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Breeding for Plant Resistance to Pests and Diseases

editors:

A. Nicholas E. Birch and Bogumil Leszczynski

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IOBC / WPRS

Working Group "Breeding for Plant Resistance to Pests and Diseases"

Proceedings of the meeting

Breeding for Resistance to Pests and Diseases

at

Bialowieza, Poland

15 – 19 September, 2004

Editors:

A. Nicholas E. Birch and Bogumil Leszczynski

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Preface

Convenor: Dr A.N.E. Birch
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Deputy Convenor and Local Organiser: Dr Bogumil Leszczynski
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Dr Iwona Łukasik
Dr Agnieszka Wójcicka
Sylvia Golawska
Robert Krzyżanowski
Henryk Matok
Teresa Piesio

Background to the WG:

The 10th meeting of the Breeding for Resistance to Pests and Diseases WG was held at Bialowieza, Poland The meeting was attended by 45 people (double the attendance at the last WG meeting in December 2001), including 13 entomologists, 8 biochemists, 8 plant physiologists, 6 molecular biologists, 1 plant geneticist, 2 phytochemists, 2 plant breeders and 5 other persons.

General discussion in the closing session

It was provisionally proposed to hold the next working group meeting in Trentino, Italy. This still has to be agreed with local organizers and then confirmed to the WG.

Dr Bogumil Leszczynski has kindly agreed to act as Deputy Convenor until a new Convenor and Deputy can be formally elected.

The Polish Organizing Committee were warmly thanked by the Convenor and all participants for their excellent organization of a very enjoyable scientific and social working group programme.

Contents

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PROGRAMME

10th meeting of the IOBC/WPRS working group on 'Breeding for Resistance to Pests and Diseases'.

Bialowieza, Poland
15-19 September 2004

Session timetable

Wednesday 15 September 2004

Travel to Bialowieza (accommodation in "Bialowieski Hotel", Waszkiewicza 218 B Str. tel. +48 85 7444380; FAX +48 85 7444534;
e-mail: incoming@hotel.bialowieza.pl)

15.00 – 19.30 Registration

20.00 Welcome Reception

Thursday 16 September

7.30 – 8.30 Breakfast

8.00 – 9.00 Registration

9.00 – 9.30 Welcome & Opening (*A.N.E. Birch, B. Leszczynski*)

9.30 - 10.00 Studies on plant resistance to nematode and arthropod pests in Poland – A historical perspective (Z. T. Dąbrowski - Poland) – plenary lecture

10.00 –10.30 Coffee Break

SESSION I (Chair: Z.T. Dąbrowski)

10.30 – 11.00 Constitutive and expressed gene interactions between the Russian wheat aphid, *Diuraphis noxia* Mordvilko and aphid resistant wheat plants (C. M. Smith, E. V. Boyko, Y. Deng, S. Starkey, P. Voothuluru (USA)

11.00 – 11.30 Breeding for resistance to the large raspberry aphid: An update on durability of current genes and future prospects (A.N.E. Birch, S.C. Gordon, R. Brennan, A.T. Jones – United Kingdom)

11.30 – 12.00 Apple tree resistance against codling moth (*Cydia pomonella*) Lepidoptera: Tortricidae egg laying due to plant surface metabolites (Lombarkia N., Derridj S.) (France)

12.00 – 12.20 Discussion

12.30 – 13.30 Lunch

13.30 – 15.00 POSTER SESSION

15.00 - Visit to Bialowieski National Park Museum and Bison Range

20.00 Conference Dinner with traditional singing and dancing display

Friday 17 September

7.30 – 8.30 Breakfast

SESSION II (Chair: M. Smith)

9.00 – 9.25 Mechanisms involved in induced resistance with extracts of *Reynoutria sachalinensis* (A. Schmitt – Germany)

9.25 – 9.50 Effect of natural monoterpenes on the behaviour of the peach potato aphid *Myzus persicae* (Sulz.) (B. Gabryś, K. Dancewicz, E. Janusz - Poland)

9.50 – 10.15 Role of jasmonates in defence reactions in plants under biotic stresses (M. Saniewski, A. Saniewska, H. Urbanek – Poland)

10.15 – 10.30 Discussion

10.30 –11.00 Coffee Break

SESSION III (Chair: S. Derridj)

11.00 – 11.25 Saponin as a source of alfalfa resistance towards pea aphid *Acyrthosiphon pisum* Harris (Leszczynski B.,Golawska S., Oleszek W. – Poland)

11.25 – 11.50 Assessment of partial resistance to anthracnose in water yam (*Discorea alata*) using tissue culture generated whole plants (J. Onyeka – Guadeloupe)

11.50 – 12.15 Full modification of coding sequence for enhancing potato expression of insect control protein Cry3a gene and prediction of its expression in plant using east transformation (G.R. Salehi Jozani, I.V. Goldenkova, E.S. Piruzian – Russia)

12.15 – 12.40 The art of making things simple. Insect resistance tests and their practical implementation (S. Sutterlin – The Netherlands)

12.40 – 12.55 Discussion

13.00 – 14.00 Lunch

14.30 - Visit to Białowieski National Park

19.30 Open fire with BBQ

Saturday 20 September

7.30 – 8.30 Breakfast

SESSION IV (Chair: M. Saniewski)

9.00 – 9.20 Some biochemical and physiological aspects of cucumber resistance to spider mites induced by plant growth promoting *Rhizobacteria.* (A. Tomczyk – Poland)

9.20 – 9.40 RAPD analysis of Russian and Polish isolates of *Sclerotinia sclerotiorum* from crucifers. (W. Irzykowski, V. Soldatova, E. Gasich, N. Razgulaeva, M. Jędryczka – Russia, Poland)

9.40 – 10.00 Molecular level of potato resistance to Colorado beetle and pathogens. (J. Szafranek, Poland)

10.00 – 10.20 Prospects of native entomogenous fungus *Metarhizum anisopliae* var major for integrated control of termite pests of tea in North East India. (S. Debnath – India)

10.20 – 10.40 Effects of host plant on infection of aphids by the fungus *Pandora neoaphidis.* P.A. Shah, C. Tkaczuk, S.J. Clark and J.K. Pell (United Kingdom, Poland)

10.40 –10.55 Discussion

10.55 – 11.15 Coffee Break

11.15 – 11.40 Pest resistant GM crops: A chemical ecology viewpoint. (A.N.E. Birch, United Kingdom)

11.40 – 12.05 Breeding for resistance: An option not only for growers and industry, but also for policy markers. (S. Sutterlin – The Netherlands)

12.05 – 13.00 Summing up the Poster Session and Closing Remarks (A.N.E. Birch & B. Leszczynski)

13.00 – 14.00 Lunch

14.30 – Post conference tour (10-15 EURO per person, according to number of participants)

20.00 Dinner

Sunday 19 September

6.30 - 7.30 Breakfast

7.45 - Departure for Warsaw

Presentations

Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.3 - 9

Studies on plant resistance to nematode and arthropod pests in Poland: A historical perspective

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Abstract: The paper reviews development of modern research activities on plant resistance to nematode and arthropod pests in the last 35 years in Poland. Since the 1970's the inter-institutional and inter-discipline team research has concentrated on selection and mechanisms of moderate level of resistance. The research started with behavioural, physiological and biochemical relationships between host plants and pest species. Later various plant properties (morphological, anatomical and biochemical) responsible for constitutive resistance were identified in a number of crop plants; finally has included factors involved in the induced resistance in tomato, apple and cereals. Three research groups has already initiated studies on molecular basis of plant resistance to nematode and arthropod pests. The active groups of Polish scientists in various academic and research institutes form a critical mass, having knowledge and experience to be involved in any EU project on resistance selection and breeding as well as on mechanisms of constitutive and induced resistance.

Keywords: Plant resistance to cyst nematode, cultivar resistance to spider mites, cereal resistance to aphids, resistance screening to insects, induced resistance.

Introduction

First advanced studies on plant resistance to nematode and arthropod pests have in Poland been started in the late 1960's by the staff of Institute of Plant Protection (IPP), Poznań, where a group of nematologists examined potato plant resistance to the potato cyst nematode (*Globodera rostochiensis* Woll.). Another group of entomologists and chemists started experiments on biochemical mechanisms of alfalfa and lupine resistance to the pea aphid [*Acyrthosipphon pisum* (Harris)]. Four techniques were introduced in their experiments:

1. Confirmation of field resistance under greenhouse bioassays by measuring fecundity and survival of individually caged aphids on single plants;
2. Using P^{32} to measure food intake by aphids on resistant (R) and susceptible (S) varieties;
3. Infiltration of sap taken from R and S varieties to prove their biochemical effect on the aphid feeding activity and survival;
4. Treatment of S varieties with 5 different alkaloids to demonstrate their toxic effect on the aphid survival (Węgorek & Czaplicki 1969).

Rapid expansion of research projects on resistance screening and mechanisms of varietal resistance to arthropod pests in Poland took place in the 1970-ties through international exchange of methodology and techniques as well as advanced training of Polish scientists conducted abroad. Achievements in plant resistance breeding in wheat, maize and alfaalfa in the USA presented in the books of Painter (1968) and Maxwell & Jennings (1980) were especially stimulating.

The results from research on plant resistance to arthropod pests were regularly presented by Polish scientists on international forum since the 3rd international meeting on Host plant –

insect relationships held in Tihany (Hungary) (3 persons). Two other scientists participated in the 5th meeting in 1982 in Wageningen (The Netherlands), 6 in the 7th meeting in Budapest (Hungary), and 2 to 3 persons in the 8 to 10th meetings. Poland was represented by three institutions in the 1st meeting of the EUCARPIA/IOBC Working Group "Breeding for Resistance to Insects and Mites", 1976, Wageningen (IOBC, 1977) among 61 participants from 16 countries. Interdisciplinary and inter-institutional research projects established for the execution of research projects on plant resistance in the 1970-ties in the USA were used as a model in Poland. The first such programme was implemented by entomologists from the Department of Applied Entomology, Warsaw Agricultural University (WAU) and biochemists from Agricultural and Teacher College (now Academy of Podlasie - AP), Siedlce (Niraz & Dabrowski 1990). A number of M.Sc. and Ph.D. theses were prepared in both institutions on screening for resistance and on biochemical and physiological relationships between resistant and susceptible cultivars of strawberry, apple and cucumber to spider mites (Acarina), and cereals to aphids. Their approach in the identification of plant properties responsible for moderate level of cultivar resistance has basely differed from the majority of projects carried out at that time in leading world research centers (USA, IRRI), which concentrated mainly on high level of resistance.

Plant resistance to nematodes (Nematoda)

Anatomical factors involved in the potato plant root tissues response (local lignin deposition in the induced cells) to the infestation by the potato cyst nematode (*G. rostochiensis*) had initiated detailed biochemical studies by biochemists of IPP (Poznań). The differences in interaction between β-glucosidase secreted by nematode larvae and a non-active form of plant glycosides affecting IAA-oxidase level in infected cells had been identified as the susceptible-resistant plant reaction to nematode infestation (Giebel 1970). Identification of resistance mechanisms and a breeding programme have resulted in the release by Institute of Plant Breeding and Acclimatization (IPBA) of new potato cultivars resistant to the pathotype Ro 1 of *G. rostochiensis*, meeting various consumer and industry preferences. Eight resistant cultivars were registered in the 1970-ties (6% of the total registered cultivars) and 46 (36 for food use and 12 for industrial use) in the 1990's (47% of the total).

Another group of nematologists and agronomist from Institute of Pomology and Floriculture based in the Brzezna Experimental Station (southern Poland) screened 25 - 33 strawberry cultivars for resistance to a number of pest species such as: *Meloidogyne hapla, Aphelenchoides fragariae and A. ritzemabosi, Longidorus elongatus, Pratylenchus penetrans.* Some preliminary studies had confirmed the role of phenolic compounds in the resistance (Szczygieł & Danek 1984).

The molecular basis of syncytia formation induced by root infection by various species of cyst nematode genera *Globodera* and *Heterodera* in resistant and susceptible host plants are presently under investigation by a team of botanists lead by Prof. W. Golinowski, WAU. The same group of scientists is also involved among 8 institutions from 7 countries in the European Union involved in the project on "Durable resistance against *Meloidogyne chitwoodi* and *M. fallax*" (QLRT5-1999-1462). The following studies are carried out in cooperation with the WAU Department of Plant Genetic, Breeding and Biotechnology: (a) interaction between plants and nematodes at cytological level; (b) the presence of virulence genes, their genetics and host specificity populations of in populations of both nematode species (e.g. Golinowski *et al.*, 1997; Grymaszewska & Golinowski 1998; Golinowski *et al.*, 2003).

Plant resistance to spider mites (Tetranychidae)

Basic research on selection and acceptance of host plants (using olfactometers and artificial diets with ^{32}P) by *Tetranychus urticae,* a field and screenhouse screening of strawberry, cucumber and rose cultivars and related wild species resulted in identification of resistant germplasm used by biochemists to identify preliminary and secondary plant compounds affecting host nutritional suitability or antibiosis to *T. urticae* (Dabrowski & Rodriguez 1972; Rodriguez *et. al.,* 1976; Dąbrowski & Bielak 1977).

Using large collection of crosses between old German and Polish apple cultivars with Russian cultivars we were at WAU able to select a cross resistant to the apple scrab and *Panonychus ulmi* and to relate the differences in the mite development and fecundity to the leaf morphology, histology and chemical composition of various apple cultivars (Bielak & Dabrowski 1985). The studies on apple susceptibility (inluding scab resistant cultivars) to other species of acari as: *Eotetranychus uncatus* Garman, *Tetranychus vienensis* Zacher, *Tetranychus urticae* Koch, *Aculus schlechtendali* (Nalepa) were continued by the staff of IPP (Kozłowski 1998; Skorupska 1993, 2003). The staff of Institute of Pomology and Floriculture (IP&F), Skierniewice in their recent studies on morphological and anatomical characters of apple leaves associated with cultivar susceptibility to *Tetranychus urticae* Koch used a digital camera and a computer program for image analysis of leaf damage (Warabieda *et al.,* 1997; Warabieda & Olszak 2003).

Regular screening of existing and newly introduced strawberry and raspberry cultivars under natural field infestation for susceptibility to *T. urticae* have been lead by Łabanowska of IP&F (Fabanowska & Pala 1986; Łabanowska & Bielenin 2002).

Another approach to research on plant tolerance and compensation mechanisms to spider mite infestation had been initiated by a team of acarologists and plant physiologists at WAU in the middle of the 1970-ties by a series of projects on the physiological response of infested and damaged susceptible/resistant cultivars of chrysanthemum and strawberry plants. Carbon metabolism in photosynthesis by using ^{14}C showed important quantitative changes in the synthesis of the primary assimilation products such as: soluble sugars, amino acids, organic acids and starch in chrysanthemum leaves in the initial and later stages of plant infestation by *T. urticae* (Tomczyk & Kropczyńska 1985). Biochemical and cytological studies on strawberry cultivars damaged by *T. urticae* had demonstrated the involvement of some constitutive chemicals of primary and secondary metabolism in the interaction between mites and plant cultivars, e.g. phenols (Kiełkiewicz 1988). Recent achievements in identification of factors responsible for induced resistance in some selected cultivars of host plants to spider mites are the results of many years of team work in this area (Tomczyk 1989; Kiełkiewicz 2003; Warabieda *et al.,* 2003; Puchalska 2003).

Plant resistance to insects

Extensive research by the entomologists of WAU and plant breeders of IPBA on cereal species/cultivars resistance to the cereal aphids, first concentrated on germplasm selection had built the foundations for extensive research projects (internationally recognized) on biochemical bases of cereal resistance to aphids by the team of UP biochemists (Niraz & Dabrowski 1990). Field screening for resistance had included 1429 spring wheat cultivars and breeding lines, 4232 -winter wheat, 2350 – rye, 2300 – barley. Nutritional factors and secondary metabolites as the biochemical factors responsible for cereal aphid resistance in wheat were identified and published in numerous papers and summarized in two theses for doctorate level degrees - Leszczyński (1987) and Ciepiela (1990). Enzymatic defense of the

grain aphid and the bird cherry oat aphid towards cereal secondary metabolits had been extensively studied by Urbańska (Urbanska *et al.*, 2004). The impressive presentation of papers and posters by the UP group during the 2004 Bialowieza meeting confirms the dynamic and creative approach in their biochemical studies on the host plant – insect pest interactions.

A number of projects had been carried out in various reseach institutions on crop plant resistance to insect pests such as: (a) maize to the European corn borer (*Ostrinia nubilalis* Hübn. by Prof. Cz. Kania (Agricultural Academy, Wrocław) under a programme internationally coordinated by dr H.C. Chiang (1974); (b) pea to the pea weevil (*Sitona lineatus* (L.) and white clover to *Sitona hispidulus* (F.) (Wnuk & Wiech 1980; Wiech & Wnuk 1985); (c) sugar beet to the leaf miner *Peogmyja hyoaciami* Panz. (Łuczak 1986); (d) grain and legume seeds to the stored product pests under the leadership of Prof. D. Ciepieleska at the Warmia-Mazurien University (e.g. Ciepielewska & Fornal 1994); and especially by the interdisciplinary team of entomologists of IPP (lead by Prof. J. Nawrot)) and chemists lead by Prof. J. Szafranek of the Gdansk University (e.g. Warchalewski & Nawrot 1993; Szafranek et al. in this volume).

Summary

Research on plant resistance to nematode and arthropod pests is well established in Poland since the 1970-ties. Two academic text books (Dabrowski 1976 and 1988; Leszczyński 1996) are being used for advanced courses on "Plant resistance to pests" and "Breeding resistance to stresses" for M.Sc students in "Plant protection" and "Plant breeding". Additional information on the methods, techniques and status of research on resistance in Poland had been published in the proceedings of the 2-nd National Symposium on "Plant resistance to diseases, pests and unfavorable environmental factors", organized in 1995 by the Institute of Plant Breeding and Acclimatization, Radzików, 12 – 14 September 1995 (Arseniuk *et al.*, 1995).

The major achievements in these studies were related to morphological, anatomical and biochemical factors involved in the moderate level of varietal resistance to pests as a part of integrated pest management approach. The research started first on constitutional resistance to evolve into studies on the biochemical and physiological mechanisms involved in induced plant resistance to pests. Two scientists have represented Poland in the first international conference on induced resistance (Tomczyk 2004; Kiełkiewicz – Szaniawska 2004) as three others had participated in the past and presented two papers during the 1st EUCARPIA/IOBC Working Group meeting – "Breeding for Resistance to Insects and Mites" held in Wageningen, 7 –9 December 1976 (Kowalewski & Robinson 1977; Dabrowski & Bielak 1977). Presently two research groups (WAU and AP) have upgraded the studies to the molecular level.

The European Union's present emphasis on breeding pest resistant cultivars for ecological agriculture/horticulture should invigorate breeding and screening programmes also in Poland. The Polish breeders and plant protection specialists are well aware of methods and techniques used in resistance screening and breeding.

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Differential Expression of Genes in Wheat, *Triticum aestivum* L. Controlling Resistance to the Russian Wheat Aphid, *Diuraphis noxia* (Mordvilko)

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Abstract: The Russian wheat aphid (RWA), *Diuraphis noxia* Mordvilko, is a major economic pest of wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., in all continents except Australia. Several genes controlling *D. noxia* resistance have been identified and aphid resistant cultivars have proven to be a viable tactic for *D. noxia* management. Experiments with near isogenic lines, segregating F_2 populations and wheat microsatellite markers have shown that numerous *D. noxia (Dn)* resistance genes are inherited as single, dominant traits located on wheat chromosomes 1D or 7D. *Dn1, Dn2, Dn5, Dn6* and *Dnx* occur in a cluster on the short arm of wheat chromosome 7DS. *Dnx* is involved in *D. noxia* recognition and defense response elicitation in wheat. *Dn4* and *Dn9* are also single dominant genes and are located in defense gene-rich regions on the short arm *(Dn4)* and the long arm *(Dn9)* of wheat chromosome 1D, respectively. Since the beginning of *Dn* gene deployment, several *D. noxia* populations have been identified that are virulent to *Dn4.* A population from the U. S. central plains is virulent to all known *Dn* genes except *Dn7.* In the current study, plants expressing *Dn4* and *Dn6* were evaluated for plant tolerance of aphid-induced chlorophyll loss and antibiosis (reduced aphid population development). Both sources of resistance exhibited both antibiosis and tolerance. Measurement of leaf chlorophyll loss was a less accurate indicator of tolerance than proportional dry weight loss measurements, because of innate chlorophyll content differences in the two *D. noxia* resistant wheat genotypes. The data for *Dn6* antibiosis and tolerance are the first reports of either resistance category existing for plants expressing this *Dn* gene.

Keywords: plant resistance, gene mapping, *Triticum aestivum,* wheat, Russian wheat aphid, *Diuraphis noxia* Mordvilko, virulence, antibiosis, tolerance

Introduction

Aphids (Order Homoptera) are major pests of world agriculture, damaging crops by removing photoassimilates and vectoring numerous devastating plant viruses. Many pest aphid species, along with several hundred other insect pests, are resistant to insecticides (Devonshire & Field 1991). These arthropod traits have necessitated the development of insect resistant crop cultivars. However, arthropods have been equally resilient to plant resistance genes, developing biotypes in over 20 species of pest arthropods (Smith 1999).
A recent and significant example is the Russian wheat aphid, *Diuraphs noxia* Mordvilko, which has spread globally to become a major pest of wheat and barley production in Central Asia, Europe, North America and South America since 1950.

D. noxia is indigenous to Central Asia (Hewitt *et al.,* 1984) and was first discovered as a pest of barley in Russia and the Mediterranean region in 1900 and 1912 (Blackman & Eastop 1984).

