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/m^2) 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/m^2. 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 CO_2 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.

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

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		normal	conditional	abnormal	
Experiment 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
Experiment 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

*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.

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

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

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* МГ-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).

Table 1. Influence of biopreparations on seedlings of *Picea obovata*.

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 *Streptomyces lateritius*	9,650	6,280
trichodermin-c + 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.

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

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

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).

Table 1. Plant protection indicators within the REPRO concept.

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

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 deltamethirn 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 ß-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 into 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.

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