-ml-glass vessels containing 200 ml of application solution. Using glass fibre application systems the time course of input of water-solved, bioavailable pollutants (TNT, RDX) to the soil/tree system is precisely quantifiable (Schoenmuth & Pestemer, 2004). For uptake studies, uniform ring-labelled ¹⁴[C]-TNT and ¹⁴[C]-RDX were permanently applied via glass fibre wicks. After exposition to ¹⁴[C]-TNT and ¹⁴[C]-RDX overall radioactivity of tree compartments was determined (Biological Oxidizer OX 500, Zinsser Analytik GmbH, Frankfurt/M, Germany). Extractability of radio-labelled explosives from plant tissues was calculated by radioactivity determination of plant extracts, using a Multipurpose Liquid Scintillation Counter (Beckman Instruments GmbH, Munich, Germany). Radio-labelled extracts were separated by radio thin layer chromatography (TLC). TLC plates were evaluated quantitatively using a Linear TLC Scanner (Bertholdt, Germany).

RESULTS AND DISCUSSION

Evaluating the mass distribution of radio-labelled compounds showned that pines as well as spruces are able to reduce the content of 14 [C]-TNT and 14 [C]-RDX in soil. Substrates containing conifer plants clearly indicate less explosive equivalents than unplanted variants.

Both TNT and RDX are accumulated in pines and spruces. For TNT, highest concentrations of 14[C]-TNT equivalents (eq) are found in roots. Concentrations up to 308 mg TNTeq kg-1 root

dry matter were determined for Pinus. Relative mass distribution shows that 96% of 14[C]-TNT equivalents taken up by both tree species remain in roots. Only a very small percentage is transported to above-ground tree compartments, i.e. wood (3%) and needles (2%). For RDX, however, highest concentrations of 14[C]-RDX equivalents are observed in above-ground tree compartments. Roots contain only 21–22 mg RDXeq kg-1 DM. In wood concentrations are 32 mg RDXeq kg-1 DM for Picea and 39 mg RDXeq kg-1 DM for Pinus. At the time of tree harvest after three weeks, highest concentrations were detected in needles of Pinus (94 mg RDXeq kg-1 DM). RDX is obviously translocated by the transpiration stream in conifers. This is supported by the finding that more than 60% of needle accumulated RDXeq are located in the youngest needles, where transpiration normally is most extensive.

For ultrasonic extraction procedures different extractants were tested. Extraction efficiency of is given by the following range: 50% (v/v) acetic acid > methanol > acetonitrile for both, TNTeq and RDXeq. Extractability of TNTeq was very low in roots (c. 10%) but higher in wood (25–30%) and highest in needles (30–40%). This leads to the conclusion that the bulk of TNTeq is non-extractable bound in root tissue, and only very low amounts of metabolites are translocated to above-ground tree parts. This interpretation is confirmed by radio TLC analysis which indicates that extractable TNTeq residual portions contain neither TNT nor known metabolites (e.g. ADNTs, DANTs), but only very polar (unknown) compounds. In contrast to TNTeq, extractability of RDXeq is very high when applying acetic acid as extractant. It reaches 80% or more in almost every tree compartment. High extractability of RDXeq in Pinus and Picea obviously causes good mobility via the transpiration stream. Moreover, in all tissue extracts RDX itself is the predominating compound. This low degree of RDX metabolisation seems to be a prerequisite for mobility and accumulation of RDXeq in above-ground tree compartments.

CONCLUSIONS

Conifers are excellent helper components to reduce the content of TNT and RDX in soils, and they contribute as a remarkable sanitation potential. This dendroremediation potential opens a wide range of future sustainable sanitation possibilities for explosive-contaminated areas.

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

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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 μ m 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.

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

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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*).

Species	Ecolo	ogical	para	meters								
		А			С			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

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

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

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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 ($60 \text{ cm} \times 90 \text{ cm}$). 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

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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.

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
<i>Oulema</i> 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 1. Occurrence of the more important pests of crops in Poland.

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

Pest/disease	Crops infected	Crops infected	Long-term	Regions of
	in 2006 (%)	in 2005 (%)	average (%)	greatest
				occurrence
Blumeria graminis o	22.3	22.6	27.1	north, locally
winter wheat				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	5.3	7.0	15.6	south-east
destructor				
Phoma lingam	2.5	3.4	4.4	north, north-east
Pseudomonas	9.7	5.4	14.9	north-west, central
lachrymans				
Phytophthora	17.6	27	48.9	south-east
infestans				
Phytophthora infestat	7.9	9.4	18.0	central
on tomato				
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

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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.