Susceptible wheat plants react to injection of *D. noxia* saliva by rolling the leaves longitudinally around the main leaf vein, forming a tubular refuge that protects aphids

from predators. *D. noxia* feeding significantly reduces chlorophyll and carotenoid content in susceptible plants that develop white or purple longitudinal leaf streaks (Fouche *et al.,* 1984, Burd & Eloitt 1996, Reidell & Blackmer 1999, Heng-Moss *et al.,* 2003). Chlorophyll reductions retard photosynthetic and chlorophyll fluorescence rates, photosynthetic efficiency and ultimately lower grain yield (Walters *et al.,* 1980). Yield losses of up to 80% are prevalent in susceptible commercial cultivars. In resistant commercial cultivars, yield losses of approximately 20% are routinely reported with severe infestation. El Nino-related drought conditions have exacerbated the severity of *D. noxia* damage to wheat production in North America. The cumulative losses to all U. S. small grain production due to *D. noxia* control, grain losses, and lost economic activity from 1986 to 1993 was valued at $850 million (Legg & Amosson 1993).

To date, about 30,000 accessions of wheat and related Triticeae have been evaluated for *D. noxia* resistance since 1987 (Souza 1998). Twelve genes from barley, rye, or wheat are known to confer *D. noxia* resistance (Table 1). The *Diuraphis noxia (Dn)* resistance genes *Dn1* and *Dn2* were identified in South Africa in the common wheat accessions PI 137739 *(Dn1)* and PI 262660 *(Dn1),* from Iran and Azerbaijan, respectively (du Toit 1987, 1988, 1989, Smith *et al.,* 1991). The recessive gene *dn5* is present in the *Aegilops tauschii* line SQ24, a parent in the amphiploid wheat derived from crosses between *Ae. tauschii* and *T. turgidum* (Nkongolo *et al.,* 1991a). *Dn5* was identified in Bulgarian wheat accession PI 294994 (du Toit 1987) and characterized by Saidi & Quick (1996) and Zhang *et al.,* (1998). *Dn4* and *Dn6* originated from Russian (PI372129) and Iranian (PI243781) bread wheats, respectively (Nkongolo *et al.,* 1989, 1991b, Saidi & Quick, 1996). *Dn7,* a rye gene, was transferred to chromosome IRS of the 1RS•1BL translocation in "Gamtoos" wheat (Marais & du Toit 1993, Marais *et al.,* 1994). *Dn8* and *Dn9* are co-expressed with *Dn5* in PI 294994 (Liu *et al.,* 2001). *Dnx* is from PI220127, a winter wheat accession from Afghanistan (Martin & Harvey 1997, Liu *et al.,* 2001). The chromosome location of *Dny,* an unnamed resistance gene in 'Stanton' is yet to be established. All *Dn* genes except *dn3* are inherited as dominant traits.

Table 1. Genes expressing resistance to the Russian wheat aphid, *Diuraphis noxia* Mordvilko.

Dn Gene	Crop	Plant Introduction	Geographic Origin	Chromosome Location
Dn1	Wheat	PI 137739	Iran	7DS
Dn2	Wheat	PI262660	Azerbaijan	7DS
dn3	*Ae tauschii*	Line SQ24	Iran	?
Dn4	Wheat	PI372129	Turkmenistan	1DS
Dn5	Wheat	PI294994	Bulgaria	7DS
Dn6	Wheat	PI243781	Iran	7DS
Dn7	Rye	Turkey	Turkey	IRS
Dn8	Wheat	PI294994	Bulgaria	7DS
Dn9	Wheat	PI294994	Bulgaria	7DS
Dnx	Wheat	PI220127	Afghanistan	7DS
Dny	Wheat	Lin-Yuan207	China	?
Dnz	Wheat	PI220350	Russia	7DS

Dn1, Dn2, Dn5, Dn6, Dnx and several uncharacterized Dn genes are tightly linked to microsatelite markers on the short arm of chromosome 7DS (Liu *et al.*, 2001, 2002, 2004). Both *Dn4* and an uncharacterized *Dn* gene in PI151918 are located on chromosome IDS and are also either linked or allelic (Liu *et al.*, 2001, 2004).

Dn8 and *Dn9* are linked to wheat microsatellite markers on the short and long arm of wheat chromosome 7D, respectively (Liu *et al.*, 2001). Results of Liu *et al.*, (2004) indicate that *Dn1, Dn2, Dn5, Dn6* and *Dnx* are a cluster of completely linked resistance genes or alleles on the short arm of wheat chromosome 7D. The arrangement of these genes is characteristic of the genome organization of disease resistance genes (Bergelson *et al.*, 2001, Boyko *et al.*, 2001), suggesting that a family of resistance genes is involved in *D. noxia* recognition and defense response elicitation in wheat. Boyko *et al.*, (2004a,b) have demonstrated that at least one member of this gene family, *Dnx*, is elicited by *D. noxia* feeding. Boyko *et al.*, (2004) used suppression subtractive hybridization (SSH) to construct a cDNA library containing both *D. noxia* and *Dnx* sequences. A *D. noxia*-derived elicitor of *Dnx* resistance is similar to three directly repeated sequences in *avrXa10*, an avirulence protein from *Xanthomonas oryzae* pv. *oryzae* that belongs to an *avrBs3* group of proteins including several avr proteins from *Pseudomonas syringae* pv. *tomato*. The structure-function relationship of this group of proteins suggests the possibility that these gene products are secreted into the plant by *D. noxia* and act to elicit *D. noxia* resistance in *Dnx* plants.

D. noxia resistant wheat cultivars are produced in the U.S. (Quick *et al.*, 1996, Souza *et al.*, 1997a,b, Martin *et al.*, 2001) and South Africa (Marais & duToit 1993, Marais et al. 1994, Prinsloo et al. 2000). Nevertheless, *D. noxia* biotypes have developed in response to *Dn* genes in Europe, North Africa, North America and South America (Table 2) (Bush *et al.*, 1989, Puterka *et al.*, 1992, Basky 2002, Haley *et al.*, 2004, Smith *et al.*, 2004).

Table 2. Reaction of *Dn (Dimaphis noxia)* resistance genes in wheat to feeding by populations of *D. noxia* from different geographic locations, V - virulent, R - resistant, V/R - intermediate reaction.

D. noxia Origin	*Dn* Resistance Gene										
	Dn1	*Dn2*	*dn3*	*Dn4*	*Dn5*	*Dn6*	*Dn7*	*Dn8*	*Dn9*	*DnX*	*DnZ*
Argentina	V/R	V/R		V/R	V/R	V/R					
Chile	R	V		V	R	R					
Czech Republic		R		V	R	R				V	R
Ethiopia		R		V	R	R			V	V	V
Hungary	V/R	V		V	V						
Mexico	R	R		R	R	R	R				
South Africa	R	R			R						
Syria				V							
USA	V	V	V	V	V	V	R	V	V		V

Virulence to *Dn4* from Turkmenistan has now been documented in *D. noxia* populations in many of the major cereal producing areas of the world. Virulence to *Dnz*, originating in Russia and bred into the U. S. cultivar 'Stanton', also exists in Ethiopian and U. S. populations. One population in the Central Plains of the U. S. is virulent to all *Dn* genes except *Dn7*, derived from rye. These results have major implications for North American wheat production, as *Dn4* and *Dnz* are the only *D. noxia* resistant genes in current North American wheat cultivars. The *D. noxia* virulence to these genes suggests that this narrow genetic base of aphid resistance should be strengthened by the addition of with new *Dn* genes.

Resistance in plants possessing *Dn1*, *Dn2*, *Dn4*, *Dn5* and *Dnx* is based on plant tolerance of aphid-induced chlorophyll loss and antibiosis (reduced aphid population development) (Smith *et al.*, 1992, Hein 1992, Haile *et al.*, 1999, Boyko *et al.*, unpubl.). The category(s) of resistance in plants containing *Dn6* are not known. *D. noxia* feeding induces greatly increased polypeptide levels in resistant plants (Porter and Webster 2000, van der Westhuizen & Pretorius 1995, van der Westhuizen & Botha 1993) that in turn, elicit higher levels of intercellular chitinases, peroxidases and glucanases in resistant plants than in susceptible plants (van der Westhuizen *et al.*, 1998a,b, 2002). However, the relationship of these enzyme activities to tolerance and antibiosis resistance is not known. In comparing members of the wheat chromosome ID and 7D *Dn* gene families described previously, Liu *et al.*, (2002) noted phenotypic differences in the symptoms exhibited by plants expressing *Dn4* (chromosome ID) and *Dn6* (chromosome 7D). Leaves of infested *Dn4* plants exhibit very little rolling along the mid-vein but develop moderate chlorosis after infestation by *D. noxia*. Leaves of infested *Dn6* plants exhibit moderate leaf rolling but very little chlorosis. This observation, coupled with the fact that *Dn6* retains more resistance to various *D. noxia* populations than *Dn4* (Table 2) led us to hypothesize that these *Dn* genes have different modes of action. In addition, the categories of resistance in plants expressing these genes have not been compared in the same experiment. The objectives of this study were to quantify antibiosis and tolerance resistance in wheat plants containing the *Dn4* or *Dn6* genes for *D. noxia* resistance.

Materials and methods

Plant materials

Seeds of wheat PI 372129 *(Dn4)* and P243781 *(Dn6)* were obtained from Dr. Harold Brockelman of the USDA/ARS National Small grains Repository in Aberdeen, ID USA. Seed of the susceptible cultivar Wichita were obtained from the Kansas Crop Improvement Association in Manhattan, KS USA.

Phenotypic analyses

The method of Smith & Starkey (2003) was used to determine antibiosis to *D. noxia* population accumulation on ten replicates (plants) of *Dn4*, *Dn6* and the susceptible control Wichita. Seedlings of each genotype were grown to the two-leaf stage in 10-cm-diameter plastic pots filled with Pro-mix® BX (Hummert International). Each plant was infested with four late instar *D. noxia* biotype A nymphs and covered with a plastic cylinder cage with a mesh top. When leaves of Wichita plants became 70 - 80% chlorotic, (~21 days after infestation), the numbers of *D. noxia* small nymphs, large nymphs nymphs, apterate adults and alate adults on each plant were recorded.

To assess tolerance, 20 plants each of *Dn4*, *Dn6* and Wichita were grown individually in 10-cm-diameter plastic pots to the two-leaf stage of development, and pairs of similar sized

plants were then caged individually. For each pair of plants, a control plant remained un-infested and a treatment plant was infested with four *D. noxia* biotype A adults. When susceptible Wichita plants became 70% - 80% chlorotic, the shoots of all plants were cut at the base (soil level), placed in an aluminum foil pouch and dried at 60°C for 48 h. Tolerance was measured as the percent change in proportional plant dry weight (DWT), where DWT = [(dry wt. uninfested plant - dry wt. infested plant)/ dry wt uninfested plant] x 100 (Reese *et al.,* 1994).

Plant tolerance to *D. noxia* - related chlorophyll loss in the three genotypes was also measured photometrically with a SPAD - 502 chlorophyll meter (Minolta Camera Co., Ltd., Japan), designed to measure chlorophyll A and B (Yadava 1986). A linear relationship exists between chlorophyll content and SPAD (chlorophyll) unit values (Markwell *et al.,* 1995). To concentrate *D. noxia* adults into an area where chlorophyll loss could be measured, a double-sided adhesive foam leaf cage (Converters, Inc., Huntington Valley, PA, USA) was placed on the top middle of each of three fully expanded leaves. Approximately 20 *D. noxia* were released onto the caged leaf surface area (0.5 cm diameter) and a small piece of organdy cloth (2.5 x 2.5cm) was then placed on the adhesive cage surface. Aphids were allowed to feed for 4 days and were then removed. Differences in chlorophyll content of infested and non-infested leaf tissue were compared on each leaf. A SPAD-based chlorophyll loss index (CLI) was calculated as: CLI = [(SPAD meter reading, uninfested plant - SPAD meter reading, infested plant) / SPAD meter reading, uninfested plant] x 100 (Deol *et al.,* 1997). Five representative SPAD unit measurements were taken at each leaf cage site and averaged, yielding a mean cage site SPAD unit measurement. These measurements were used to calculate a mean cage CLI, and CLI measurements from each cage were averaged to calculate a mean plant CLI value. The plant CLI value for each of 10 plants was then used to calculate a mean genotype CLI value for each genotype.

In each experiment, plants were arranged in completely randomized designs in the greenhouse at a photoperiod of 14:10 (L:D) h, 24°C (mean day) and 19°C (mean night), and 40-65% relative humidity. Data were subjected to analysis of variance using the SAS PROC GLM (SAS Institute 1999) and differences between treatment means were evaluated using least squares difference tests at $P = 0.05$.

Results and discussion

Both *Dn4* and *Dn6* plants were antibiotic to *D. noxia,* as evidenced by significantly reduced *D. noxia* populations on these plants. Comparatively, populations on plants of Wichita were significantly higher than those on *Dn4* and *Dn6* plants by 21 days after infestation (Table 3). These antibiosis data verify those of Hein (1992) for *Dn4* and provide new evidence that *Dn6* plants also have antibiotic effects on *D. noxia* population development. Smith *et al.,* (1992) demonstrated that *D. noxia* reared on plants containing *Dn1, Dn2* or *Dn5* exhibited moderate antibiosis in the form of reduced mean daily nymph production which was significantly less than nymph production on plants of the susceptible cultivar 'McKay'. Antibiosis in the form of reduced *D. noxia* population development was detected in plants containing *Dn1, Dn2* or *Dn5* by Budak *et al.,* (1999) and in plants of near isogenic lines derived from each of these genes (Tang *et al.,* 2004).

There were few differences in the age distributions of the *D. noxia* populations assessed. The proportion of the total population collected as small nymphs was 12% on plants of Wichita, 12% on *Dn4* plants, and 22% on *Dn6* plants. The proportion of the total population collected as large nymphs was 86% on Wichita and *Dn4* plants, and 74% on *Dn6* plants. The proportion of the total population collected as adults (apterous or alate)

ranged from 1% to 4% among the three genotypes evaluated. This lack of differences suggests that *Dn4* and *Dn6* plant defenses are not focused on any particular stage of *D. noxia* development, and that the overall effect of antibiosis resistance in *Dn4* and *Dn6* is to reduce *D. noxia* population development by lowering fecundity.

Plants of both *Dn4* and *Dn6* had significantly less dry weight loss (16 and 10%, respectively) than plants of Wichita (49%) (Table 3). These results confirm those of Hein (1992) and *Haile et al.,* (1999) for *Dn4,* and indicate that this genotype also displays *D. noxia* tolerance using the proportional dry weight loss method of tolerance determination. Smith *et al.,* (1992) determined that *D.* wox/a-related dry weight loss in plants expressing *Dn1, Dn2* and *Dn5* ranged from 0 to 6%, and was significantly less than dry weight loss in plants of the susceptible cultivar 'McKay' (18.5%). Results of Budak *et al.,* (1999) support these finds for *Dn1* and *Dn2,* but not *Dn5.*

Table 3. Mean ± standard error (SE) *D. noxia* population, percent proportional dry weight loss and percent chlorophyll loss of PI372129 *(Dn4),* PI243781 *(Dn6)* and Wichita (susceptible control) wheat plants after *D. noxia* biotype A infestation for 21 days.

Wheat Genotype (*Dn* Gene)	Mean *D.noxia* Population ± SE	Mean % Proportional Dry Weight change (DWT) ± SE [1]	Mean % Chlorophyll Loss Index ± SE [2]
Wichita	323.8 ±36.9 a[3]	+ 49.4± 7.5 a	53 ± 6 a
PI372129 *(Dn4)*	50.4 ± 13.2 b	+ 16.0± 4.5 b	53 ± 7 a
PI243781 *(Dn6)*	22.5 ± 3.9 b	- 10.0 ± 14.4 b	21± 2b

[1] DWT = [(dry wt. uninfested plant - dry wt. infested plant)/dry wt. uninfested plant] x 100 CLI = [(SPAD meter reading, uninfested plant - SPAD meter reading, infested plant)/SPAD meter reading, uninfested plant] x 100 [3] Means followed by the same letter are not significantly different (P>0.05; LSD)

The leaf chlorophyll loss index measurements of *D. noxia* - infested *Dn6* plants (21%) and Wichita plants (53%) were similar to the proportional plant dry weight loss of *Dn6* and Wichita plants. However, plants of *Dn4* had significantly more chlorophyll loss (53%) than *Dn6* plants. In addition, there was no difference in chlorophyll loss between *Dn4* plants and those of the susceptible control Wichita (Table 3). As in earlier observations of Liu *et al.,* (2002), the leaves of *D. noxia* - infested *Dn4* plants rolled slightly along the mid-vein, and leaves of infested *Dn6* plants did not roll. Plants of the susceptible variety Wichita, in contrast, sustained heavy leaf rolling, which resulted in several trapped leaves, as well as severe chlorosis. This is likely due to the fact that leaves of infested *Dn4* plants express moderate chlorosis after *D. noxia* infestation, while leaves of

infested *Dn6* plants exhibit very little chlorosis, as reflected in the chlorophyll loss data. Thus, in this comparison of *Dn4* and *Dn6* plants, the SPAD-based measurements of chlorophyll loss were a less accurate measurement of plant tolerance than proportional dry weight loss measurements.

Our hypothesis was that phenotypic differences in symptom expression of *Dn4,* on wheat chromosome ID, and *Dn6,* on wheat chromosome 7D, indicated that they have different modes of resistance. However, our results do not support that hypothesis, since plants with either gene expressed both antibiosis to *D. noxia* population development, as well as plant tolerance of *D. noxia* - induced plant dry weight loss. The explanation for the sustained resistance of *Dn6* resistance to *D. noxia* populations where *Dn4* has become susceptible remains difficult to explain. For each resistance category, *Dn6* plants had a higher (though not significant) level of resistance than *Dn4* plants. It is possible that different growing environments could favor *Dn6* plant growth and related resistance over *Dn4* plants. Plants possessing *Dn6* (originating from Iran) may have been co-evolutionarily exposed to greater levels of biotic stresses, resulting in more durable *D. noxia* resistance, compared to plants containing *Dn4,* originating from Turkmenistan. Nevertheless, both genes remain viable for wheat improvement for *D. noxia* resistance in many wheat production areas, and in areas where virulence has occurred, if deployed in combination with new genes for *D. noxia* biotype B resistance. The data for *Dn6* antibiosis and tolerance are the first reports of either resistance category existing for plants expressing this *Dn* gene.

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Breeding for resistance to the large raspberry aphid: An update on durability of current genes and future prospects.

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Abstract: Breeding for host plant resistance to insects (e.g. raspberry aphids) has been a successful strategy deployed by SCRI's plant breeders and entomologists, together with other research centres, for over 40 years. Breeding resistance into crops has reduced the need for synthetic pesticides and has controlled spread of aphid-borne viruses. Breeding is generally a long-term approach (e.g. 10-15 years for new pest-resistant raspberry varieties to reach growers), throughout which time pest insects are constantly adapting and overcoming plant resistance genes in a co-evolutionary "arms race". Examples of pests' counter-adaptations to introduced pest resistance genes seen in SCRI's raspberry varieties will be presented. Several types of aphid resistance genes (minor gene = multigenic; A1, A10 = single, major genes) with different mechanisms (antixenosis, antibiosis) were deployed against *Amphorophora ideai* in sequence by raspberry breeders. Each type of major gene resistance has now been broken in the U.K. Each gene has been effective for between 10-30 years, but with sequentially decreasing periods of durability against the three main virulent aphid biotypes. To date, minor gene based aphid resistance has remained durable in raspberry, but only provides partial resistance. The pest adaptation process takes as little as one third of the time taken to breed a new pest-resistant variety, so the "arms race" is generally skewed in favour of the counter-adapting pest, not the plant breeder. New research has been initiated at SCRI to find new sources of durable aphid resistance (wild species and cultivars) and to combine different types of resistance genes (e.g. minor gene and major genes) using molecular markers to speed up selection.

Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.23 - 26

Apple tree egg laying resistance against codling moth (*Cydia pomonella) Lepidoptera Tortricidae* and implication of plant surface metabolites

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Abstract: An apple tree egg laying resistance against *C. pomonella* has been found in orchard on X65-11. Insect egg laying on substrates impregnated with water leaf surface washing of X65-11 and a susceptible cultivar P5R50A4 stimulated egg laying in the same way as in the orchard. Chemical analyses of sugars of the leaf surface washings of both cultivars showed lower quantities and different ratios of several metabolites on X65-11 leaf surfaces. A blend of them, 6 already known as influencing egg laying found on each cultivar was experimented on egg laying in laboratory. The blends reproduced the resistance effect of X65-11 observed in orchard. Bioassays with increasing concentrations of the X65-11 blend showed that quantities could eliminate resistance when rather high (multiplied by 10 000). Comparison of these results with egg laying ratios obtained between the two cultivars led to the hypothesis that X65-11 resistance would be explained by low quantities of leaf surface metabolites but also to the composition and particularly ratios of fructose and sorbitol within the blend.

Keywords: *Cydia pomonella,* apple tree, resistance, egg laying, plant surface, sugars

Introduction

Development of crop plant resistance against pests has been based mostly on antixenosis and antibiosis. Antixenosis denote the presence of morphological or chemical plant factors that adversely alter insect behaviour. Among them resistance by lack of sufficient levels of phytochemicals that stimulate acceptation and egg laying are rather scarce. Furthermore those which are based on chemical stimuli which are on the plant surface (Fiala *et al.,* 1990) are not known. We already demonstrated that primary metabolites, which are coming from the plant tissues (Derridj 1996), influence *Cydia pomonella* egg-laying (Lombarkia & Derridj 2002). Here we project to study the resistance of apple tree cultivar against *C. pomonella* egg-laying and look at plant surface metabolites concerns.

Materials and methods

In a first step resistance observations were carried in Gotheron orchard (France) in 2000 where there is a high *C. pomonella* population level. The apple tree cultivar X65-11 was chosen for its low fruit pest damages and compared to the susceptible P5R50A4, which was cultivated on the same row. Trees were 10 and 17 years old respectively. Egg numbers were recorded at first and second moth flights on a sample of 51 branches per tree (three trees of P5R50A4 and two of X65-11).

