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.

- Florez J A; Guzmán M A; Ricketts T H; Vandame R (2006). Café y abejas: Conservación y producción. In: *El cafetal del futuro: Realidades y Visiones*, eds J Pohlan, L Soto & J Barrera, pp 299-331. Aachen, Shaker Verlag.
- Gamboa W; Pohlan J (1997). La importancia de las malezas en una agricultura sostenible del trópico. *Der Tropenlandwirt / Beiträge* **98**, 117-123.
- Pérez Romero A; Pohlan J (2004). Practicas de cosecha y poscosecha del Rambután, en el Soconusco, Chiapas, México. *LEISA, Revista de Agroecología*, diciembre 2004, **20** (3), 24-26.
- Pohlan J (2002). Begleitwirkungen von Unkräutern im Ökosystem Kaffee in Chiapas, Mexiko. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, Sonderheft XVIII, 175-182.
- Pohlan J; Borgman J; Eiszner H (1997). Potentials of sustainable agricultural systems in tropical hill regions of Central America. *Plant Research and Development* **45**, 51-60
- Vanderlinden E J M; Pohlan H A J; Janssens M J J (2004). Culture and fruit quality of rambutan (*Nephelium lappaceum* L.) in the Soconusco region, Chiapas, Mexico. Fruits, 59 (5), 339-350.

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.

REFERENCES

Matyjaszczyk E (2006). Registration of Plant Protection Products in Poland: Feedback on Problems and Experiences to Date. *The 13th International Conference on Registration of Agrochemicals in Europe 23-24 May 2006, Brussels.*

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.

REFERENCES

Iljinskaja L I; Vasjukova NI; Ozerechkovskaya O L (1991). Biochemical aspects of induced resistance and a susceptibility of plants. *Results of a science and technics (technical equipment). A series: Protection of plants. M. : VINITI*, pp. 4-71.

Levitsky A P (1979). Extraction methods of trypsin inhibitor from seed cereal crops. Biochemical Methods of Breeding Material Research 15, 73-77.

Lutsik M; Panasjuk E; Lutcik A (1981). Lectins. Vusha shkola: Lvov.

Raskin I (1992). Salicylate a new plant hormone. Plant Physiology 99, 799-803.

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).

REFERENCES

- Pavlek P (1978). Brassicae oleraceae cabbage plants. Center for the studies of Mediterranean agriculture, Zagreb-Split (in Serbian).
- Pajović I (2005). Some interesting facts about growing traditional cultivars of rashtan (*Brassica* oleracea var acephala) in Montenegro. Savremena poljoprivreda **3-4**, 449-455.
- Sivčev I (1981). Biological monitoring of *Mamestra brassicae* L. (Lepidoptera, Noctuidae) and role of light traps in monitoring of population density. University of Belgrade, Agriculture faculty, Beograd-Zemun. (In Serbian).

Southwood T R E (1977). Ecological Methods. Chapman and Hall: London.

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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- Dorner J W; Cole R J; Sanders T H; Blankenship P D (1989). Interrelationship of kernel water activity, soil temperature, maturity and phytoalexin production in pre-harvest aflatoxin contamination of drought-stressed peanuts. *Mycopathologia* **105**, 117-128.
- Griffin G J; Garren K H (1974). Population levels of *Aspergillus flavus* and the *A. niger* group in Virginia peanut field soils. *Phytopathology* **64**, 322-325.
- Mehan V K; Mayee C D; Jayanthi S; McDonald D (1991). Preharvest seed infection by *Aspergillus flavus* group fungi and subsequent aflatoxin contamination in groundnuts in relation to soil types. *Plant and Soil* **136**, 239-248.
- Sanders T H Cole R J; Blankenship P D; Hill R A (1985). Relation of environmental stress duration to *Aspergillus flavus* invasion and aflatoxin production in pre-harvest peanuts. *Peanut Science* **12**, 90-93.

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 as substrate were less resistant to insects compared with ecotypes containing methylsulfinyl 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.

- Agrawal A A; Kurashige N S (2003). A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae. Journal of Chemical Ecology* **29**, 1403-1415.
- Raybould A F; Moyes C L (2001). The ecological genetics of aliphatic glucosinolates. *Heredity* **87**, 383-391.
- Renwick J A A (2002). The chemical world of crucivores: lures, treats and traps. *Entomologia Experimentalis at Applicata* **104**, 35-42.
- Rodman J E; Karol K G; Price R A; Sytsma K J (1996). Molecules, morphology, and dahlgren's expanded order Capparales. *Systematic Botany* **21**, 289–307.
- Wittstock U; Kliebenstein D J; Lambrix V M; Reichelt M; Gershenzon J (2003). Glucosinolate hydrolysis and its impact on generalist and specialist insect herbivores. In: J T Romeo (ed.), *Integrative phytochemistry: from ethnobotany to molecular ecology*, pp. 101–125. Amsterdam: Elsevier.

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.

- Schoenmuth B W; Pestemer W (2004). Dendroremediation of trinitrotoluene (TNT). Part 2: Fate of radio-labelled TNT in trees. *Environmental Science & Pollution Research* 11, 331-339.
- Schröder P; Fischer C; Debus R; Wenzel A (2003). Reaction of detoxification mechanisms in suspension cultured spruce cells (*Picea abies* L. KARST.) to heavy metals in pure mixture and in soil eluates. *Environmental Science & Pollution Research* 10, 225-234.
- Talmage S S; Opresko D M; Maxwell C J; Welsh C J E; Cretalla M F; Reno P H; Daniel F B (1999). Nitroaromatic munition compounds: Environmental effects and screening values. *Review of Environmental Contamination & Toxicology* 161, 1-156.