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

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

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

Date of	Developmental		Sośnicowice	Remarks
observation	stage	(Wielkopolska)	(Śląsk))	
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	21 larvae/100 stem		Winna Góra – number of
	hatching			larvae 4 mm long below
	-			threshold - treatment not
				recommended
10 June	mass of eggs			Sośnicowice – see above
	hatched			

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

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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 ($\emptyset = 3.7$ 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*

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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).

Fungicide	Wild-type EC ₉₀ (µg ml ⁻¹)		Relative growth at 10 μ g ml ⁻¹							
				P. inf	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		

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

^{*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.

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Workshop

Chairman: Volker Schick Unilever, Hamburg, Germany

The Concept of Best Agricultural Practice

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INTRODUCTION

Since 2003, the concept of *Good* Agricultural Practice (GAP) is being implemented in EU policies and legislations following the so called CAP reform (Common Agricultural Practice). Basicly, the CAP reform is geared towards the framework of GAP of the FAO (FAO, www.fao.org/ag). The vision of common agricultural practice policy is starting to being realized by the political instrument of compulsatory cross compliance (Council Regulation No. 1259/1999; Council regulation No 1782/2003 and Commission Regulation EC No 796/2004), directing agricultural practices by financial incentives.

Because of the increasing political regulation of agricultural practices using the term "Good Agricultural Practice" as a defined operating instrument for policies on EU and UN level, since 2004 the term "Best Agricultural Practice" (BAP) arises (e.g. www.undp.org/gef). BAPs in that sense summarize criteria for agricultural practices which are not yet politically regulated and, therefore, are often used simply as synonym for "Good Agricultural Practice" because they reflect common practices for special cases (see www.undp.org/gef).

We propose an integrative concept of existing terms (and underlying approaches) to ease communication between all stakeholders concerned with agricultural practices. It bases on scaled production quality, takes into account the importance of increasing sustainability and defines the role of science, administration, industry, trade and producer of agricultural goods. and fits into the goals of Agenda 21.

PRODUCTION QUALITY

It is common knowledge that agricultural practices influence the interest of several groups along the food chain including the producer with his employees, producer associations, individual retailers, retailer organizations, supply-chain driven systems, industry and – last not least the consumers. Their specific interests can be summarized in three main fields: societal demands, environmental demands and economic demands. Consequently, Meier (2002) asked for "social, environmental and economic compatibility" of production systems with "cultural compatibility as the central dimension of sustainable development".

Societal demands (including e.g. social, sociological or cultural components), environmental demands (including aspects of e.g. soil, water, air, biodiversity or landscape protection) and economic demands (including e.g. healthy food for all and not restricted to those who can pay high prices) are in direct relationship to each other like the length of the sides of a triangle. High prices influence societal demands, societal demands may lead to high prices, both may negatively or positively influence environmental demands and vice versa.

On that background, it is easy to demonstrate how those fields of demands influence sustainability: a circle within the triangle touching each side in one point differs in size when the length of the triangle sides changes. The size of the circle serves as a measure for

sustainability. In case of satisfaction of all demands an equilibrated, equilateral triangle develops with momentary optimal sustainability.

Basing on the requirements following from Agenda 21 the term "production quality" can be defined as "combination of factors resulting in a certain value of sustainability of a specific product chain". For the quantification of sustainability already several indicators are available (see e.g. www.sustainabilityindicators.org). A very easy measure for sustainability in practice is the length of the time period of unchanged use of a certain level of production quality.

Because of the determination of sustainability by the cited sets of demands, logically, the search for higher sustainability is passing lack of equilibration between the fields of demands demonstrating the fields of necessary actions.

GAPs fix a certain degree of production quality with a certain value of sustainability. GAPs evaluate and, mostly, certify a specific combination of production factors by more or less extended catalogues of criteria (overview see www.fao.org). Certification following defined standards is or recently becomes characteristic of GAPs: transparency of production, reliability of producer, trade and retailer, cross compliance of production techniques are the main goals behind the introduction of standards which should lead to traceability of agricultural production. GAPs may initiate improvements to production techniques and to supply chain infrastructure (e.g. processing, storage, transportation) but they are more and more fixed regulatory standards and policy instruments. They leave behind the not or only partially legalized concepts of Good Farming Practice (GFP), Good Plant Protection Practice (GPP), Integrated Agriculture, Integrated Production (IP), Integrated Farming Systems, Integrated Crop Management (ICM) and Integrated Pest Management (IPM). These concepts will have important function as part of "codes of conduct" in the future.

THE BAP CONCEPT

The concept of Best Agricultural Practice (BAP) bases on the directed disequilibrium between the sides of the outlined triangle of economical, ecological and societal demands.

The driving force is the expressed wish to overcome changed external or internal demands and the introduction of a continual improvement system. *Audit* followed by search for possibilities of *improvement*, conceptualised in concrete *plans* and final implementation move the circle of amelioration of production systems up to momentary best practices with momentary equilibrium of satisfied demands.