Leaf surface washings collected with ultra-pure water at the second flight were then tested in no choice conditions on insect acceptance and egg-laying stimulation in laboratory at

25°C ± 2, 70%±10 RH (30 replicates of single female per cage). The leaves chosen were those of the corymb.

Three egg laying parameters were considered, the acceptance of the plant or substrate which was calculated by the number of egg laying females after 3min of contact with substrate at the scotophase period, time during which 50% of females lay eggs on control without substances (Derridj *et al.*, 1999), the egg laying stimulation for calculated by the numbers of eggs laid by females which accepted to lay eggs, and egg numbers calculated on all experimented females.

Metabolites known as influencing *C. pomonella* egg laying (Lombarkia & Derridj 2002) were analysed in the leaf surface washings of both cultivars and reproduced in artificial substrates submitted to egg laying. Ultra-pure water solution were composed of six metabolites: sucrose, D (+) glucose, D (-) fructose, D-sorbitol, *myo*-inositol, L-Quebrachitol. The egg laying substrate was a white square nylon cloth 200 cm² area and 5µm mesh that was soaked in the test solution and then dried horizontally under the hood at ambient temperature during 30 minutes.

The 6 metabolite blend collected on X65-11 was then tested, 30 replicates of single females for each concentration: x100, x1000, x10,000, to evaluate the quantity effect in egg-laying resistance.

Numbers of eggs laid per female were compared by Student t-test. The percentages of females laying eggs in the different treatments were compared by the χ^2 and Kruskal-Wallis tests. For both tests the level of significance taken was $p<0.05$.

Results

In orchard

Egg numbers recorded in orchards were 7 to 4 times less on X65-11 than on P5R50A4 at each flight respectively (Fig. 1).

Effect of leaf surface washings from the two cultivars on C. pomonella *egg laying*

Egg laying bioassays with corymb leaf washings showed the same egg-laying differentiation of the 2 cultivars as in orchard but with a smaller ratio about 2,4. (Fig. 1). Water leaf washings did not influence differentially the substrate acceptance which was maintained as on water control at 50%.

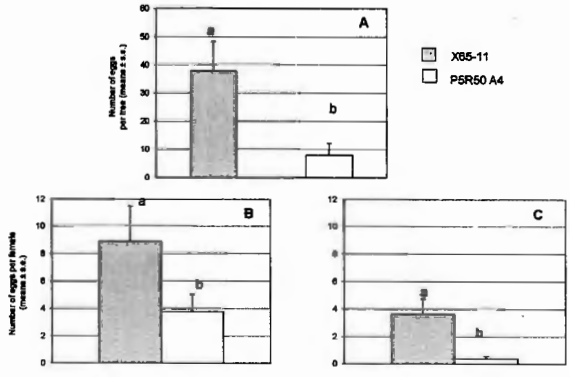

Fig. 1: Numbers of *C. pomonella* eggs on trees (A) of X65-11, P5R50A4 cultivars (2nd flight) and on their leaf surface washings (B) and the 6 metabolite component of their washings (C).

Chemical analyses of leaf surface washing metabolites of the two cultivars
The 6 metabolites (sugars and sugar alcohols) in the washings were in fewer quantities on X65-11 (ratios between each metabolite varied from 5 to 2 times) and particularly fructose, sorbitol and *myo*-inositol (Fig. 2). Ratios of fructose and sorbitol were the most different between the two cultivars.

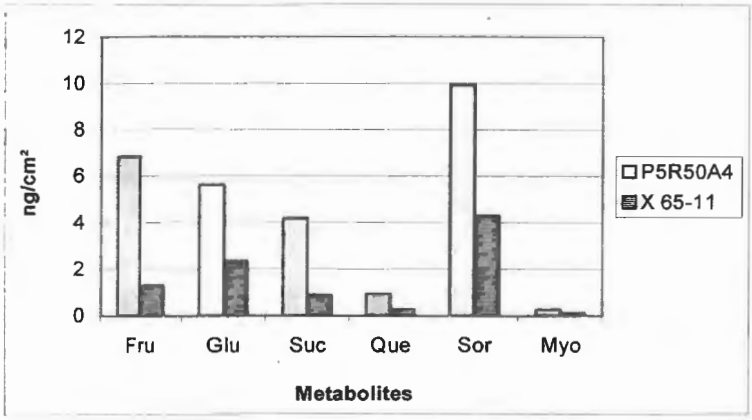

Fig. 2: Chemical analyse of the leaf surface washings collected from X65-11 and P5 R50A4.

Activity of the 6 metabolite blend found on leaf surface of X65-11 and P5R50A4 on* C. pomonella *egg laying
The activity of the blends reproduced the resistance observed in the orchard (Fig.1). The ratio of eggs between the two cultivars, was closer to that observed in orchard than with the washings. The egg-laying stimulation was generally lower with the blend than with the washing itself. Acceptance of the substrate by females diminished with the blend from X65-11 vs. PR50A4 and water control (18% vs. 40%).

***Dose effect of X65-11 metabolite blend on the acceptance and egg laying stimulation of* C. pomonella**
Numbers of eggs per female increased progressively with the 6 metabolite blend doses (Fig. 3) and was multiplied by 6 at the end (concentration multiplied by 10.000) The increase of concentrations did not affect the acceptance which was lower than with water control and maintained under 25%.

Conclusions

We observed egg laying resistance of an apple tree cultivar X65-11 against *C. pomonella* in orchard. This effect was well reproduced in laboratory by the blends of 6 metabolites from the leaf surfaces. The blends acted on acceptance of the substrate and egg laying stimulation. The effect of leaf surface washings on egg laying differentiation between both cultivars was smaller than those observed on trees in orchards and blends.

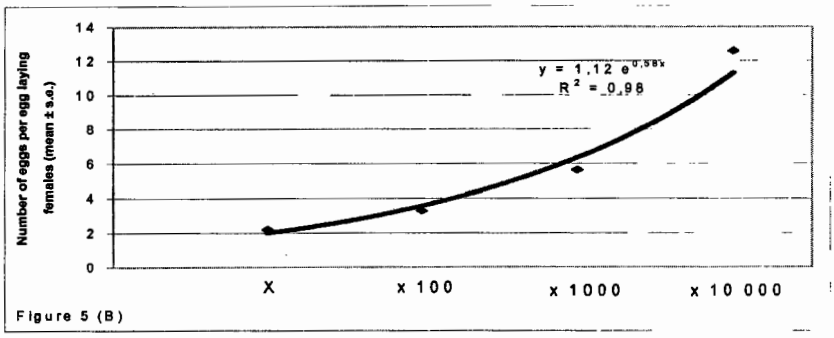

Fig. 3: Numbers of eggs laid by *C. pomonella* on substrate impregnated with different concentrations of the 6 metabolite blend found on X65-11 leaf surface.

The higher stimulation of washings vs. blends shows that other water-soluble components than the 6 metabolites might stimulate egg laying. We cannot exclude the influence on egg laying of products due to degradation of the washing during its conservation. Increase of the 6 metabolite blend concentrations from X65-11 showed that egg-laying resistance response could diminish with the increase of quantities of metabolites even though acceptance was not modified. But low quantities of metabolites present on X65-11 cannot explain entirely the egg laying resistance effect. Ratios between components and particularly fructose and sorbitol within the blend have to be studied to precise the modalities of activities of leaf surface metabolites in the resistance of X65-11.

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Mechanisms involved in induced resistance with extracts of *Reynoutria sachalinensis*

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Abstract: Plant extracts of *Reynoutria sachalinensis* are inducing resistance in a variety of crops. Extract application leads to effective disease control of powdery mildew fungi on e.g. cucumber, tomato or grape vine as well as against e.g. *Botrytis cinerea* on young ornamental or vegetable plants.

The induction by *R. sachalinensis* is characterised by a variety of processes following treatment with the extract. In non-infected cucumber leaf discs, the development of reactive oxygen species after treatment with the extract were found by Müller et al. (1998). Six hours after treatment, levels of hydrogen peroxide were increased from 3 µM in water treated leaf discs to 22 µM in extract treated. When in addition to the treatment with *R. sachalinensis* Plantacur E (formulated vitamin E preparation) was infiltrated into leaves, the level of hydrogen peroxide was 11 µM, while Plantacur E applied alone did not induce any increase in H_2O_2. At the same time, Plantacur E treatment of plants before the extract application reduced the efficacy of *R. sachalinensis,* indicating that the development of ROS plays a major role in this induction process. In barley coleoptiles treated with *R. sachalinensis* extract, Moch et al (2000) identified increased papilla formulation at the penetration sites of *Blumeria graminis* f.sp. *hordei* together with the accumulation of hydrogen peroxide in the papillae.

Other processes, such as enhanced lignification of cell walls in cucumber leaves were observed by Schneider-Müller (1991) together with enhanced activities of enzymes belonging to the phenolic pathway. These are also involved in the production of phytoalexins, which were qualitatively and quantitatively determined in cucumber plants infested with *S. fusca* and treated with *R. sachalinensis* extract. Treatment of conidia of *S. fusca* with these phytoalexins resulted in a significant decrease in germination (Daayf et al., 1995 and 1997). Deformation of powdery mildew haustoria in cucumber leaves treated with *R. sachalinensis* extract have been reported by Herger (1991) and Wurms et al. (1999). The results show that the mechanisms involved in resistance induced by *R. sachalinensis* extract are manifold and are covering processes occurring directly after extract treatment, as well as processes associated at a later stage, i.e. at the time of pathogen attack.

Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.29 - 34

Effect of natural monoterpenes on the behaviour of the peach potato aphid *Myzus persicae* (Sulz.)

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Abstract: The effect of selected acyclic (citral, linalool), monocyclic (*p*-cymene, α-ionone, β-ionone, S-limonene, R-limonene, S-pulegone, R-pulegone, α-terpineol) and bicyclic (camphene, β-pinene) monoterpenes on the behaviour of the peach potato aphid *Myzus persicae* during settling on plants was studied by direct observation of aphid behaviour. Citral and linalool had repellent activity, which was manifested in the significant decrease in time spent on leaves, decrease in total and mean time of penetration, and reduced number of probes as compared to control. Citral, linalool, S-limonene, α-ionone, and camphene were feeding deterrents and reduced the total and mean probing time of aphids and their settling on the leaves. Taking into account both, the repellent and the feeding deterrent effect of monoterpenoids on the behaviour of *M. persicae*, the following order of relative activity may be proposed: citral > linalool > camphene > S-limonene = α-ionone > β-ionone > R-pulegone > p-cymene > β-pinene > R-limonene > S-pulegone > α-terpineol. There was a difference in activity between the isomers of a given compound: α-ionone was more active than β-ionone, R-pulegone was more active than S-pulegone, and S-limonene was more active than R-limonene.

Keywords: Peach potato aphid, *Myzus persicae*, feeding deterrent, plant resistance.

Introduction

Plant resistance against insect pests, whatever mechanism is involved – antibiosis, antixenosis, or tolerance, is a consequence of their close association, that is, it is a result of the effects they have on each other. The progress in breeding of the resistant plant varieties or cultivars depends on the understanding of mechanisms of host plant selection and host plant acceptance by the pest. These mechanisms often involve plant secondary metabolites that affect the behaviour of the herbivorous insect. The disruption of the established pattern of behaviour during host plant selection process may lead to the decrease or complete decline of feeding. Accordingly, the reduced feeding may lead to the abandonment of a plant, may affect the longevity of the insect, or may lead to its death. In the case of aphids, which are very efficient vectors of plant viruses, the reduction of their feeding may also protect plants from pathogen infection. Secondary plant metabolites have multiple functions in the herbivore-plant relationships, which makes them an attractive target for plant breeding (Dawson *et al.*, 1989; Verpoorte & Memelink 2002). Molecular strategies leading to the modification or supplementation of the existing biosynthetic pathways of secondary metabolites in plants are under investigation (Pickett 1991; Verpoorte & Memelink 2002).

Terpenoids are one of the major classes of secondary metabolites synthesized by plants that are toxic, unpalatable or at least repellent to herbivores, and as such acting as defense compounds (Harrewijn *et al.*, 2001; Pickett 2001, Wittstock & Gershenzon 2002). Monoterpenoids that are major components of many plant essential oils have a strong effect

on insects. There are reports on their insecticidal, feeding deterrent and repellent activity (Simmonds 1998). The repellent activity of linalool and α-terpineol to *Myzus persicae* was proved by Hori (1998). Gutierrez *et al.,* (1997) found that geraniol inhibited settling of this aphid.

The aim of this study was to evaluate the effect of selected acyclic, monocyclic and bicyclic monoterpenes on the behaviour of the peach potato aphid *Myzus persicae* (Sulzer) during the settling on plants.

Materials and methods

The activity of the following monoterpenes (Fig. 1) was evaluated:acyclic monoterpenes: citral, linalool

- monocyclic monoterpenes: p-cymene, α-ionone, β-ionone, S-limonene, R-limonene, S-pulegone, R-pulegone, α-terpineol
- bicyclic monoterpenes: camphene, β-pinene

The compounds were kindly provided by the Department of Chemistry, Agricultural University in Wroclaw.

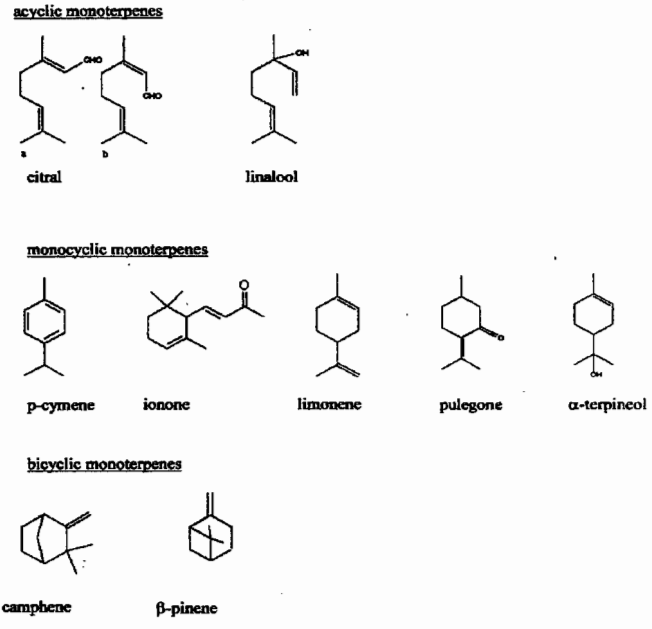


Fig. 1: Chemical structure of tested natural monoterpenoids

Aphids (*Myzus persicae)* and plants (Chinese cabbage) were reared in laboratory at 20°C, 65% r.h., and L16:8D photoperiod.

The compounds were applied to adaxial surface of a leaf as a 0.1 % ethanolic solution, 0.01ml/cm^2 of the leaf according to a method desribed by Polonsky *et al.,* (1989). All

biological tests were performed 1 hour after the application of the compounds to allow the evaporation of the solvent.

Aphid settling

This parameter was assessed using the half-leaf choice-test: compounds were applied on one half of the leaf, the other side of the midrib was coated with ethanol and acted as a control. Aphids that settled on each side of the midrib were counted at 15', 30', 1h, 2h, and 24h intervals after access to the leaf (8 replicates, 20 adult apterous aphids/replicate). The results were statistically analysed using analysis of variance.

Aphid behaviour

This parameter was studied by direct observation of the freely moving aphids on a leaf treated with the tested compounds, using a video camera. The experiment was carried out for 15 min (16 aphids/compound). The time spent on the leaf and the duration of probing were recorded basing on the relationship between antennal and body movements and penetration of the stylets as described by Hardie *et al.*, (1992). The position of antennae parallel to the abdomen and the cessation of body movements were associated with stylet penetration.

Results

All tested monoterpenoids had a deterrent effect on aphid settling. However, the deterrent activity was manifested with a varied intensity (Fig. 2). Citral, linalool, α-ionone, S-limonene, and camphene inhibited aphid settling during the entire time of the experiment.

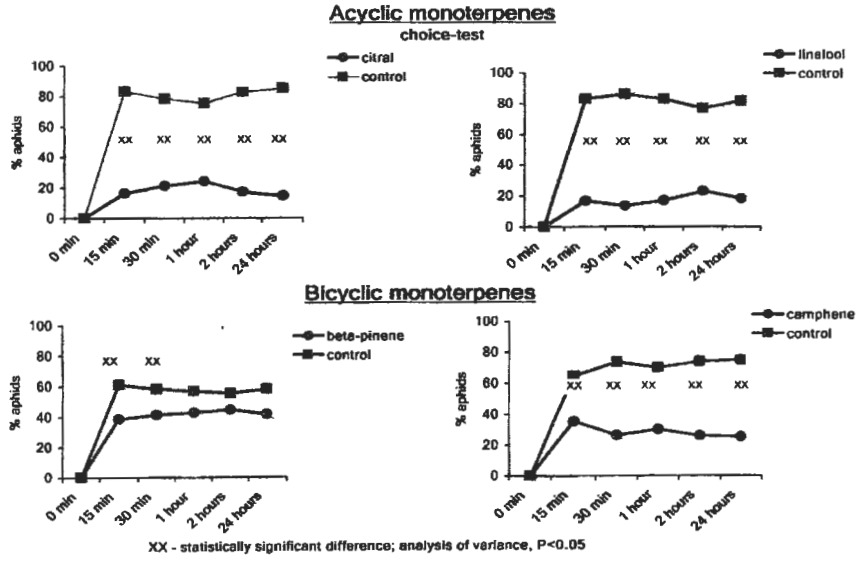
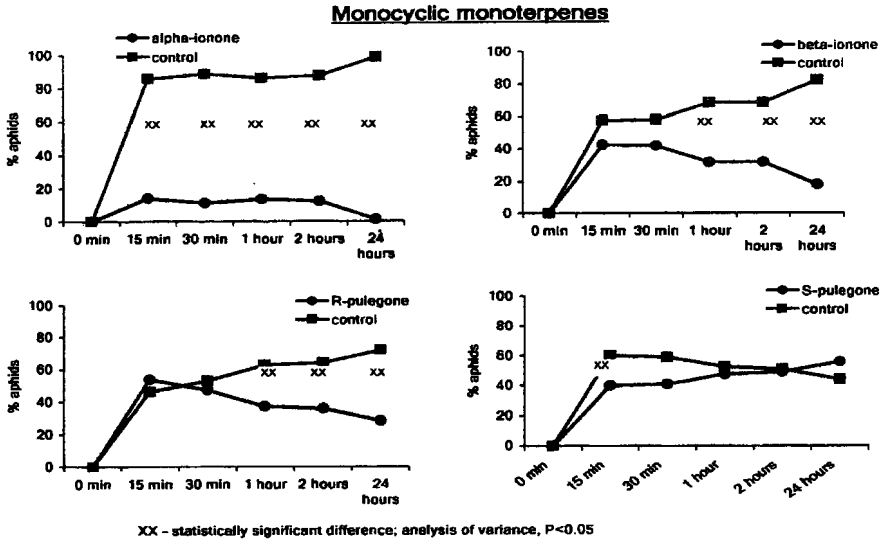


Fig. 2: Effect of natural monoterpenoids on the settling of *Myzus persicae* on plants in a choice-test.



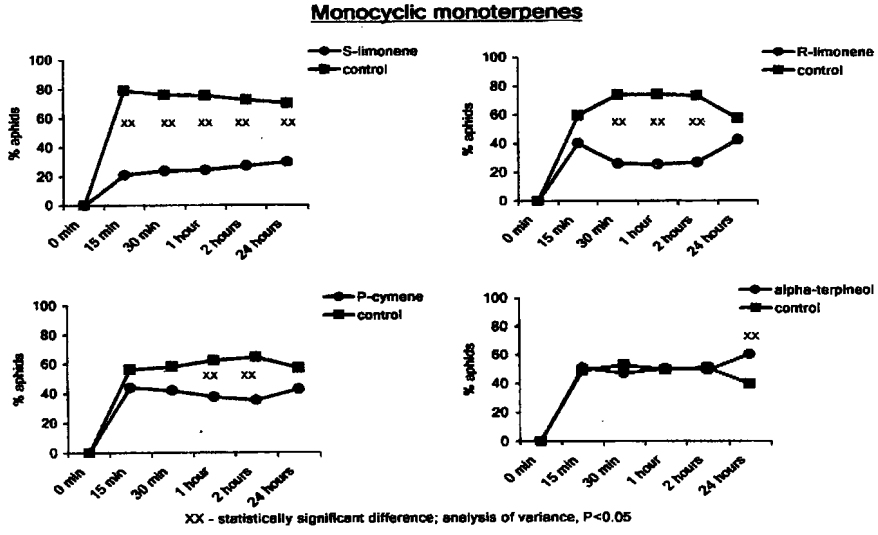


Fig. 2 (continued): Effect of natural monoterpenoids on the settling of *Myzus persicae* on plants in a choice-test.

The deterrent effects of these monoterpenes were found as soon as 15 minutes after aphids had had access to the treated leaves and lasted for at least 24 hours, i.e. until the termination of the observation. The settling deterrent effect of S-pulegone, R-limonene, p-cymene, and β-pinene was short-lived, as aphids were deterred from settling only during the first two hours of the experiment. The deterrent effect of β-ionone and R-pulegone occurred 1 hour after aphids had access to the treated leaves, and the activity of α-terpineol was manifested only at the 24th hour of the experiment. There was a difference in activity between the isomers of a given compound: α-ionone was more active than β-ionone, R-pulegone was more active than S-pulegone, and S-limonene was more active than R-limonene. S-pulegone had the weakest effect on aphid settling of all tested compounds.

The monoterpenoids had also effect on aphid behaviour during the 15 minutes after they had contact with the treated leaves (Fig. 3). The time aphids spent on the leaf was significantly shorter on leaves treated with citral and linalool as compared with the untreated leaves. Total time of probing was reduced by citral, linalool, R-pulegone, β-pinene and camphene. Mean probing time was shorter when aphids penetrated leaves treated with citral, linalool, R-pulegone and β-pinene.

Discussion

The significant decrease in time spent on the leaves, decrease in total and mean time of penetration, and the reduced number of probes as compared to control may be attributed to the repellent activity of the monoterpenes to the peach potato aphid. In this view, only citral had a strong repellent activity of all the tested compounds. A little weaker repellent action was shown by linalool.

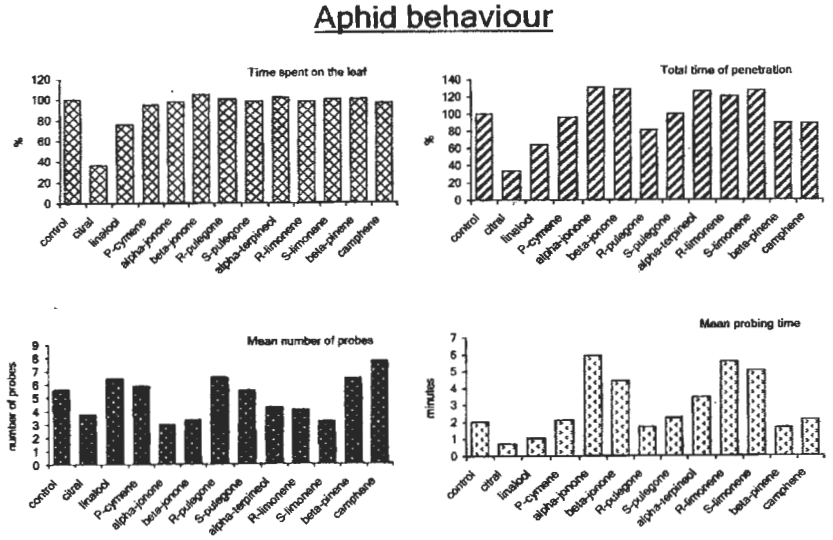


Fig. 3: Effect of natural monoterpenoids on the behaviour of *Myzus persicae* during settling on plants.

The reduced level of aphid settling on the plants as compared to the control, and the decrease in total and mean probing time might have been the effect of the feeding deterrent activity of the monoterpenes. A strong – lasting for 24 hours – feeding deterrent activity was exhibited by the following monoterpenes: citral, linalool, S-limonene, α-jonone, and camphene.The feeding deterrent activity of R-limonene, p-cymene, and β-pinene lasted less than 24 hours. β-jonone and R-pulegone were active after 1 hour. S-pulegone and α-terpineol had a very weak deterrent activity to *M. persicae*.

Taking into account both the repellent and the feeding deterrent effect of monoterpenoids on the behaviour of *M. persicae*, the following order of relative activity may be suggested: citral > linalool > camphene > S-limonene = α-ionone > β-ionone > R-pulegone > *p*-cymene > β-pinene > R-limonene > S-pulegone > α-terpineol.

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The role of jasmonates in defense reactions in plants under biotic stresses

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Abstract: Rapid increase in endogenous levels of jasmonates, mainly jasmonic acid (JA), occurs after wounding of different plant organs, which may be caused by physical injury, pathogen infection, herbivore or insect attack, as well as different natural elicitors – systemin, oligogalacturonides, chitosan, chitin. Wounding induces an expression of defense-related genes whose products are mostly involved in defense responses against pathogen and insect attack. Endogenous and exogenous jasmonates could act as signalling substances in the transduction chain between stress and molecular stress response, in most cases of the induction of gene expression and the accumulation of defense specific proteins and secondary metabolites. In different biological tests coronatine and coronalon were shown to be similar to jasmonates.

Keywords: jasmonates, defense reaction, biotic stresses

Introduction

Jasmonic acid (JA), methyl jasmonate (JA-Me) and their related compounds which are designated as jasmonates, are widely distributed in the plant kingdom and show various important biological activities in the regulation of plant growth and development, resulting in the consideration that they are putative new plant hormones (Ueda & Kato, 1980; Koda, 1992; Sembdner & Parthier, 1993; Creelman & Mullet, 1995, 1997; Murofushi *et al.,* 1999; Saniewski & Czapski, 1999). Endogenous levels of jasmonates, mainly JA, increase rapidly and transiently in plants or their organs under both abiotic stress, such as mechanical wounding, osmotic stress, water deficit, dessication stress, heavy metals (Cu^{++}, Cd^{++}, Ag^{+}) and touching, as well as biotic stress, such as pathogen infection and insect attack, and caused by different natural elicitors – systemin, oligogalacturonides (i.e. cell wall of yeast or pathogens), chitosan and chitin (Creelman & Mullet, 1995). The octadecanoid pathway appears to be a general signaling pathway for many plant physiological processes involved in plant development and response to biotic and abiotic stresses.

In this paper the role of jasmonates in defense reactions in plants under biotic stresses is reviewed.

Jasmonates and defense mechanism in plant protection against pathogens and herbivores

Plants have developed numerous defense mechanisms which are activated locally in case of pathogen infections or insect infestations, for example the hypersensitive death of affected cells, generation of reactive oxygen species (ROS), enhancement of ethylene production, of release elicitors, synthesis of phytoalexins, induction of many pathogenesis – related proteins, induction of secondary metabolites, processes strengthening cell walls (formation of wax, suberin, phenolics compounds, proteins rich in hydroxyproline, lignification, synthesis of

cellulose and cross linking). A plant defense response is not limited to the sites directly treated with pathogen or elicitor but occurs systemically all over the plant (de Bruxelles & Roberts, 2001; Pieterse *et al.*, 2001).

Table 1. The stimulatory effect of methyl jasmonate and fragments of yeast cell wall on production of secondary metabolites in various plant cell suspension cultures

Species	Elicitor	Induced metabolite	References
Crotolaria californica	JA-Me	isobavachalcone	Gundlach et al., 1992; Mueller et al. 1993
Glycine max	- " -	genisteine	- " -
Lactuca sativa	- " -	lettucenin A	- " -
Rauvolfia canescens	- " -	raucaffricine	- " -
Rubia tinctorum	- " -	rubiadin	- " -
Ruta chalapensis	- " -	rutacridone	- " -
Eschscholtzia californica	- " -	benzo[c]phenantridine alkaloids	- " -
Taxus canadensis	- " -	paclitaxel	Linden and Phisalaphong 2000
Taxus cuspidata	- " -	taxol	Mirjalili and Linder 1996
Taxus baccata	- " -	taxane	
Nicotiana tabacum	- " -	capsidiol	Laskaris et al. 1999
Lithospermum erythrorhizon	- " -	rosmarinic acid	Mandujano-Chavez et al. 2000
Coleus blumei	- " -	rosmarinic acid	Mizukami et al. 1993
Alkanna tinctoria	- " -	alkannins	
Centaurium erythraea	- " -	1-hydroxy-3,5,6,7-tetramethoxyxanthone	Szabo et al. 1999
Centaurium littorale	- " -	1-hydroxy-3,5,6,7-tetramethoxyxanthone	Urbanek et al. 1996
Petroselinum crispum	- " -		Beerhues and Berger 1995
Oryza sativa	Yeast cell wall	coumarin derivatives	- " -
Eschscholtzia californica		momilacton A	Kauss et al. 1992
		benzo[c]phenantridine alkaloids	Nojiri et al. 1996 Gundlach et al. 1992

Table 2. The stimulatory effect of jasmonates on production of secondary metabolites in intact plants

Species	Elicitor	Induced metabolite	References
Nicotiana sylvestris	JA-Me	nicotine	Baldwin et al. 1994
Catharanthus roseus	JA-Me	tabersonine, vindoline, catharanthine	Aerts et al. 1994
Cinchona	JA-Me		- " -
Brassica napus	JA, JA-Me	cinchonine	Bodnaryk 1994
Brassica rapa	JA, JA-Me	glucosinolates	- " -
Oryza sativa	JA	- " -	Tamogami and Kodama 2000
		momilactone A,	
Lupinus luteus	JA-Me	sacuranetin	
Phaseolus lunatus	JA		Kneer et al. 1999
		genisteine	Ozawa et al. 2000
		β-ocimene	

The earliest known events detected in wounded plant organs include ion fluxes across the plasma membrane, changes in cytoplasmic calcium concentration, generation of reactive oxygen species, and changes in protein phosphorylation patterns. These early events occur in the first few minutes following damage and are probably not directly responsible for inducing defense gene expression.

Jasmonates are considered to be central molecules in signal transduction pathways that induce expression of defense related genes. In common with many pathogen derived elicitors, wounding, glycans and systemin peptide all cause a rapid depolarization of the plasma membrane electrical potential. This depolarization event is associated with an effux of K^+ ions, a concomitant influx of protons, and alkalization of the extracellular medium (de Bruxelles & Roberts, 2001).Several studies have shown rapid increases in cytoplasmic Ca^{++} concentrations following wounding.

Reactive oxygen species (ROS), as defense factors, can directly inhibit pathogen growth, limit the spread of pathogen infection by strengthening plant cell wall, activate defense genes acting as signaling molecules and be involved in hypersensitive response (HR). The HR, a well-known resistance mechanism, is characterized by rapid, localized cell death around the site of infection and so it can play inhibiting role in the spread of pathogen. HR has also been suggested to release signals that activate variety of defense-related genes and systemic resistance. Some data suggest that HR may function more by releasing signaling molecules involved in the induction of defense genes than as a direct defense mechanism. Especially H_2O_2 and $O_2^{\cdot -}$ have been suggested to be diffusible signals that activate genes in local and systemic acquired resistance. The accumulation of jasmonic acid in hypersensitive response tissues of tobacco plants was documented (de Bruxelles &Roberts, 2001).Wound-induced MAP kinases activate phospholipase A_2, releasing linolenic acid from the plasma membrane that then acts as a substrate for jasmonic acid (JA) biosynthesis. Jasmonates have been implicated as intermediate signals in elicitor-induced secondary metabolite accumulation and other defense reactions against pathogen infections and insect attacks.

Secondary metabolites play an important role in plant protection against pathogens and pests. Due to their toxic or deterent activity they directly counteract pathogen infection and

herbivore damages. There is growing evidence demonstrating that JA induces synthesis of secondary metabolites of various classes, that are associated with resistance to biotic stresses. It has been shown that JA induced phenolics, flavonoids, terpenoids, alkaloids and glucosinolates. Cell suspension cultures of many plant species could be elicited with respect to the accumulation of specific secondary metabolites by exogenously supplied jasmonic acid or methyl jasmonate (Table 1). Treatment of some intact plants also induced accumulation of secondary metabolites (Table 2). Plant defense reactions against pathogens include the induced synthesis of phytoalexins.

Numerous volatile metabolites, especially terpenoids and phenol derivatives emitted by plants in response to pest attack and JA treatment may also play indirect defense role as attractants for herbivore predators. JA-Me treatment induces local and systemic increase in the emission of many volatiles, similar to those which are observed in the case of herbivore attacked plants (Baldwin & Preston, 1999; Baldwin *et al.,* 2002; Gatehouse, 2002; van Poecke & Dicke, 2004).

Glucosinolates, occurring in species of *Brassicaceae* family, are a potentially important source of defense against herbivores and pathogens. After damage of tissues glucosinolates are degraded into highly toxic products such as isothiocyanates, thiocyanates and nitriles via myrosinase. Isothiocyanates and thiocyanates are inhibitors of insect and pathogens development. Induced expression of the glucosinolates was shown to be dependent upon jasmonates signaling. Exogenous JA and JA-Me greatly stimulated accumulation of indole glucosinolates in some species of *Brassicaceae*, i.e. *Brassica rapa*, *B. napus* (Bodnaryk, 1994) and *Tropaeolaceae*, i.e. *Tropaeolum majus* (Wielanek & Urbanek, 1999).
Some strains of rhizobacteria colonizing plant root surface trigger and induced systemic resistance against pathogens in above-ground plant parts. This induced systemic resistance is mediated by signaling pathways, in which JA play a key role.

JA controls the enzyme activities in the metabolic chains leading to accumulation defense related products of various chemical structures. It was reported that JA increased activity of phenylalanine ammonia lyase, chalcone synthase, lipoxygenase, polyphenol oxidase, β-glucosidases, cytochrome P450, peroxidase, hydroxymethylglutaryl CoA reductase and induced many specific proteins, i.e. osmotins, thionins, proteinase inhibitors and others (Reymond & Farmer, 1998).

Proteinase inhibitors common in plants counteract insect damages by inhibiting proteinase activity in the herbivore digestive track. Insect damage-induced expression of proteinase inhibitors in tomato was correlated with increase in JA, which caused the transcriptional activation of genes encoding these inhibitors. The interaction of jasmonates with ethylene was reported, in which these compounds acted together to regulate proteinase inhibitors gene expression during wound response (de Bruxelles & Roberts, 2001).Combination of jasmonates and ethylene caused synergistic induction of pathogenesis-related gene expression osmotin RNA in tobacco seedlings (Xu *et al.,* 1994). There was documented synergy between jasmonates and ethylene for the induction of the plant defense gene of *PDF1.2* in *Arabidopsis thaliana* infected by *Alternaria brassicicola* (Penninckx *et al.,* 1998).

Table 3. Inhibitory effect of jasmonates on the development of disease on different species caused by plant pathogens

Pathogen	Plant host	References
Pythium ultimum	*Picea abies*	Kozlowski et al. 1999
Verticillium dahliae	*Gossypium hirsutum*	Li et al. 1996
Phytophthora infestans	*Lycopersicon esculentum*	Cohen et al. 1993
- " -	*Solanum tuberosum*	- " -
Erysiphe graminis f. sp. hordei	*Hordeum vulgare*	Schweizer et al. 1993
Penicillium digitatum	*Citrus paradisi*	Droby et al. 1999
Botrytis cinerea	*Rosa hybrida*	Meir et al. 1998
- " -	Strawberry fruits	Moline et al. 1997
- " -	*Arabidopsis thaliana*	Thomma et al. 2000
Pyricularia oryzae	*Oryza officinalis*	Neto et al. 1991
Alternaria brassicicola	*Arabidopsis thaliana*	Thomma et al. 2000
Plectosphaerella cucumerina	- " -	- " -

Polyphenol oxidase (PPO) activity increases systemicly in leaves in response to wounding and is induced in tomato plants supplied with systemin or methyl jasmonate. This enzyme oxidizes phenolic compounds to quinones, reactive molecules which can interact with many biological molecules. During feeding by folivore insects, PPO is mixed with phenolic substrates and the resulting quinones alkylate essential amino acids of the dietary protein, making them nutritionally unavailable to the insect (Constabel & Ryan, 1998). PPO has been considered to have a possible defensive role against pathogen too. Inhibitory effect of jasmonates on the development of disease symptoms caused by different pathogens on various plant species is well documented (Table 3). It should be mentioned that jasmonic acid and other related compounds were detected in some pathogenic fungi, i.e. *Botryodiplodia theobromae* (syn. *Lasiodiplodia theobromae*), *Fusarium oxysporum* f. sp. *matthiolae* (Aldridge *et al.*, 1971; Miersch *et al.*, 1999).

Nitric oxide (NO) is shown to control host responses to infection, it regulates early events after infection, triggers expression of several defense genes, modulates H_2O_2 action and play a role in pathways leading to systemic acquired resistance. JA as an integral component of signal transduction pathways may mediate H_2O_2 and NO induced reactions in local and systemic resistance (Wendehenne *et al.*, 2004).

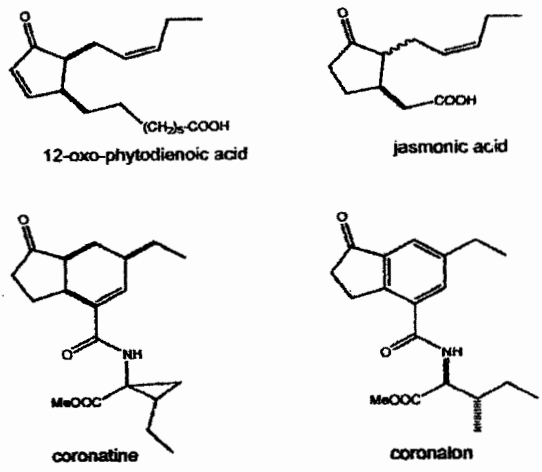

Fig. 1: Structures of octadecanoid-related compounds (Schüler *et al.*, 2004).

Thus, jasmonates are an integral part of the signal transduction chain between stress signal(s) and stress response(s) in plants. Cooperative cross-talk among jasmonates and various hormonal signals, especially ethylene and salicylic acid, occurs in defence responses against a wide variety of abiotic and biotic agents (Pieterse *et al.*, 2001). Many of plant responses to jasmonates, can also be elicited by a bacterial phytotoxin coronatine (Fig. 1), including induction of secondary metabolites. Coronatine is an amide of coronafacic acid and coronamic acid (1-amino-2-ethylcyclopropane-1-carboxylic acid). Coronatine was isolated for the first time from a fermentation broth of the phytopathogenic bacterium *Pseudomonas syringae* var. *atropurpurea* but is also produced by several other pathovars of *P. syringae*, e.g. *tomato* and *glycinea* (Schüler *et al.*, 2004). Coronalon (Fig. 1), a synthetic 6-ethyl indanoyl isoleucine conjugate (2-[(6-ethyl-1-oxo-indane-4-carbonyl)-amino]-3-methyl-pentanoic acid methyl ester), has been designated as a highly active mimic of octadecanoid phytohormones that are involved in insect and disease resistance (Schüler *et al.*, 2004). The spectrum of biological activities that is affected by coronalon was investigated in nine different plant systems specifically responding to jasmonates and/or 12-oxo-phytodienoic acid. The results obtained showed the induction of defense-related secondary metabolite accumulation in both cell cultures and plant tissues, specific abiotic and biotic stress-related gene expression, and root growth retardation. In all bioassays analyzed, coronalon demonstrated a general strong activity at low micromolar concentrations.

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.45 - 50

Saponins as a source of alfalfa resistance towards pea aphid, *Acyrthosiphon pisum* Harris

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Abstract: The pea aphid considerably less accepted the alfalfa with higher content of saponins. Aphids fed on such cultivars showed reduction in reproduction, survival and disturbance in population development. Highly saponin alfalfa also exerted a strong negative influence on feeding behaviour of the pea aphid. Prolonged non-penetration and probing of the peripheral tissues (epidermis and mesophyll) and almost three times shorter phloem sap ingestion was observed. While probing of the peripheral tissues the pea aphid showed a longer total duration, shorter single activity and more aphid probes on the high-saponin cultivar. In addition, during initial period of the peripheral tissues penetration (the first hour) more potential drops (pds) of longer duration was found for the aphids performed on the high-saponin alfalfa. Role of the saponins in partial resistance of the alfalfa to the pea aphid is discussed.

Keywords: pea aphid, *Acyrthosiphon pisum,* saponins, alfalfa, aphid feeding behaviour

Introduction

Secondary plant metabolites are involved in plant – herbivorous insect chemical interactions. Many of them including phenolics, hydroxamic acids, indole alkaloids, glucosinolates, cyanogenic glycosides and/or surface waxes are known as sources of natural plant resistance towards various species of aphids (Argandona *et.al.*, 1983, Leszczynski *et.al.*, 1989, Ciepiela & Chrzanowski, 1999, Wójcicka *et. al.*, 2004). They seriously affect the aphids behaviour, physiology and metabolism and as a result reduce the aphid populations on the resistant plants. When mechanisms of the partial resistance is studied, antixenosis and antibiosis was usually determined.

Pea aphid, *Acyrthosiphon pisum* (Harris) is one of the most important insect pests of various *Fabaceae* including alfalfa (*Medicago saliva* L.). Previous study suggested that the aphid feeding cause a foliar damage and an increase in coumestrol content within the infested alfalfa plants (Loser, 1968, Kain & Biggs, 1980). On the other hand, saponins are suggested as possible chemicals involved in defence of alfalfa against the phytophagous insect pests. So far, there are only some information on influence of commercial saponins (Baker) on the aphid (Horber, 1972).

The present paper reports on effects of alfalfa cultivars high and low in saponin content on the pea aphid performance.

Material and Methods

Plant material

High and low-saponin cultivars of alfalfa, Radius and Sapko, respectively were obtained from Institute of Plant Breeding and Acclimatisation (IHAR), Radzików/Błonie, near Warsaw. Seeds of the studied lines were germinated in a climatic chamber kept at 20-25°C and under a 16 h daylight and 8 h darkness. The plants were grown in a medium nutrient fine structure compost with sand, in 7 cm x 7 cm x 9 cm plastic pots, one per pot. The pots were regularly

watered and no extra fertiliser was added. The six months old plants of alfalfa were used in the experiments.

Aphids
The aphids used in the experiments came from a stock culture kept at Department of Biochemistry, University of Podlasie at Siedlce. The aphid population was reared on a broad bean plants and transferred to alfalfa plants for one generation, before using the adult apterous females to run the experiments (Apablaza & Robinson, 1967).

Population tests
The adult apterous females were caged individually on the high and low-saponin cultivars of the alfalfa and allowed to deposit nymphs. After 24 hs, one nymph remained on a single plant and other offsprings and the adult were removed. The experiment was run in 10 independent replicates for each cultivar of the studied alfalfa. Development time and reproduction of the individuals were observed daily until their death. Prereproductive period (time from birth until maturity), reproduction time and total fecundity were determined after (Leszczynski *et.al.* 1989). In addition, an intrinsic rate of natural increase (r_m), net reproduction (R_o) and multiplication rate of the population growth (λ) on the high- and low-saponin cultivars was calculated.