BAPs have the character of models first and can become common agricultural practice after being evaluated as advantageous for the production system. BAPs demonstrate and exemplarily realize visions. Important character of BAP is the use of Best Available Techniques (BAT).

The actors of the optimisation process are manifold: stakeholders from economy, ecology and society formulate questions and modify demands. Research looks for answers. Authorities evaluate the whole process, often co-ordinate it and integrate new developments to standards supported by consultation. The mutual interaction between all stakeholders finally leads to increased sustainability of the production system.

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Criteria-based and value-oriented agricultural practice in crop-growing companies and its societal benefit

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INTRODUCTION

The voluntary introduction of environment management systems – as well as ISO EN 14001 (1996), the Eco-Audit of the EU (Regulation EC 761/2001), the social management system SA 8000, and the development of practical criteria for the assessment of companies in view to their ecological and social competence and performance – were the results of the 'Conference for environment and development' in Rio de Janeiro in 1992 and the connected and guiding principles of the Rio-declaration within the framework of Agenda 21. The guiding principles of a globally sustainable development in this Agenda apply to the relationships between humans and nature, and (equally) to the relationships between individual societies. Although ecologically oriented, integrated plant cultivation in agriculture has been discussed for many years and corresponding results have already (partly) been achieved in practice. Further, international discussions (in commercial industries and in food trading) are expanding towards including voluntary assessment and evaluation of social and, lately, cultural standards. The aim is an evaluation of the extensive social performance of a company, according to ethical-ecological criteria. The 'Co-operative Pahren' (in Germany) and the 'Flor-Verde-Programme' (in Columbia) are examples.

ETHICAL-ECOLOGICAL CRITERIA

In comparison with already existing systems, and also in regard to adaptability in agricultural enterprises, the Guideline Frankfurt-Hohenheim (FHL) of Hoffmann *et al.*, (1997) has proved particularly helpful. This is, at present, the most extensive 'criteria catalogue' for management according to ethical-ecological standards.

The voluntary integration of environment management systems, and the use of ethical-ecological criteria in the production, trade and service industries is gaining increasing significance in manufacturing. On a voluntary basis, internationally rating agencies and certification organizations assess ethical-ecological performance and supervise the adherence to principles and criteria.

The search for a peaceful solution to conflicts requires new forms of dialogue between the various interest groups, who currently oppose one another in ecological and socio-economical areas. Economic ethics and, correspondingly, agricultural ethics, are guided by the idea that the actions of all persons involved in the market economy and in the design of legal-political outline conditions should be oriented towards ethical principles. This includes the responsibility to preserve nature, and to respect human dignity and the life of future generations. Therefore, economic ethics are, according to Kersting (1994), an area where the strategic rationality – characteristic of modern economy – meets ethical reason.

The most substantial and, up to now, most precise and subtle catalogue of criteria (the FHL), which claims to contain a complete description of potentially relevant aspects required for an ethical assessment system, was developed by Hoffmann *et al.*, (1997) on behalf of German banks. Thus, on the basis of epistemological considerations (together with practical possible applications) they developed 'ethical-ecological criteria'. FHL focuses on entire society-related company results, by taking the cultural compatibility of actions into consideration, as well as considering environmental and social compatibility. Apart from ecological and social dimensions, the cultural dimension was also introduced to the discussion on ethical conduct.

In the meantime, the ethical-ecological assessment model became established in economic practice, and is increasingly used by nationally (Kohlhof et al., 2006) and internationally operating companies which employ ranking methods (www.oekom research).

The cultural dimension of FHL was added to the entrepreneurial efficiency evaluation because (for the design and establishment of ecologically compatible and socially benign products, production processes and innovations, and for the development of techniques) knowledge about the social order of civilizations is of major importance. Moreover, the FHL assumes that the capability of a society to solve its occurring social and ecological problems depends on the knowledge about the order of civilizations, which is based on traditions and conscience. Economic actions have to rely on the continued existence of this knowledge of order. However, they threaten it simultaneously every time economic and commercial forms of thought are set in an absolute way. As a result, they affect non-commercial areas of life (Hoffmann *et al.*, 1997).

Under the condition that economic actions have to rely on the continued existence of knowledge about order, threatening it at the same time, there is the possibility to supervise and assess companies as to their ethical-ecological performance, by requiring a guaranteed commitment to moral standards (Wieland, 1993).

Ecologically and socially compatible actions are always related to economic compatibility of commercial entrepreneurial activities. Our examples (Co-operative Pahren and Flor Verde Programme) fit ideally into this scheme. The activities also form part of the knowledge about the order of civilizations. In this respect, cultural compatibility of ethical-ecological actions can be seen as the central dimension in the field of tension (called 'sustainable development') and are the basis for descriptions of Best Management Practice guidelines.

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