EPG tests
Feeding behaviour of the pea aphid on the studied high and low-saponin cultivars of alfalfa was monitored with help of EPG (electrical penetration graphs) method after Tjallingii (1988). The EPG recordings were carried out during 8hs, for each alfalfa cultivar on 10 different plants and aphids. Duration and number of such aphid activities as: non-probing (EPG pattern Np), penetration of peripheral plant tissues (pattern C), salivation into sieve elements (pattern E1), phloem sap ingestion (patter E2) and xylem sap ingestion (pattern G) were measured. Obtained results of the EPG recordings were analysed using computer programme Stylet (DOS PCs).

Statistics
The obtained results were subjected to one-way ANOVA, followed by Duncan's test.

Results and discussion

Pea aphid performance on high and low-saponin cultivars of alfalfa
Alfalfa plants contained high level of saponins were less accepted by the pea aphid than those with lower level of the secondary plant metabolites. Such cultivars negatively influenced growth, development and reproduction of the pea aphid population (Table 1). Particularly they prolonged larval development (prereproductive period) of the pea aphid and shortened its reproduction time. Moreover, reduction of the aphid fecundity and value of intrinsic rate of natural increase (r_m) was observed (Table 1). On such plants reproductive time of the pea aphid began later and finished earlier than on the low-saponin ones.

Table 1. Effect of the alfalfa cultivars on population development of the pea aphid.

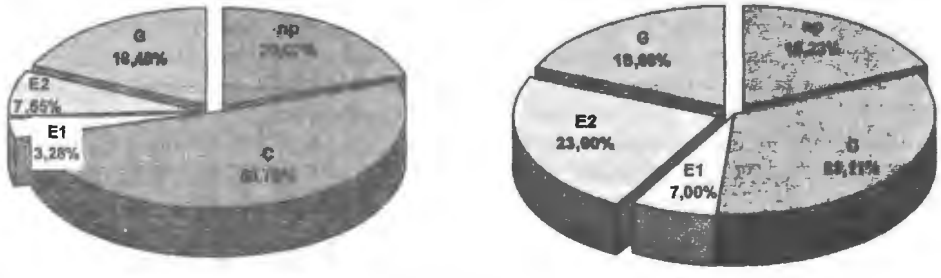
Studied parameters	Alfalfa cultivars	
	High-saponin	Low-saponin
Prereproductive time (days)	13,50[a]	11,20[b]
Reproductive time (days)	11,60[b]	15,10[a]
Total fecundity	32,90[b]	46,20[a]
r_m	0,1902	0,1998
R_o	32,89	46,10
λ	1,21	1,22

Values in rows followed by different letter are significantly different at $p < 0,01$ (Duncan's test)

The high-saponin cultivars of alfalfa also caused reduction of the other studied population parameters such as net reproduction (R_o) and multiplication rate of the population growth (λ).

Pea aphid feeding behaviour on high and low-saponin cultivars of alfalfa

EPG recordings showed clear differences in the pea aphid feeding behaviour on alfalfa cultivars with low and high content of saponins. The high-saponin cultivar prolonged penetration of epidermis and mesophyll (peripheral tissues) and as a result significantly reduced the aphid feeding by shortening of salivation into sieve elements and the phloem sap ingestion (Fig. 1).



High-saponin cultivar Low-saponin cultivar

Fig. 1: Percentage of pea aphid probing activity on high and low-saponin cultivars of alfalfa, during 8 hs of the EPG recordings.

During the initial period (the first hour) of the pathway phase, aphids showed more short probes while penetrating tissues of the high saponin alfalfa line than the low saponin line (Fig. 2). During penetration of the alfalfa peripheral tissues, where the saponins are mostly located, aphids performed numerous potential drops (pds), corresponding to cell wall punctures and short insertions of the aphid stylets inside of cells. The aphids which had fed on alfalfa

cultivars with higher content of saponins showed more of these events, and they were clearly longer in comparison to those performed on the low-saponin cultivars (Fig. 3).

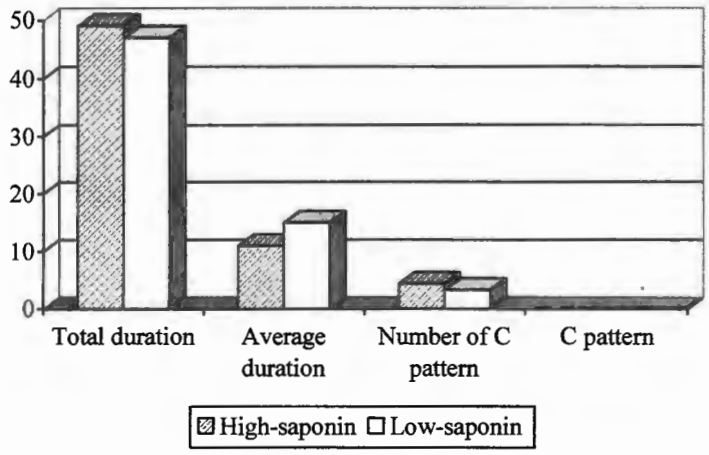


Fig. 2: Duration (min) and number of peripheral tissues probing by the pea aphid on high and low-saponin cultivars of alfalfa, while the first hour of penetration.

Previous works indicated that alfalfa saponins form a chemical barrier for feeding of the phytophagous insects, and that resistance mechanisms of legumes plants such as red clover (*Trifilium pratense* L.), white clover (*T. repens* L.) and birdsfoot trifoil (*Lotus corniculatus* L.) towards whitegrubs (*Meldontha vulgaris* F.) was related to high content of saponins (Applebaum & Birk 1979, Sutherland *et. al.,* 1982, Potter & Kimmerer, 1989, Nozzolillo *et. al.,* 1997, Matsuda *et. al.,* 1998, Adel *et. al.,* 2000,). The saponins slowed down passage of food through the alimentary canal, reduced digestibility, and negatively influence food uptake by the herbivorous insects (Ishaaya, 1986). In addition, saponins block the sterols uptake which are essential for development of phytophagous insects (Shany *et. al.*, 1970). On the other hand, it was discovered that performance of various clones of the pea aphid on legumes is related to the host-plant quality (Caillaud, 1999, Bournoville *et. al.,* 2004a, b). Results presented here suggest that saponins are important metabolites in chemical interactions between alfalfa and the pea aphid, since they strongly reduced its growth, development, fertility and feeding behaviour. Thus, these secondary metabolites might be an important factor in some alfalfa genotypes because they confer resistance to the pea aphid.

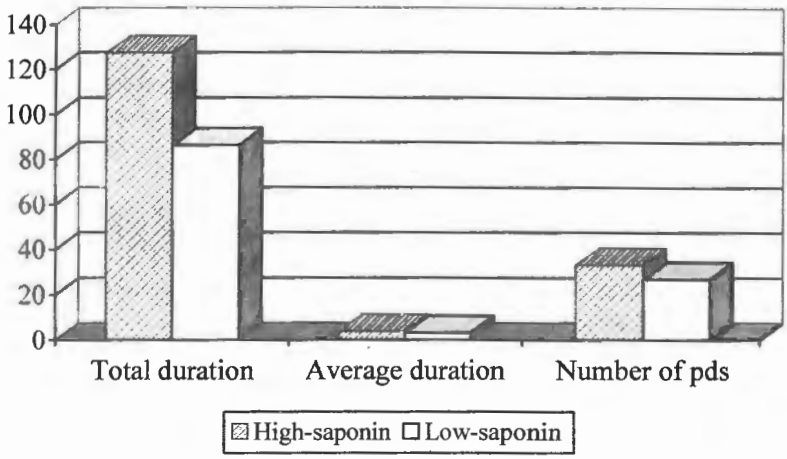


Fig. 3: Duration (min) and number of potential drops (pds) on high and low-saponin cultivars while the first hour of the tissues probing by the pea aphid.

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.51 - 57

Assessment of partial resistance to anthracnose in water yam (*D. alata*) using tissue culture generated whole plants

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Abstract: A study was conducted to evaluate components of partial resistance that will allow the analysis of quantitative trait loci (QTL) for anthracnose resistance in water yam (*D. alata*) using tissue culture generated whole plants in growth chamber. Five variables for assessing resistance (incubation period-IP, infection frequency - IF, single point severity score-SD7, disease progress rate-R_d and area under the disease progress curves-AUDPC) were compared. Also two methods of scoring (percentage whole plant area and percentage of individual leaf area) were compared for reproducibility of results on eleven genotypes of *D. alata* inoculated with 3 isolates of *Colletotrichum gloeosporioides*. All the variables and the two scoring methods identified continuous variation among the genotypes. However, there were differences in the degree of discrimination among genotypes by the variables and the reproducibility of the scoring methods. Consistent results across trials were obtained by scoring the percentage of whole plant area with symptom, but not with the mean percentage of individual leaf area. The components of partial resistance based on the two scoring methods were highly correlated ($r \geq 0.82$). AUDPC consistently showed a high correlation with all other components of partial resistance, and also with IP ($r = -0.77$) and IF ($r = 0.72$). AUDPC based on whole plant scoring method was recommended as the main variable for evaluating rate reducing resistance for QTL analysis in *D. alata*.

Keywords: Yam anthracnose, partial resistance, disease evaluation, scoring method

Introduction

Yam anthracnose caused by *Colletotrichum gloeosporioides* is globally the most important foliar disease of the food yams (*Dioscorea* spp). In the Caribbean, anthracnose is particularly important because of regional preference for *D. alata* which is the most susceptible of all the yam species. Yield losses of up to 90% have been reported from this region (McDonald *et al.*, 1998). The use of resistant varieties is believed to be the most reliable management approach to the disease. There have been concerted efforts to identify resistant varieties based on field evaluations (Ano *et al.*, 2002). Field evaluation is however limited by time and cost, and also by the spatial and temporal dynamics of the *C. gloeosporioides* isolates which make it difficult for selection under optimal conditions. Consequently, for analysis of the host-pathogen interaction and identification of quantitative trait loci (QTL) associated with resistant to anthracnose in yam, there is need for evaluation under controlled environment that will ensure optimal conditions for disease development. Previous studies suggest the existence of genotype by pathogen interaction for yam anthracnose disease (Abang, 1997; Mignouna *et al.*, 2001), the nature of this interaction is however poorly understood.

Various *in-vitro* methods have been described for assessment of yam response to anthracnose (Moura-Costa *et al.*, 1993, Green *et al.*, 2000, Abang *et al.*, 2001). Most of these methods are based on a single score at the time of maximum differential disease expression and in some cases involve inoculation of detached plant part. Evaluation at the point of critical disease expression has been criticised for its inability to identify the quantitative

nature of partial resistance (Walther *et al.*, 1999). Partial resistance is believed to be non-race specific and effective against a large number of pathogen genotypes (Peever *et al.*, 2000). An ideal method for assessment of partial resistance must take into consideration the beginning of disease development, the rate of disease progression and the final level of diseased area (Walther *et al.*, 1999).

In this study, we evaluated five disease assessment variables and two scoring methods for identification of partial resistance in inoculated tissue culture generated whole plants. Also we compared two scoring methods previously described for field assessment of quantitative resistance, for reproducibility of results.

Materials and methods

Plant material

In vitro plantlets of eleven genotypes of *D. alata* (Plimbite, Pyramide, AIA443, Cing, Cuba 1, Divin 1, Divin 2, Cross Lisbon, Noumea, Pacala Guyane and Wenefela bis) were raised from nodal explants culture from *D. alata* tissues culture bank of the Institut National de Recherche Agronomique (INRA). Plimbite which has been described as highly resistant in early studies in the Caribbean (Plumbley & Sweetmore, 1994) and Pyramide which is moderately susceptible under field conditions were used as resistant and susceptible checks respectively.

The potted plants were grown for 3 weeks in a bioclimatic chamber under controlled temperature (28/25 °C day/night) and 16-h photoperiod before inoculation. Each pot was treated as a sampling unit and prior to inoculation the pots were transferred into mini-serres (portable greenhouse). The mini-serres were arranged in blocks in the growth chamber. We used a completely randomized design with 2 blocks (i.e. 2 mini-serres) each with 4 replicates per genotype per isolate. The experiment was conducted twice in the same growth chamber.

Plant inoculation and disease assessment

Inoculation was performed with three isolates (Cg78, Cg118 and Cg206) representing different levels of virulence from INRA collection of *C. gloeosporioides* isolates from different agroecological zones of Guadeloupe. Inoculum was prepared from 7-day old single spore cultures of the fungal isolates grown on potato dextrose agar (PDA) in Petri plates at 25°C. Spore suspension was prepared by washing sporulating culture plates with sterile distilled water. The spore suspension was filtered through double-layered cheesecloth and spore concentration was adjusted to 10^5 spores per ml. Inoculation was carried out with hand sprayer and plants in the mini-serres were sprayed to run off, the mini-serres were then covered and the growth chamber was maintained at temperature of 28/25 °C day/night, 16-h photoperiod and 80-85% RH.

Plants were assessed for disease development daily from day 1- 5, then at 7 and 9 days after inoculation (DAI). Two methods of scoring (whole plant and individual leaf methods) described by Simons and Green (1994) were used to assess disease severity. In these methods, percentage of individual leaf area or whole plant area affected by disease was scored on a scale of 0–6 following a diagrammatic key as follow: 0 (0%), 1 (1%), 2 (2%), 3 (5%), 4 (10%), 5 (25%) and 6 (≥50%). Five variables (IP, IF, SD7, AUDPC and R_d) were used to assess resistance. Incubation period (IP) was defined as time in days required for 50% of plants in a treatment to show disease symptom. Infection frequency (IF) was defined as the percentage of plants in a treatment with disease symptom at 4 DAI which is a day after the appearance of disease on susceptible host. SD7 was defined as a single severity score at 7 DAI based on the scale above. AUDPC is the cumulative area under disease progress curve and was computed from the mean disease severity ratings as Area = $\sum_{i=1}^{n} [(x_i + x_{i+1})/2]t$, where x_i is the percentage plant area/mean percentage leaf area infected at time i, n is the

number of assessments made and t is the time between assessments (Shaner & Finney, 1977). Disease progress rate (R_d) was defined as the slope of regression line for disease severity against time.

Statistical analyses

Analyses of variance (ANOVA) were performed with general linear model (GLM) procedure of SAS statistical package, version 6.12 (SAS, 1989). All the variables were analysed for significant differences among the genotypes, pathogens, trials and interaction between genotypes and pathogens. Relationship between diseases parameters were tested by Person correlation coefficient analysis. Significantly different means were separated using Duncan's multiple range test.

Results

Incubation period (IP) and Infection frequency (IF)

The IP and IF showed variation among the genotypes and the pathogens, there was no variation between the trials. Also there was no differential interaction between host genotypes and pathogen isolates (Table 1). The IP ranged from 3-7 days depending on the pathogen isolate. For the most virulent pathogen isolate, the shortest IP was 3 days and the longest was 5 days, while for the less virulent isolate the shortest IP was 5 days and the longest was 7 days.

Components of partial resistance

Three components of partial resistance (SD7, AUDPC and R_d) were assessed based on the individual leaf and the whole plant scoring methods. With the whole plant scoring method, the three variables showed high variation among genotypes and pathogens. Also differential interactions were detected between host genotypes and pathogen isolates. The results from different trials were consistent for all the variables (Table 2a). Assessment based on scoring of individual leaf area showed variation between trials for all the three variables, also there was no difference among the genotypes based on the R_d (Table 2b).

Association between variables

The correlation analysis showed a strong relationship ($r \geq 0.70$) between the two scoring methods and among components of partial resistance based on each method. The incubation period showed significant association with only two components of partial resistance (SD7 and AUDPC), while the infection frequency only showed significant relationship with the AUDPC (Table 3).

Comparison of the genotypes across the three pathogen isolates showed that genotype rankings were similar for the components of partial resistance. The SD7 and R_d identified three genotypes (Noumea, Plimbite and Divin 2) with significantly lower disease levels relative to the susceptible check, while the AUDPC identified five genotypes (Noumea, Plimbite, Divin 2, Cross Lisbon and Divin 1) with significantly lower disease levels relative to the susceptible check (Table 4). Ranking based on IP identified two of these genotypes as being significantly better, while ranking based on IF indicated that all the genotypes had significantly lower infection frequency than susceptible check.

Table 1. Analysis of variance for incubation period (IP) and infection frequency (IF) on eleven *D. alata* genotypes inoculated with three isolates of *C. gloeosporioides*.

		IP		IFD4	
Source	**DF**	**MS**	**F-Val**	**MS**	**F-Val**
Genotype	10	0.71	5.57**	1358.85	3.68**
Pathogen	2	15.56	48.90**	10009.47	27.10**
Trial	1	1.23	3.38	151.52	0.41
Genotype x Pathogen	20	0.52	1.66	717.80	1.94
Genotype x Trial	10	0.13	0.40	609.85	1.65
Error	22	0.32		369.32	

** *F*-values are significant at $P \leq 0.01$

Table 2. Analysis of variance for single disease score at 7 DAI (SD7), area under disease progress curve (AUDPC) and disease progress rate (R_d) on eleven *D. alata* genotypes inoculated with three isolates of *C. gloeosporioides*.

			SD7		AUDPC		R_d	
	Source	**DF**	**MS**	**F-Val**	**MS**	**F-Val**	**MS**	**F-Val**
A	Genotype	10	1.76	3.48**	23.36	4.43**	0.01	2.74*
	Pathogen	2	163.82	323.75**	1586.07	300.59**	0.28	118.60**
	Trial	1	0.61	1.21	0.03	0.01	0.01	3.92
	Genotype x Pathogen	20	6.15	12.16**	67.84	12.86**	0.02	10.00**
	Genotype x Trial	10	0.51	1.01	5.72	1.09	0.01	0.72
	Error	143	0.51		5.28		0.01	
B	Genotype	10	1.31	4.13**	13.29	3.28**	0.01	1.76
	Pathogen	2	131.37	317.51**	958.47	260.79**	0.13	86.48**
	Trial	1	2.67	6.46**	60.01	16.33**	0.02	33.75**
	Genotype x Pathogen	20	4.11	9.93**	36.79	10.01**	0.01	6.08**
	Genotype x Trial	10	0.32	0.76	2.47	0.67	0.00	1.57
	Error	143	0.41		3.68		0.00	

A – Assessment based on whole plant scoring method;
B – Assessment based on individual leaf scoring method.
** - Significant at $P \leq 0.01$

Table 3. Correlation coefficients for relationships of variables estimating reactions of eleven *D. alata* genotypes to anthracnose disease in growth chamber.

			Whole plant (WP)			Individual leaf (IL)		
	IF	**IP**	**SD7**	**AUDPC**	**R_d**	**SD7**	**AUDPC**	**R_d**
IF	1.00							
IP	-0.68							
WP								
SD7	0.58	-0.72						
AUDPC	0.72	-0.77	0.97					
R_d	0.63	-0.64	0.94	0.94				
IL								
SD7	0.66	-0.75	0.98	0.97	0.92			
AUDPC	0.76	-0.78	0.94	0.98	0.92	0.98		
R_d	0.66	-0.59	0.82	0.85	0.86	0.89	0.91	1.00

Values ≥0.70 were considered significant

Table 4. Mean groupings for five disease parameters[1] of *D. alata* genotypes inoculated with 3 isolates[2] of *C. gloeosporioides* in the growth chamber.

Genotypes	**SD7**	**R_d**	**AUDPC**	**IP**	**IF**
Cubal	4.67a	0.13a	12.83a	4.70bc	37.50ab
Cing	4.54a	0.12ab	12.44ab	4.50c	37.50ab
Pyramide (CHK-1)	3.96bc	0.1bc	12.15ab	4.30c	50.00a
AIA443	4.25ab	0.1bc	11.88ab	5.00abc	33.33abc
Pacala Guyane	4.50a	0.1bc	11.17bc	5.00abc	8.33bcd
Wenefela bis	4.46a	0.12ab	11.15bc	4.70bc	29.17abc
Divin 1	4.25ab	0.12ab	10.13cd	5.00abc	12.50bcd
Cross Lisbon	3.75c	0.08c	9.50d	5.00abc	16.67bcd
Divin 2	3.33d	0.04d	7.54e	4.80abc	16.67bcd
Plimbite (CHK-2)	2.13e	0.02de	5.19f	5.30ab	0.00e
Noumea	1.71f	0.01e	4.02f	5.50a	16.67bcd

[1] SD7 – disease severity 7 DAI on a scale of 0 – 6 using the whole plant scoring method (Simons and Green, 1994); R_d – disease progress rate; AUDPC – Area under disease progress curve; IP – Incubation period; IF – Infection frequency

[2] Values are means across the three pathogen isolates, means in the same column followed by the same letter are not significantly different ($p = 0.05$)

CHK-1 – Susceptible check

CHK-2 – Resistant check

Discussion

Five parameters and two scoring methods were compared for identification of differential response in genotypes that will allow the analysis of quantitative trait loci (QTL) associated with anthracnose resistance in water yam (*D. alata*). The parameters and the two methods identified continuous variation among the genotypes. However, there were differences in the

degree of discrimination among genotypes by the parameters and the reproducibility of the scoring methods.

Both the individual leaf and the whole plant scoring methods identified high significant differences among genotypes and there was a strong correlation between parameters generated based on the two methods. This is in agreement with the result of Simon & Green (1994) which identified good association between individual leaf and the whole plant scoring methods under field conditions. Although, previous studies with the individual leaf scoring method (Simon & Green, 1994; Abang *et al.*, 2001) did not mention reproducibility of the method, in this study we did not obtain reproducible result with the individual leaf scoring as indicated by highly significant trial effect. Reproducibility is very important to assessment methods that will relate crop response to disease. In addition to the consistent results we obtained with the whole plant scoring method, it is relatively rapid and appropriate for monitoring severity of anthracnose on individual small yam plants (Simon & Green, 1994) typical of the plant size used in this study.

The incubation period (IP), infection frequency (IF), and the three components of partial resistance (SD7, AUDPC and Rd) generated based on the whole plant scoring method, effectively identified differences in the genotypes and in the virulence of the isolates, and results were consistent across trials. There were however significant interactions between genotypes and pathogens for all the components of partial resistance. The variation in pathogen isolates and their significant interactions with the genotypes confirmed previous results by Green *et al.*, (2000), and this is an indication that the reaction of genotypes can vary depending on the isolate used. Consequently there is need to ensure that the genotypes are exposed to all potential variations in pathogen population during screening (Peever *et al.*, 2000). There were strong correlations between the various components of partial resistance, which shows that resistance factors identified by these parameters may be under the same genetic control. Therefore any one of these parameters could be used for selection. Ranking the mean responses of the genotypes across the three isolates showed that the AUDPC identified five genotypes with significantly lower disease reaction compared to the susceptible check. The single point scoring and disease progress rate identified only three of these genotypes as significantly better than the susceptible check. This is an indication of the sensitivity of multiple points scoring in identifying small but distinct variation between genotypes. This, in addition to the good correlation of AUDPC with all other disease variables, showed that AUDPC will be a good parameter for identifying resistance to yam anthracnose.

The IP has been identified as indirect parameter for selecting for partial resistance in other crops (Broers & Lopez-Atilano, 1994); this is in line with the good association established between IP and partial resistance in this study. The non significant interaction between host genotypes and pathogen isolates obtained for IP tends to imply that QTL identified with this parameter will not be strain-specific. However, this requires more investigation to further understand the importance of incubation period to anthracnose resistance.

In conclusion, we recommend the use of AUDPC based on whole plant scoring method as the main variable for evaluating partial disease resistance for QTL analysis in yam anthracnose.

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Full modification of the coding sequence for enhancing potato expression of insect control protein cry3a gene and prediction of its expression in plants using yeast transformation

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Abstract: Current efforts to develop genetic pools of crops through biotechnology are based primarily on transforming plants with a single gene encoding new protein (insecticidal, herbicidal and fungicidal enzyme or toxin). *Bacillus thuringiensis* is characterized by the production of parasporal crystals composed of proteins that have highly specific insecticidal activity against larvae of certain Lepidoptera, Coleoptera, or Diptera, and several other invertebrate groups including nematodes, mites and protozoans. After ingestion by a susceptible insect, crystalline inactive δ-endotoxin is solubilized and proteolytically cleaved to an active toxin form. The activated toxin binds to receptors in the midgut and ion channels are formed, causing midgut cells to lose their membrane potential.The insecticidal protein from the *B.t var. tenebrionis* (Cry3A) is a specific toxin for coleopteran insects (the yellow mealworm, the Colorado potato beetle, and the cottonwood leaf beetle) yet exhibits no toxicity toward humans, other vertebrates or beneficial insects. This gene contains some sequences that reduce their expression in plants. These are splicing and polyadenylation sites, the ATTTA sequences, mRNA degradation signals and transcription termination sites as well as the codons that are rare in plants. The truncated *cry3a* gene was obtained from the *B.t* genome (*var tenebrionis*) by means of PCR. Our preliminary results on expression of the truncated gene in eukaryotic cells (yeast) showed that the expression level was low. To enhance the expression, we redesigned and modified the *cry3a* gene serquence. The purpose of our work was to create a synthetic gene that would be highly expressed in potato cells and thus confer resistance to Colorado potato beetle larvae. The modified *cry*3A coding region has a codon usage pattern altered to resemble that of the average dicot gene. The dinucleotide frequencies used at the second and third positions in codons of dicot and monocot genes were considered in the *cry*3A gene design. The CG dinucleotide is strongly avoided in plant genes possibly due to regulation involving methylation. The dinucleotide TA is also avoided in codon position 2 and 3 in most eukaryotes. In parallel with codon usage analysis of the native *cry*3A gene revealed that the coding region has 64% A-T. This level of A-T is about 10% higher than that found in a typical plant gene's coding region. High A-T content is found in plant intergenic and regulatory regions. The A-T content of synthetic *cry*3A gene was decreased from 64% to 51%. All known DNA sequences that might contribute to RNA instability in plants were eliminated from the synthetic gene. Finally a single modification, to introduce guanine in lieu of adenine at the forth nucleotide position in the *cry*3A coding sequence was made to form a consensus plant initiation sequence. The redesigned gene differs from the native gene in 21% of its nucleotides. A new strategy was used for the chemical synthesis of the modified gene. First the gene sequence was divided into four fragments about 500 bp long and then, every fragment was synthesized by means of phosphoramide method. The native and synthetic genes were then fused with the reporter gene *licB* which codes for a *Clostridium thermocellum* thermostable lichenase, and cloned into bacterial and yeast expression vectors. A comparative analysis of expression in *E.coli* cells has shown that the synthetic gene was 1.5 times less active than the native gene, which may reflect the fact that the codon composition of the synthetic gene was optimal for eukaryotes. Quantitative and qualitative assays of lichenase in the hybrid proteins and of gene expression in yeast cells has shown that the synthetic gene was expressed approximately 10-fold greater than the truncated gene. These results suggest that a high level of expression of the synthetic *cry3a* gene can be expected in plant cells.

The art of making things simple: Insect resistance tests and their practical implementation

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Abstract: In tests developed for detecting resistance to *Frankliniella occidentalis* in rose varieties, the parameter for quantifying silver damage showed a consistent picture, when ranking cultivars in series of experiments. Silver damage is a type of feeding damage by thrips, on leaves of the non-flowering stage of plants. The consistency also holds for other cut flowers under glass, such as gerbera and chrysanthemum. Feeding damage by *F. occidentalis* on flowers, however, is considered much more important by growers (and breeders) because of aesthetic injury levels and consequently market value. Flower damage caused by *F. occidentalis* depends on season and numbers of thrips in the flowers. We undertook a project to describe the relationship between silver damage, flower damage and thrips population level in flowers, in order to make tests easier to perform (using fewer parameters) under 'field conditions'.

Because the relationship between the three selected parameters were found to be complex, we developed a way to show all test results per cultivar simultaneously, in a visualized form. Breeders and growers can draw their own conclusion from a simple pie diagram and judge for themselves the relative importance of each parameter for selecting overall resistance to this important pest.

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Some biochemical and physiological aspects of cucumber resistance to spider mites induced by plant growth promoting rhizobacteria (PGPR)

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Abstract: The influence of plant growth promoting rhizobacteria (PGPR), developing in root system of glasshouse cucumber, on interaction between host plant and spider mites was studied. Seeds of cucumber plants were inoculated with *Pseudomonas fluorescens*, known as an inductor of plant resistance to some diseases, insects and spider mites. Intensity of photosynthesis and some biochemical changes of spider mite infested and bacteria treated plants were studied in comparison with non-bacterized and not mite-infested plants. Total phenol and cucurbitacin content as well as activity of poliphenoloxidase (PPO) and peroxidase (POX) were estimated in mature and young cucumber leaves of bacterized and non-bacterized plants. Intensity of photosynthesis was decreased in the mite infested plants, but this negative effect of mites was Increase in phenol and cucurbitacin content was observed in mite- and bacteria-treated plants as compared to control plants. It was more expressed in young leaves. The activity of PPO strongly increased in young leaves of mite-infested and bacteria-treated plants. The plant response was connected with induction of cucumber resistance to spider mites. Changes in the level of secondary components in cucumber leaves, induced by PGPR, can be responsible for the decrease in mite population and have some importance in spider mite – cucumber plant interactions.

Keywords: induced resistance, cucumber, spider mites, PGPR

Introduction

Plant growth promoting rhizobacteria are known as the inducers of resistance to many patogens and also to some arthropod pests (Wei *et al.,* 1991; Borowicz *et al.,* 1992; Zehnder *et al.,* 1997; Van Loon *et al.,*1998; Tomczyk & Kielkiewicz, 2000). Rhizobacteria-mediated induced systemic resistance (ISR) can occur in many plant species. Induced systemic resistance is connected with jasmonate acid and ethylene, in contrast with systemic acquired resistance (SAR), which is related to endogenously synthesized salicylic acid (SA). Some rhizobacteria can induce resistance through the SA-dependent SAR pathway (Van Loon *et al.,* 1998), but it was, however, indicated that plant growth promoting rhizobacteria can also induce SAR which does not require SA (Pieterse *et al.,* 1996).

Both; salicylic and jasmonic acids affect the processes of photosynthesis, transpiration, respiration and plant growth (Popova *et al.,* 2003). Besides SA, also other phenolic compounds can be accumulated in plants infested with herbivores (Tomczyk, 1989; Bi & Felton, 1995) or treated with rhizobacteria (Sarma *et al.,* 2002). A large number of plant enzymes such as: lipoxygenaze (LOX), peroxidase (POX), poliphenol oxidase (PPO) and phenylalanine ammonia-lyaze (PAL), have been associated with ISR (Silva *et al.,* 2004).
Biochemical changes in plant tissues induced by PGPR are responsible for a decrease of plant value for patogens and herbivores and can change development of their population on the host (Zehnder *et al.,* 1997).

Materials and methods

Cucumber plants cv. Aramis were grown in two glasshouse chambers. In each chamber, plants were cultivated either with or without rhizobacteria. Bacteria *Pseudomonas fluorescent* (isolate 112 derived from cucumber seedling rhizosphere) was used in the experiment according to the method described earlier (Tomczyk & Kielkiewicz, 2000).
In one of the chambers, all plants (with and without bacteria) were infested with 10 *Tetranychus urticae* (Koch.) females. This way 4 groups of plants were obtained: control (without bacteria and without mites), control B (with bacteria and without mites), infested (without bacteria but with mites), infested B (with mites and with bacteria). Six weeks after plant infestation with the mites, the intensity of photosynthesis in the leaves of plants from every experimental group was measured and leaf samples were collected for biochemical analyzes.

Photosynthesis measurements
The intensity of photosynthesis was measured in mature leaves of plants from every group. Measurements were conducted in the glasshouse, using CO_2 analyzer LICOR- 6200.
Five replications were done for every group of plants.

Estimation of total phenol and cucurbitacin content
Phenols were extracted with 80% methanol and estimated according to Johnson and Schaal (1957). Chlorogenic acid was used as a standard. Cucurbitacins were extracted with methanol, separated on TLC plates 254 F, removed from plates according to Górski at al. 1985 and the total amount of cucurbitacin was estimated according to Metcalf et al. (1987). Cucurbitacin E was used as a standard.

Estimation of PPO and POX activity
Activity of enzymes was measured in the extracts made on fresh young (top) and older (mature) leaves, according to Brenneman and Black (1979). The plant material was homogenized in 0.1 M sodium phoshate buffer pH = 7, at 4°C. The activity of enzymes was measured at 24-25 °C, spectrophotometrically: PPO at 420 nm, POX at 495 nm.

Results and discussion

The data of photosynthesis measurements are presented in Fig. 1. The lowest intensity of photosynthesis was found in mite-infested leaves of cucumber plants cultivated without bacteria. The presence of PGPR in root system of cucumber decreases negative effect of spider mites on the process of photosynthesis by about 17 %. It was probably connected with lower level of injures occurred on the leaves of bacteria treated plants, as compared to untreated, rather than with stimulation of photosynthesis. However it is known that the presence of PGPR in root system of different plants affects growth of plants as well as the activity of photosynthesis and respiration (Popova *et al.*, 2003).

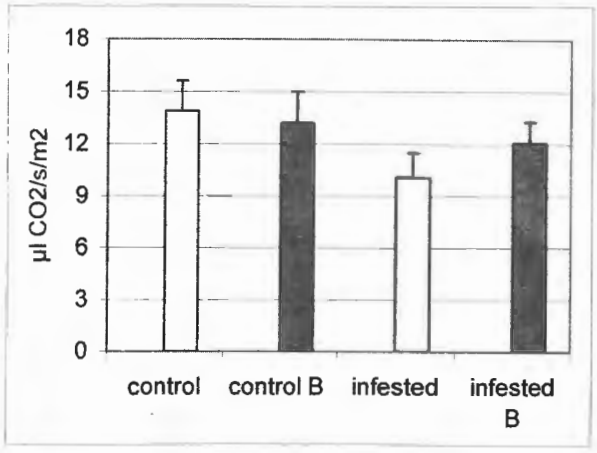

Fig. 1: Intensity of photosynthesis in cucumber leaves. Standard errors (SE) are presented.

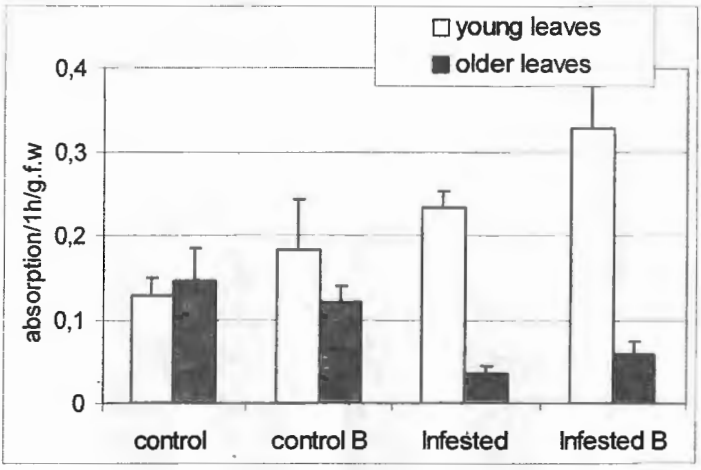

Fig. 2: Intensity of PPO in cucumber leaves. Standard errors (SE) are presented.

The influence of PGPR and spider mites on the activity of enzymes is presented in Fig. 2 and Fig. 3. Strong increase in the activity of PPO was detected in young (top) leaves of the mite infested plants in contrast to mature, damaged leaves. This phenomenon indicates a possibility for induction of systemic resistance in both mite-infested and bacteria-treated plants. The increase in PPO activity was often detected as the effect of plant infestation by different herbivores or by presence of plant growth promoting rhizobacteria in the root system of different plants (Tomczyk, 1989; Bi & Felton, 1995.). The activity of POX increased as a result of leaf injury by spider mites but this increase had a local character. It occurred only in mite-infested (older) leaves in the contrast to young (top) leaves of the infested plants. PGPR

had no influence on this enzyme, however, it could be dependent on the age of plants and the time of leaf sampling.

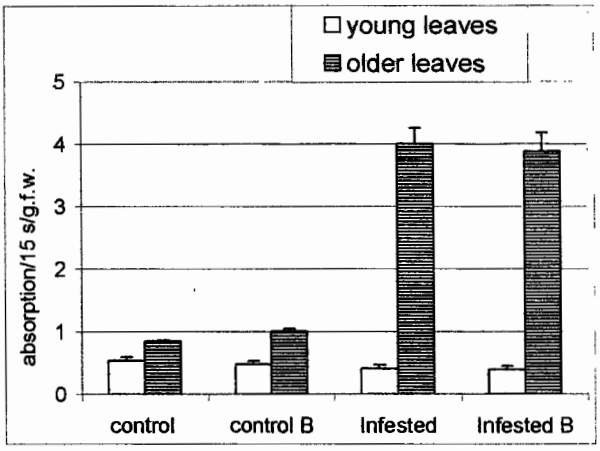

Fig. 3: Intensity of POX in cucumber leaves. Standard errors (SE) are presented.

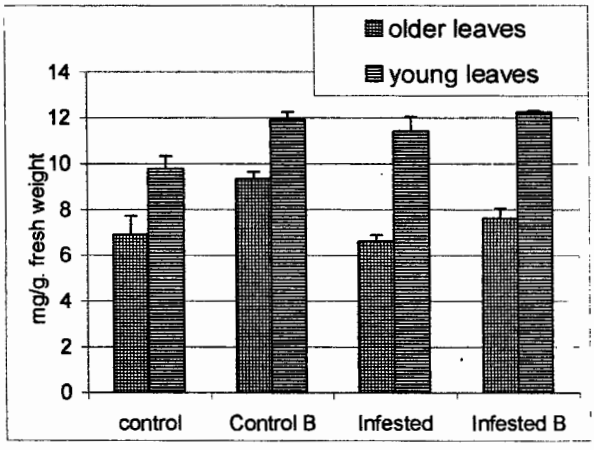

Fig. 4: Content of total phenols in cucumber leaves. Standard errors (SE) are presented.

In Figures 4 and 5 the total contents of phenols and cucurbitacins in the leaves of experimental plants are presented. The amount of phenols increased in mite infested and Bacteria-treated plants in both mature and young leaves. The phenomenon was more evident in young leaves. The increase in concentration of phenolic compounds in the tissues of plants injured by different herbivores is well known and described as a defence mechanism of infested plants (Bi & Felton, 1995). Accumulation of phenols in plants is also known as a

result of induced resistance by different inducers, especially by plant growth promoting rhizobacteria (Sarma *et al.*, 2002).

Inoculation of plants with plant growth promoting rhizobacteria as well as the infestation with spider mites increased concentration of cucurbitacin in cucumber leaves to the same level. The combination of two factors - bacteria and mites - gave the same data as treatment with each factor separately.

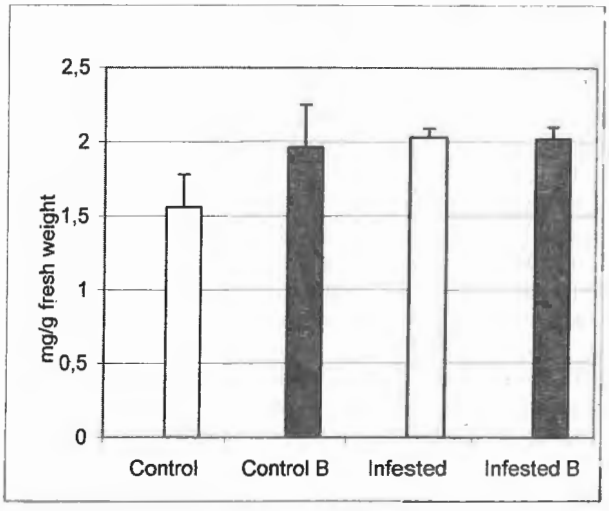

Fig. 5: Content of cucurbitacins in cucumber leaves. Standard errors (SE) are presented.

In the experiments conducted on cucumber in field conditions, opposite influence of PGPR on concentration of cucurbitacin in the leaves was detected (Zehnder *et al.*, 1997). Probably different strains of bacteria, conditions of cultivation and cucumber varieties can have influence on the process of induction and on the level of secondary metabolites in plant tissues.

Biochemical changes observed in young cucumber leaves (increase in phenol concentration and activation of PPO) can be responsible for increase of plant resistance to spider mites.

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RAPD analysis of Russian and Polish isolates of *Sclerotinia sclerotiorum* from crucifers

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Abstract: Oilseed rape in Poland and in Russia share a common pathogen - *Sclerotinia sclerotiorum* (Lib.) de Bary, the cause of sclerotinia stem rot or grey mould disease. The fungus attacks both spring and winter forms of *Brassica napus* and many other plant species, including cruciferous weeds. We report the characterization and comparison of Russian and Polish field populations of the fungus originating from oilseed rape, using RAPD analysis. In total, 108 isolates originating mostly from Russia (49 isolates) and Poland (36), but also from China (16), Czech Republic (3), Austria (2), Slovakia (1) and Croatia (1) were studied using seven decamer primers. Most of the isolates originated from winter oilseed rape (64 isolates), some from spring oilseed rape (35 isolates), 7 isolates from other crucifers, 1 isolate from lupin and 1 isolate from sunflower. In addition to RAPD analysis, the isolates were compared based on the sequence similarities of the ITS1-5.8s-ITS2 region. The RAPD method allowed differentiation of the 108 isolates into 83 groups, based on 80 polymorphic DNA fragments. Isolates from different geographical regions in Russia and the isolates from China formed distinct clusters, with only a few exceptions. Half of Polish isolates belonged to three clusters, the others were highly polymorphic. One Russian and one Chinese isolate showed a single basepair change in the ITS1 region, and one isolate from Austria showed a single basepair change in the ITS2 region. None of Polish isolates showed variation in the ITS sequences studied. The results showed a high genetic variation of *Sclerotinia sclerotiorum* isolates. Not all isolates from the same geographic origin or host plant belonged to the same cluster. Differences between *S. sclerotiorum* populations from geographic origins were much greater than differences between the isolates derived from various host-plants.

Key words: *Brassica napus*, oilseed rape, sclerotinia stem rot, Internal Transcribed Spacer, ITS sequence, DNA polymorphism

Introduction

Oilseed rape is the most important source of domestic plant oil in Poland, with an increasing acreage of around 0.5 mil ha. Other oilseed crops like soybean and sunflower are not important. Recently the cultivation of oilseed rape became even more popular due to the possibility of using the oil as a biofuel. At present oilseed rape is already used in Europe on large scale in the UK and Germany and also produced in substantial amounts by Italy, Austria, Czech Republic and Slovakia (Seedlink 2002). Forecasts predict a doubling of the cultivation area in Poland, if biofuel production is introduced successfully. Some authors are even more optimistic and forecast the potential cultivation area of oilseed rape in Poland to approximately 2 mln ha (Kozłowski *et al.* 2004).

In contrast, the popularity of oilseed rape in Russia is not very high and the growing area in recent years averaged between 0.1-0.2 mil ha. The main oilseed crops in Russia are sunflower and soybean at the moment. However, similar to Poland, oilseed rape is regarded as a crop with great prospective and an increase in cultivation of up to 2-3 mil ha has been suggested (Artiomov 1998, Garejev 1996). The main reason for the small acreage of oilseed rape in Russia at present is its unsatisfactory profitability, due to low yielding of this crop. This is a result from the cultivation of lower yielding spring oilseed rape rather than high yielding winter oilseed rape cultivars, which in most cases cannot survive the severe and frosty Russian winters. However, in some areas in Central Chernozem region the average yield of spring rape can reach 19 dt/ha (Artiomov *et al.* 2000) and with well balanced fertilization it can be as high as 27 dt/ha (Savenkov and Shevtchenko 1999). Spring oilseed rape constitutes 72 % to 97 % of the overall oilseed rape grown, depending on the year (Jedryczka *et al.* 2002). Most of spring rape is cultivated in the Volga region (30 % of area under oilseed rape) and also in Central and West Siberia regions, *ca.* 15 % area in each region. Other main areas of importance are East Siberia, the Ural and the North West region, with 6%, 5 % and 1 % of the area cultivated with oilseed rape, respectively. The rest of the fields are dispersed in other areas. There is one region where oilseed rape can survive the winter, and where winter oilseed rape can make up 80 % of the total oilseed rape grown. This is a region located on the east coast of the Black Sea, comprising the Krasnodar region (Kuban) and the Stavropol region (Proizvodstvo 1998). The total area of oilseed rape cultivation in this region ranges from 10 % to 15 %, depending on the year.

In Poland winter type of *Brassica napus* predominates over the spring type. It is a relatively new crop introduced to larger growing areas in Poland in 1996, following great frost damage of the winter type. Since then, the spring type remained in cultivation, but the primary acreage of 0.1 mil was reduced in subsequent years by 50% (Sadowski *et al.* 2002).

The intensive cultivation of oilseed rape results in greater presence of noxious insects and pathogens. The most important diseases of oilseed rape in Poland are stem canker and sclerotinia stem rot, followed by black spot, grey mould, downy mildew and light leaf spot in some regions and years (Frencel *et al.* 1991, Karolewski 1999, Sadowski *et al.* 2002). In Russia, the most important diseases are sclerotinia stem rot, fusarium wilt and clubroot, followed by black spot, downy mildew and a root rot complex (Portenko 1997, Nikonorenkov *et al.* 1997, Portenko and Nikonorenkov 1998, Gasich and Levitin 2000, Jedryczka *et al.* 2002, Gasich *et al.* 2003).

Both in Russia and Poland, sclerotinia stem rot (white mould), is regarded as a serious disease of oilseed rape. It also attacks other crucifers and a wide range of hosts from numerous plant families. The disease is caused by the ascomycete fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, a necrotrophic pathogen with cosmopolitan distribution. The fungus has a very fast growth rate and produces oxalic acid, which readily degrades plant cells and makes them defenceless to the pathogen attack (Ziman *et al.* 1998). Due to these characters the pathogen can cause very serious disease symptoms which result in high yield loss. At present, sclerotinia stem rot is the most damaging disease of oilseed rape in China - the largest producer of oilseed rape worldwide (Liu *et al.* 2001). However, the disease is also observed in other major regions of rapeseed production, including central and eastern Europe (Ziman *et al.* 1999). Unlike in China, *Sclerotinia* epidemics are not observed every year. The disease can be damaging in seasons with wet springs, when fruiting bodies (apothecia) can be formed, resulting in the release of numerous *S. sclerotiorum* ascospores, which are distributed by the wind. Ascospores are the source of primary infection of plants and the first trigger of subsequent crop loss.

For a long time the determination of species within the genus *Sclerotinia* was based on morphological differences in the sclerotia and ascospores (Kohn 1979). So far, no

physiological specialization has been found in *S. sclerotiorum* (Mordue and Holliday 1976). However, it was demonstrated that isolates can express different levels of virulence to various host plants (Waipara *et al.* 1993). Nevertheless, in contrast to some other fungal species (Harry and Clark 1986, Køster *et al.* 1986, Ansan-Melayah *et al.* 1995, Balesdent *et al.* 2001, Wicker *et al.* 2003, Sharma *et al.* 2004) no reliable standard set of differential plant hosts has been defined. Hence, more discrimination was expected from molecular techniques, as they allow to study the genetic variation of fungal isolates in greater detail.

In this study we have used RAPD (Random Amplified Polymorphic DNA) analysis to characterise the differences between isolates of *S. sclerotiorum* originating from oilseed rape in Russia and Poland. By now, RAPD methods have successfully been used in the analysis of *S. homeocarpa* - the cause of dollar spot disease of turfgrass (Hsiang and Mahuku 1999) and *S. cepivorum* - the cause of white rot on onion (Tyson *et al.* 2002). The RAPD analysis using a few or several isolates of *S. sclerotiorum* have also been reported (Fraissinet-Tachet *et al.* 1996, Noonan *et al.* 1996, Starzycka *et al.* 2000).

In this study we used 99 isolates of *S. sclerotiorum* from oilseed rape, mainly from Russia and Poland, together with a few isolates from other crucifers or hosts belonging to other plant families. Additionally, the isolates were compared based on their ITS (Internal Transcribed Spacer) sequences. Sequence comparison of the ITS region has been used for many molecular systematic studies at the species level (Kretzer *et al.* 1996, Balesdent *et al.* 1998, Skouboe *et al.* 1999, Mendes-Pereira *et al.* 2003, Vellinga *et al.* 2003), and can be used to analyse the genetic variation within species (Curran *et al.* 1994, Morales *et al.* 1993 and 1995, Tsolaki *et al.* 1996). Some standard primers have been described which are widely used in studies of rDNA sequences in fungi (White *et al.* 1990, Vilgalys *et al.* 1994), and several taxon-specific primers have also been described (Gardes and Bruns 1993).

Materials and methods

Origin of Sclerotinia sclerotiorum isolates

Most of strains (91,6 %) were isolated from *Brassica napus*, of which 64 isolates from winter type (forma *biennis*) and 35 isolates from spring type (forma *annua*). Other cruciferous plants included *Erysimum hieracifolium* (2 isolates), *Raphanus raphanistrum* (2), *Brassica campestris* (1), *Brassica rapa* (1) and *Thlaspi arvense* (1). For comparisons, two exemplary isolates originating from other plant families (*Helianthus annus* from Compositae and *Lupinus mutabilis* from Fabaceae) were included in this study. Most of isolates from Russia were obtained in the same year (2003), but they were collected from three distant geographic regions in the European part of Russia. The isolates from the north (Byelogorka near St. Petersburg) originated from spring rape (18 isolates), the ones from the central region (near Moscow) were derived from both spring and winter rape (one isolate per each form) and the ones from the south (near Krasnodar) were obtained solely from winter oilseed rape (22 isolates). Remaining isolates from Russia originated from the other crucifers listed above. All *S. sclerotiorum* isolates from Poland were obtained from the same host plant species: winter oilseed rape (*B. napus* forma *biennis*), but they were isolated in different regions and in six years (1994, 1995, 2000, 2002, 2003, 2004) over the period of 11 years (1994-2004). The only exception was 1 isolate from *Lupinus mutabilis*. The isolates from Russia and Poland were characterised together with several selected isolates from oilseed rape obtained from other geographic origins worldwide, including 16 isolates from spring rape in China and isolates from winter oilseed rape from the Czech Republic (3 isolates), Austria (2) and Croatia (1). The details on the origin of the isolates used in this study are presented in Table 1. Isolates from oilseed rape collected at the same locality in Russia and Poland originated from the same field.

Table 1. The origin of *Sclerotinia sclerotiorum* isolates used in this study

Country	Locality	Province/Region	No. of isolates	Symbol of isolate	Host plant	Year
China	Chaohu	Anhui	1	Ch 1	*B. napus (annua)*	2003
	Laian	Anhui	2	Ch 3	*B. napus (annua)*	2003
	Anqing	Anhui	1	Ch 4	*B. napus (annua)*	2003
	Xuancheng	Anhui	1	Ch 5	*B. napus (annua)*	2003
	Wuhe	Anhui	1	Ch 6	*B. napus (annua)*	2003
	Fuyang	Anhui	1	Ch 8	*B. napus (annua)*	2003
	Chuzhou	Anhui	2	Ch 11	*B. napus (annua)*	2003
	Hefei	Anhui	1	Ch 12	*B. napus (annua)*	2003
	Hefei	Anhui	3	Ch 15	*B. napus (annua)*	2000
	Xi'ning	Qinghai	2	Ch 14	*B. napus (annua)*	2000
	Xi'ning	Qinghai	1	Ch 9	*B. napus (annua)*	2003
Russia	Byelogorka	North-West	17	P	*B. napus (annua)*	2003
	Byelogorka	North-West	1	48.1	*B. campestris*	2000
	Byelogorka	North-West	1	48.2	*B. napus (annua)*	2000
	Byelogorka	North-West	2	P5-1, P5-2	*Erysimum hieracifolium*	unknown
	Byelogorka	North-West	2	P6-1, P6-2	Raphanus raphanistrum	unknown
	Byelogorka	North-West	1	P7-1	Thlaspi arvense	unknown
	Krasnodar	Kuban	22	K	*B. napus (biennis)*	2003
	Lugovaya	Moscow Region	1	M1-1	*B. napus (annua)*	2004
	Lugovaya	Moscow Region	1	M2-1	*B. napus (biennis)*	2004
	Lugovaya	Moscow Region	1	M4-3	*B. rapa (biennis)*	2004
Poland	Cerekwica	Wielkopolskie	1	Sc-1	*B. napus (biennis)*	1994
	Rarwino	Zach.-Pomorskie	1	Sc-2	*B. napus (biennis)*	1994
	Palikije	Lubelskie	1	Sc-3	*B. napus (biennis)*	1994
	Krylow	Lubelskie	1	Sc-4	*B. napus (biennis)*	1994
	Wierszczyca	Lubelskie	1	Sc-5	*B. napus (biennis)*	1994
	Kalinowa	***Lodzkie***	1	Sc-6	*B. napus (biennis)*	1994
	Bezek	Lubelskie	1	Sc-8	*B. napus (biennis)*	1994
	Steszew	Wielkopolskie	1	Sc10	*B. napus (biennis)*	1995
	Pepowo	Wielkopolskie	1	Sc13	*B. napus (biennis)*	1995
	Gorka	Mazowieckie	1	Sc14	*B. napus (biennis)*	1995
	Sroda Slaska	Dolnoślaskie	1	Sc15	*B. napus (biennis)*	1995
	Jugowa	Dolnoslaskie	3	Sc16, Sc17, Sc22	*B. napus (biennis)*	1995
	Katy Wroclawskie	Dolnoslaskie	1	Sc-18	*B. napus (biennis)*	1995
	Olesnica Mala	Dolnoslaskie	1	Sc20	*B. napus (biennis)*	1995
	Lubcz	Dolnoslaskie	1	Sc23	*B. napus (biennis)*	1995
	Borowo	Wielkopolskie	2	SBor3, SBor4	*B. napus (biennis)*	2003
	Borowo	Wielkopolskie	3	Sc32, Sc34, Sc36,	*B. napus (biennis)*	2004
	Marchwacz	Wielkopolskie	1	LL2	*B. napus (biennis)*	2002
	Szczebrzeszyn	Lubelskie	1	LL15	*B. napus (biennis)*	2002
	Stolpie	Lubelskie	1	LL27	*B. napus (biennis)*	2002
	Grabow	Lubelskie	1	Gr17114	*B. napus (biennis)*	2004
	Osiny	Lubelskie	3	Os1-12, Os1-5, Os5-1	*B. napus (biennis)*	2004
	Gronowo	Zach. Pomorskie	1	P1	*B. napus (biennis)*	2000
	Zlotniki	Wielkopolskie	5	Zl	*B. napus (biennis)*	2004
	Przebedowo	Wielkopolskie	1	Sc31	Lupinus mutabilis	2003
Austria	Vitis	Niederösterreich	1	AUS110	*B. napus (biennis)*	1998
	Limpfings	Niederösterreich	1	AUS115	*B. napus (biennis)*	1998
Croatia	Mursko Sredisce	North	1	Sc175	*B. napus (biennis)*	1998
Czech Rep.	Jakubov	Silesia	1	Cz116	*B. napus (biennis)*	1997
	Lastany	Moravia	1	Sc143	*B. napus (biennis)*	1997
	Bolatice	Moravia	1	Sc158	*B. napus (biennis)*	1997
Slovakia	Bajany	East	1	B19	Helianthus annus	1995

Conditions of RAPD analyses

For molecular analysis, DNA was extracted with the DNeasy® Plant Mini Kit (Qiagen) from three day-old mycelium of *S. sclerotiorum* isolates, grown in shaking liquid Czapek-Dox medium supplemented with yeast extract (2 g/L).

For RAPD analysis, we used seven decamer primers OPC-02, OPC-04, OPC-15, OPJ-13, OPJ-14, OPL-11 and OPL-12 (Qiagen Operon). The choice of primers was based on a previously described experiment which investigated polymorphism in *S. sclerotiorum* isolates from China (Irzykowski *et al.* 2004). Seven primers were selected out of 60 primers studied on a subset of isolates, based on the largest number of unambiguous polymorphic bands. The sequences of these primers are shown in Table 2.

Table 2. Sequences of the RAPD primers used in the study

No.	Symbol of the primer	Sequence 5' to 3'
1	OPC-02	GTGAGGCGTC
2	OPC-04	CCGCATCTAC
3	OPC-15	GACGGATCAG
4	OPJ-13	CCACACTACC
5	OPJ-14	CACCCGGATG
6	OPL-11	ACGATGAGCC
7	OPL-12	GGGCGGTACT

The RAPD reactions were performed under mineral oil, in 4.5 µL final volumes containing 0.3 µL of template DNA, 200 µM of dNTP, 1 µM of a primer, 0.5 U of Taq polymerase (Qiagen) in 1×PCR buffer (Qiagen). Amplifications were performed in a MiniCycler™ (MJ Research) using the following programme: initial DNA denaturation step 2 min at 94 °C, followed by 45 cycles of 30 s at 94 °C, 1 min at 36 °C, and 2 min at 72 °C, with a final extension at 72 °C for 5 min. The RAPD-PCR products were stored at 4 °C.Amplified fragments were separated by electrophoresis on 2% agarose gel (Life Technologies) in 1.0×TBE buffer, stained with ethidium bromide and visualized using the digital gel documentation system Scion Image release Beta 3b (Scion Corporation). The polymorphic bands were scored and analysed by TREECON for Windows version 1.3b software (Van de Peer and de Wachter 1994). RAPD analysis was performed for all isolates listed in Table 1.

Analysis of ITS fragment sequences

For analysis of polymorphism within the ITS region, we amplified a DNA fragment consisting of the 3' end of the 18s rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 5' end of 28S rRNA gene. Primer WIRZ G1 (5'-GTAACAAGGTTTCCGTAGGTG-3') was designed as a forward primer for PCR amplification and sequencing and was based on the *Leptosphaeria maculans* Leroy 18s rRNA gene (Morales *et al.* 1995). The reverse primer PN10 (5'-TCCGCTTATTGATATGCTTAAG-3') (Balesdent *et al.* 1998) was used both for PCR amplification and sequencing and both primers were purchased from Qiagen Operon. Amplifications were performed under mineral oil, in 4.5 µL final volumes containing 0.3 µL of template DNA, 200 µM of dNTP, 2 µM of a primer, 0.5 U of Taq polymerase (Qiagen) in 1×PCR buffer (Qiagen). The reaction was performed in a MiniCycler™ (MJ Research), according to the following program: denaturation step at 94 °C for 2 min, followed by 45

cycles of 30 s at 94 °C, 30 s at 60 °C and 1 min at 72 °C, with a final extension at 72 °C for 5min. The amplified ITS fragments were stored at 4 °C. PCR products were cleaned from dNTPs using the protocol detailed in Irzykowski *et al.* (2004). Sequencing of the ITS fragments was carried out using a Perkin Elmer ABI Prism 310 Genetic Analyzer. The sequencing reaction was done using an ABI PRISM BigDye V3.0 Terminator Cycle Sequencing Ready Reaction Kit and AmpliTaq DNA Polymerase following the manufacturer's instructions. DNA sequences were viewed with Chromas 1.43 (Connor McCarthy) and compared with each other using Clustal X (1.81). A BLAST search was done to compare the sequences with sequences available at GeneBank and the EMBL Nucleic Acid Database. The ITS1-5.8s-ITS2 sequence was studied in all isolates.

Results and discussion

RAPD analysis

The seven decamer primers used in this experiment generated 80 polymorphic bands for the 108 isolates studied. Primer OPL-11 generated the greatest number of polymorphic bands (18), primer OPC-02 generated 17 polymorphic bands (Figure 1), primers OPC-04 and OPL-12 generated 13 bands each, followed by OPJ-14 with 8 polymorphic bands. The fewest polymorphic bands were generated by primers OPJ-13 and OPC-15, with 6 and 5 bands, respectively.

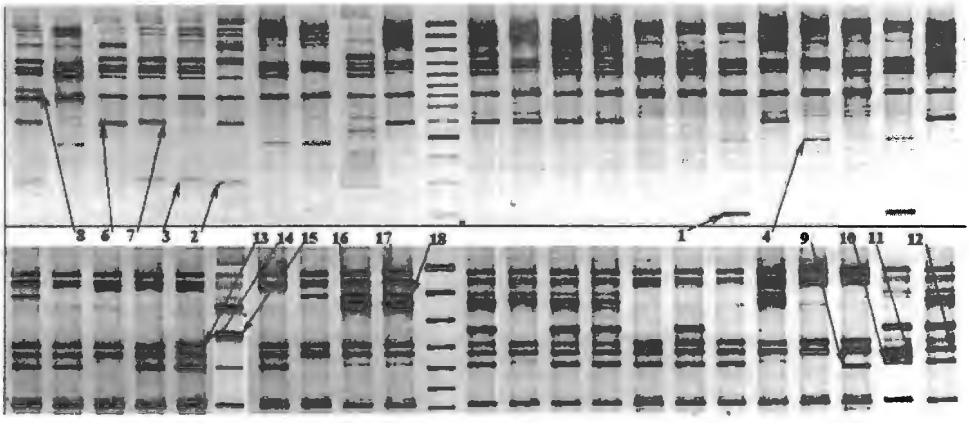

Fig. 1. RAPD-PCR products obtained using primer OPC-02 (Qiagen Operon)

The mean number of polymorphic bands was 23 per isolate. On average, one polymorphic band was present in 32 isolates. The frequency of polymorphic bands varied from 0.92 % (a polymorphic band present in 1 isolate out of 108) to 99.07 % (the same polymorphic band amplified for 107 out of 108 isolates studied). The frequency of polymorphic bands is presented in Figure 2. It demonstrates that the highest number of polymorphic fragments were generated by low number of isolates.

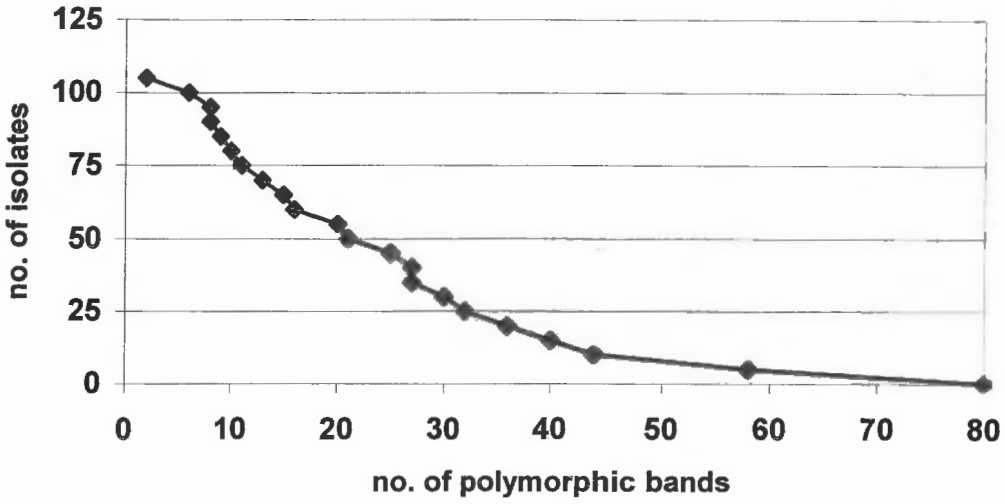

Figure 2. Frequency of polymorphic bands generated by RAPD primers used in the study

Some polymorphic bands were present only in isolates from a certain country (Table 3). The greatest number of such bands were observed in the population of *S. sclerotiorum* from China (10 bands, 12.5 % of the total number of bands). Five bands (6.25 %) were found only in Russian isolates and two bands (2.5 %) were observed only in case of the isolates from Poland. One polymorphic band was present only in an isolate from the Czech Republic and an isolate from Slovakia, but these isolates were obtained from different host plants (Czech isolate originated from oilseed rape, Slovakian isolate - from annual sunflower). The bands specific for a population originating from one country were found in some isolates only, and they were not present in all isolates from such population. In contrast, some bands were absent in all isolates originating from one country. This was observed for 24 polymorphic bands absent in a Polish population of *S. sclerotiorum* (30 % of the total number of bands), 21 bands were not present in Chinese isolates (26.25 %) and 17 bands were not observed for Russian isolates (21.25 %). Some polymorphic bands were also absent in isolates from other countries and there were 28 polymorphic bands that were not found in any isolates other than these originating from Russia, Poland and China (35 %).

The 108 isolates studied by RAPD could be differentiated into 83 groups. Most of the groups (71) were represented by one isolate only, ie. 65.7 % of the isolates generated an individual pattern of polymorphic bands or displayed an individual polymorphic band not present in other isolates. The remaining 37 isolates could be divided in 12 groups, with 2 to 6 isolates per group. Of these, the biggest group (six isolates showing identical banding patterns) were obtained from the Kuban region in Russia. In other groups with isolates sharing identical banding pattern one can find the isolates from Russia, Poland and China. The isolates originating from different countries generated different banding patterns.

Table 3. Polymorphic bands in *Sclerotinia sclerotiorum* isolates used in the study

Locality	No. of isolates	OPC-02-18	OPC-02-17	OPC-02-16	OPC-02-15	OPC-02-14	OPC-02-13	OPC-02-12	OPC-02-11	OPC-02-10	OPC-02-9	OPC-02-8	OPC-02-7	OPC-02-6	OPC-02-4	OPC-02-3	OPC-02-2	OPC-02-1	OPC-04-n12	OPC-04-n11	OPC-04-n10	OPC-04-n9	OPC-04-n8	OPC-04-n7	OPC-04-n6	OPC-04-n5	OPC-04-n4	OPC-04-n3	OPC-04-n2	OPC-04-n1	OPC-15-5	OPC-15-4	OPC-15-3	OPC-15-2	OPC-15-1	OPJ-13-5	OPJ-13-4	OPJ-13-3	OPJ-13-2
Petersburg-rapeseed	18	17	17	12	10		4	18		12	1		16	1								4	13	3	4	4	1	17		10		17	7		18	5	12	13	18	2
Petersburg-other	6	5	6	2	3			6		3			5									2	1	6			2	5		3		6	3	1	6	2	4	4	6	
Krasnodar	22						22	22		1	20		3	2		4	2		1	2				22			8	4		22	12	22	17	13	22		12	1	22	
Moscow	3	2	2		2		1	3		3			2			1							2	1			2	3		3	3		3		2	2	3	
RUSSIA	49	24	25	14	15		27	49		19	21		26	3		5	2		1	2			43	4	4	4	11	28		38	12	48	30	14	49	7	30	20	49	2
AUSTRIA	2	2	1	2	2		2	2		2			1	1									2			2		2			2			2		2	2	2	2
CROATIA	1						1	1			1					1												1		1			1		1	1		
CZECH REP.	8	2	2	1	3	1	2	2		3			2			1							1		1		3	2		3	2	1	3		1	2	1	3	
SLOVAKIA	1	1	1	1	1	1				1			1				1						1				1	1		1	1		1		1	1	1	1
Anhui	14	3	2	2	6		12	14		11	1		2	1	8		1		1				12	2			2	2	8	13	10	14	1	2	9	2	4	3	13
Qinghai	2	2			2		1	2	1	1													2						2		2			2		2	2	2	
CHINA	16	5	2	2	7		13	16	1	12	1		2	1	8		1		1				14	2			2	2	9	15	10	16	1	2	11	2	6	5	15
Wielkopolska	16	5	8	4	15		9	16		14	1		4			10				2			10		1		3	7		12	2	16	10	3	16	5	10	8	16	2
Lubelskie	10	6	6	5	10		6	8		10			6			4		2					5		1	3		6		7	2	10	4	2	10	4	6	6	10	2
Dolnoslaskie	7	1	2	1	5		6	7		3	4	1	1			2		1		1			2			1		3		6	1	7	1		7	1	3	2	7	1
Mazowieckie	1				1			1		1						1														1		1	1		1		1	1	1	
Zach.pomorskie	2	1	1	1	1		2	2		1	1		1										1					1			2			2		1	1	2	
POLAND	36	15	17	11	32		23	34		29	6	1	12	1		17		3	2	1			18		2	4	3	17		27	5	36	16	5	36	10	21	18	36	5
TOTAL	108	49	48	31	60	2	68	104	1	66	29	2	44	6	8	24	3	1	4	3	3	2	5	79	6	6	11	16	53	9	84	27	107	50	22	103	20	63	48	107	10

Locality	No. of isolates	OPJ-13-1	OPJ-14-n4	OPJ-14-n3	OPJ-14-n2	OPJ-14-n1	OPJ-14-5	OPJ-14-3	OPJ-14-2	OPJ-14-1	OPL-11-12	OPL-11-n11	OPL-11-11	OPL-11-n10	OPL-11-10	OPL-11-n9	OPL-11-9	OPL-11-8	OPL-11-7	OPL-11-n7	OPL-11-n6	OPL-11-6	OPL-11-5	OPL-11-n4	OPL-11-4	OPL-11-n2	OPL-11-2	OPL-11-1	OPL-12-n6	OPL-12-n5	OPL-12-n4	OPL-12-7	OPL-12-6	OPL-12-5	OPL-12-4	OPL-12-3	OPL-12-2	OPL-12-1	OPL-12-n3	OPL-12-n2	OPL-12-n1
Petersburg-rapeseed	18	16	1		12		2	15			18			4		6							12	10	5			18	4	2		10	6	18		17	18		2	17	4
Petersburg-other	6	6			4			5			6					1							6	6				6	3			5	2	8		6	6			5	
Krasnodar	22	22	7	4	22						20		17		10	7							3	3				4			2			22		3	5	17		22	
Moscow	3	3			3	1	1	2			3	1		2		1							2	2	1			3	1					3		2	2	1		1	2
RUSSIA	49	47	8	4	41	1	3	22			47	1	17	6	10	15							23	21	6			31	8	2	2	15	8	49		28	31	18	2	45	6
AUSTRIA	2				2		2	2			2			2		2			1				1	1	1			2	1					2		2	2			2	
CROATIA	1	1			1						1					1			1									1						1			1		1	
CZECH REP.	8	8		1	3			1			8			2		2			1	1			1	2	1			3				2		3		3	3			1
SLOVAKIA	1		1								1					1							1	1				1					1			1			
Anhui	14	14		2	13		1	2	1	4	13		1	1		13	1			5	1	6	1			2	14				1	1	7	2	5	12	2		13	2
Qinghai	2	2			2						2						2						2	1				1				1	2		1	2				1
CHINA	16	16		2	15		1	2	1	4	15		1	1		13	1	2		5	1	6	8	2		2	15				1	2	9	2	6	14	2		13	3
Wielkopolska	16	14			16	4		2			16	3		1		13			6	2			10	7	1	1		16				2		16		7	11	5		7	2
Lubelskie	10	8			10	2	1	2			10	1		4		7			1				5	5	3	1		10	2			1		10		8	9	1		1	4
Dolnoslaskie	7	6		1	7	1	1	1			7			1		4			3				5	1	2			7						7		4	2	5		4	5
Mazowieckie	1	1			1	1					1					1							1	1				1						1			1		1	
Zach.pomorskie	2	2			2						2					1			2				1	1				2						2		1		2		1
POLAND	36	31		1	38	8	2	5			36	4		6		26			12	2			22	15	6	1		36	2			3		36		20	23	13		13	12
TOTAL	108	88	9	8	98	9	8	32	1	4	105	5	18	17	10	60	1	1	7	15	6	1	56	42	14	3	2	89	11	2	2	21	10	101	2	59	74	34	2	72	24

Isolates from the Krasnodar region were the least polymorphic among the isolates studied, forming two separate distinct clusters. Isolates from these clusters were obtained from the same field, but they may originate from different cultivars of winter oilseed rape. The *S. sclerotiorum* isolates from St. Petersburg were little more diverse than those from Krasnodar. Most of them formed a cluster, but three isolates were out-grouped. The isolates from *Erysimum hieracifolium*, *Raphanus raphanistrum* and *Thlaspi arvense* belonged to the same cluster as isolates from *Brassica napus* originating from the same region. The out-

grouped isolates from north Russia were obtained from *B. napus*. Based on TREECON analysis, two isolates from the Moscow region were separated into individual groups and one isolate was clustered with Polish isolates. The isolates from Poland were the most polymorphic. They formed two distinct clusters with 11 and 10 isolates respectively, but 15 other isolates were scattered throughout the dendrogram (Figure 3). Isolates collected in 2004 from the same field in Zlotniki (Poland) belonged to different clusters. In contrast, isolates from other host- plant families were clustered with the isolates from the region they originate from, ie. the isolate from sunflower (Slovakia) was clustered with a Czech isolate (Cz116) and the isolate from lupin (Poland) was clustered with Polish isolates.

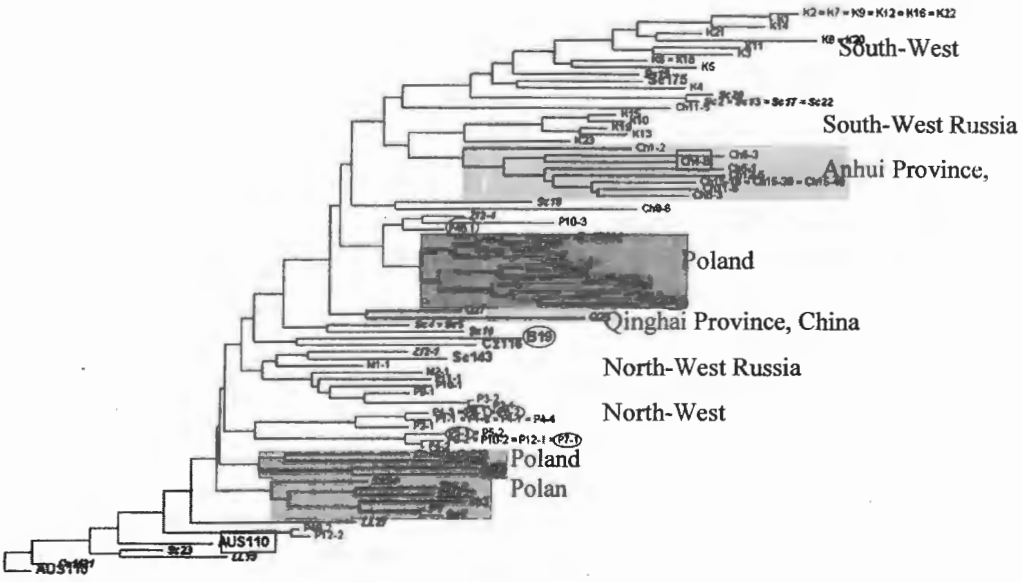

Figure 3. Dendrogram showing the relationship between *Sclerotinia sclerotiorum* isolates used in the study, based on RAPD analysis using TREECON software (Van de Peer and de Wachter 1994); main clusters are described.

⬭ - isolate from a host plant other than *B. napus*

▭ - isolate with different ITS sequence

It can be concluded that differences between the geographic origins of *S. sclertiorum* populations were much greater than differences between their host-plant origins. Most of the isolates from separated regions formed distinct clusters. However, the greater area of isolate collection yielded the higher diversity among the isolates. The samples collected in the region of Krasnodar were obtained from the smallest area and they were the least polymorphic. The isolates collected in Poland were obtained from different years and regions. Polish isolates showed the highest diversity but they had the smallest number of unique fragments generated by RAPD analysis.

Analysis of ITS sequences

All *S. sclerotiorum* isolates shared the same length of the ITS product - 448 bp, consisting of 148 bp ITS1, 143 bp ITS2 and 157 bp of the 5.8s fragment of rDNA. Amongst 108 isolates studied, three single basepair substitutions were found in three different isolates, with two basepair changes in the ITS1 region and one basepair change in ITS2 (Figure 4). The first basepair change T→C in ITS1 was found in a Russian isolate from *B. campestris* (P48.1), the second substitution in ITS1: C→G was found in a Chinese isolate (Ch4-8) originating from the Anhui Province. The substitution A→G in the ITS2 region was observed in an Austrian isolate (A-110).

48.1 (Russia) Ch 4-8

ATTA|**CAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTAC⏩GTTTGTT**
GCTTTGGCGAGCTGCTCTTCGGGGCCT⏩CTGTATGCTCGCCAGAGAATATCAAAACTCTTTT
TATTAATGTCGTCTGAGTACTATATAATAGTTA|AAACTTTCAACAACGGATCTCTTGGTTCT
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC
ATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGT
CATT|**TCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCTCTAAAAT**
CAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGC
TTCTGCCAAAA⏩GCCCAAATTTTCT|ATGGTTGACCTCGGATCAGGTAGGGATACCCGC

AUS-110

Figure 4. Sequence of ITS1-5.8s-ITS2 for *S. sclerotiorum* isolates studied

Based on the ITS sequences obtained for 108 *S. sclerotiorum* isolates from different host plants and geographic origins it can be concluded that this tool cannot be used to study intraspecific diversity within this fungal species. This is in agreement with other papers reporting that the sequence of the ITS region usually does not vary within one species but varies among different species and genera, allowing differentiation of different taxons rather than species (Barbee *et al.* 2003, Simmons and Freudenstein 2003).

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.83 - 90

Molecular aspects of potato resistance to Colorado potato beetles – a correlation with the sesquiterpene composition of ten potato varieties

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Abstract: Time courses of infestation rates of Colorado potato beetles (CPB) (Leptinotarsa decemlineata, Say) on ten Polish commercial potato (Solanum tuberosum) varieties were assigned in the field multi-choice assays. The sesquiterpene compositions in the leaf surfaces of the potato varieties were correlated with the infestation rates. Twenty two sesquiterpene hydrocarbons and alcohols were used in correlation studies. Compounds concentrations as well as chemometric classification parameters were correlated with the Colorado beetles infestation. It was found that total sesquiterpene contents and single sesquiterpene (β-caryophyllene and germacrene D) concentrations decreased the infestation rates. The group of sesquiterpenes: β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene and germacrene A decreased the CPB infestation and α-gurjunene, (E)-β-farnesene, trans-β-bergamotene, and unidentified sesquiterpene alcohol III increased.

Keywords: *Solanum tuberosum*, potato, sesquiterpenes, *Leptinotarsa decemlineata* Say, Colorado potato beetle, infestation, defoliation

Introduction

Plants can suffer numerous arthropod herbivores that differ considerably in sensitivity to various plant defence mechanisms. Defence against arthropods is provided mostly by secondary metabolites. Insect feeding often induce changes in plant metabolism providing different chemicals that can be correlated with increases in pest resistance (Baldwin, 1994).

The potato (*Solanum tuberosum*) is one of the most cultivated commercial plants in temperate zone areas. It is still an important crop in Poland although the economic transformation caused the significant decrease of potato acreage due to marked changes Pawińska, 2003). The Colorado potato beetle (*Leptinotarsa decemlineata* Say, Coleoptera; Chrysomelides) is a destructive pest of the cultivated potato in northern latitudes. The CPB is an oligophagous plant predator, which may live as long as 121 days. Colorado beetles feed, mate and oviposit on the foliage of potato plants as their habitat. Because of climate conditions in Poland favourable for pest development CPB occurs in numerousness presenting high risk for potatoes. When agro meteorological conditions are favourable two generations of the pest a year can develop in Poland, but generally only one occurs. In the years with unfavourable agro meteorological conditions the development of CPB is prolonged, and all development stages of the pest occur at the same time that is the beetles after hibernation, L_1, L_2, L_3 and L_4 larvae as well the beetles of summer generations. Economic threshold may be based on the number of adults, egg clusters or feeding larvae per potato plant. More than 15 larvae per plant may cause significant yield reduction. In some countries economic threshold for CPB is based on percentage of defoliation.

Defence responses in plants against insects are generally triggered by volatiles (Langenheim, 1994; Pare & Tumlinson, 1999; Pichersky & Gershenzon, 2002)

Sesquiterpenes act as semiochemicals, providing built-in protection against invading organisms. So it seems reasonable to search among this group of substances for components capable of controlling insect infestation.

The electroantennograph (EAG) analyses show that the Colorado potato beetle is capable of detecting not only green leaf volatiles but also the sesquiterpene fraction (Weißbecker *et al.,* 1997). The influence of sesquiterpenes on CPB behaviour is most pronounced from Dickens studies (2000a). He has found that the blends containing the green leaf volatiles (*Z*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol, and the sesquiterpene β-caryophyllene were unattractive or repellent to CPB adults. Minimal blends attractive to CPB were comprised of (*Z*)-3-hexenyl acetate, linalool and methyl salicylate. Based on EAG studies, potato plant volatiles (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, methyl salicylate, nonanal, (*Z*)-3-hexenyl butyrate, indole, linalool, decanal, β-caryophyllene and β-selinene would be expected to be important in behaviour of CPB (Dickens, 2000b).

Compositions of leaf surface sesquiterpenes in potato varieties are included in our previous papers (Szafranek et al., 1998; Szafranek et al., 2004). Potato varieties vary in their sesquiterpene composition. The main components were found to be sesquiterpene hydrocarbons such as β-caryophyllene, germacrene D and β-sesquiphellandrene. The yield of the potato sesquiterpenes varied from 28 ng/cm^2 (Aster variety) to 718 ng/cm^2 (Vistula variety).

Our research presents the CPB infestation rates on ten potato varieties and intensity of defoliation of potato plants. Colorado potato beetle chooses potatoes utilizing the volatile compounds of the plants. Thus, our studies concern CPB infestation rates and defoliation degree with comparisons of these data with sesquiterpenes concentrations. This set of data can serve as a tool to evaluate the usefulness of potato varieties in the commercial breeding.

Materials and methods

In the field experiments (Bonin, 54^{0}09'N; 16^{0}15'E) potato varieties were of the five maturity groups: very early (Aster, Drop), early (Lotos, Sumak), mid early (Mila), mid late (Bryza, Arkadia, Vistula, Wolfram) and late (Wawrzyn). The experiment plots (10 m^2 each in four replication) were subjected to standard culture and management practices such as double plough and fertilization (organic of mustard plants and minerals: urea - 100 kg/ha and Viking N:P:K 14:14:28 – 800 kg/ha). Potato tubers were planted 22 April 2002. The experiment plots were not protected against CPB, *Phytophthora infestans* fungus and weeds and no artificial irrigation to the plants was applied. Total natural precipitation during vegetation season was 310.2 mm distributed as: 25.8 mm (April), 69.8 mm (May), 66.8 mm (June), and 17.0 mm (July). Averaged air temperatures were +7.6 °C (April), +14.4 °C (May), +16.9 °C (June), and +19.4 °C (July). The field experiments were performed according to European and Mediterranean Plant Protection Organization (EPPO). The CPB observations were started after full plants emerging and was continued for three months to plant physiological desiccation. It included the percentage of the plant infested with CPB (adults and larvae) and the percentage of the damaged leaf area.

The leaves for chemical analyses were harvested sixty day after planting. The procedures of sesquiterpenes identification and quantification have been published (Szafranek *et al.,* 2004). GC-FID, GC-MS and NMR analyses were used to identify and quantify the sesquiterpenes in the leaf surfaces of ten potato varieties. The potato sesquiterpenes were: α-cubebene, α-copaene, β-cubebene, β-elemene, α-gurjunene, β-caryophyllene, *trans*-α-bergamotene, (*Z*)-β-farnesene, α-humulene, (*E*)-β-farnesene, germacrene D, *trans*-β-bergamotene, bicyclogermacrene, germacrene A, β-bisabolene, δ-cadinene, β-

sesquiphellandrene, germacrene D-4-ol, α-cadinol and three unidentified sesquiterpene alcohols. These data were used for building the models for infestation rates on different potato varieties.

Results and discussion

Primary and secondary plant compounds play a major role in host finding and acceptance by herbivores. Among them, host plant volatiles may facilitate orientation of insects to potential feeding sources or deter them (Bernays & Chapman, 1994). So, an experiment was designed herein, to correlate the CPB infestation on ten Polish commercial potato varieties with their leaf surface sesquiterpene compositions. Potato varieties such as Aster, Drop, Lotos, Sumak, Mila, Bryza, Arkadia, Vistula, Wolfram, and Wawrzyn, were used in the multi-choice field assays for CPB infestations. The design of such field screening plots allows plot-to-plot migration of the insects, which can reflect the behavioural responses to semiochemicals present on potato leaf surfaces. Thus, the number of CPB adults on the plants at the early stages can be directly used as the indicators for responses of the beetles to the potato volatiles. Although this apparent resistance of some varieties observed here is less useful in a commercial large-scale planting, the experiment threw some lights on insect-plant interaction.

Table 1 shows the infestation rates on ten potato varieties at six chosen terms after planting. At fifty-nine day after planting and twenty-one day after the plants started to grow, two potato varieties, Lotos and Wawrzyn, showed the highest infestation rates (above 60 % of the infested plants) but three others, Aster, Drop and Bryza, above 40 %. The other varieties were found to be less infested.

Table 1. Percent (%) of infestation of all development stages of Colorado beetle on potato plants.

Potato variety	Days after planting					
	59*	66**	73**	80**	87***	94***
Aster	49	30	1	1	P	P
Drop	46	44	8	9	P	P
Lotos	62	41	42	25	P	P
Sumak	26	24	11	8	P	P
Mila	39	38	16	9	6	79
Bryza	43	20	13	7	6	P
Arkadia	36	64	54	38	29	101
Vistula	20	28	12	6	9	58
Wolfram	35	49	28	19	15	63
Wawrzyn	63	59	31	15	12	75

* development stages - mostly beetles after hibernation

** development stages - mostly larvae

*** development stages - mostly beetles of summer generation

P - physiologically dry potato plants

Intensity of defoliation of potato plants by all development stages of Colorado beetle was determined, too (Table 2). The intensity of the foliage consumption measured as the degrees

of defoliation of the plants infested was the highest for Lotos variety. High rate of defoliation of Arkadia late variety in the autumn could be due to long vegetation of this cultivar.

Table 2. Intensity of defoliation of the infested plants by all development stages of Colorado beetle (% of leaf damages).

Potato variety	Days after planting					
	59	66	73	80	87	94
Aster	3	6	8	9	P	P
Drop	7	11	10	11	P	P
Lotos	13	13	26	27	P	P
Sumak	3	5	9	9	P	P
Mila	4	1	5	6	8	16
Bryza	5	4	4	5	6	P
Arkadia	5	6	8	10	13	33
Vistula	2	6	5	5	5	14
Wolfram	2	6	8	9	12	13
Wawrzyn	3	6	7	7	8	13

P - physiologically dry potato plants

Infestation rates and defoliation intensities were tested for mutual correlation. There are two factors that control intensity of feeding, the composition of potato leaves including feeding attractants and stimulants, and efficiency of resistance induction.

Induction of potato resistance to CPB feeding can be visualized in correlation of infestation rates and defoliation intensities (Fig. 1). The plants with higher infestation rates should be defoliated stronger. Thus, an important question is whether the molecular levels of plant defense to plant enemies show positive or negative correlations. If a mechanism of induced resistance exists in a potato variety, the correlation point should be significantly below the line and vice versa, the points above the line correspond to potato varieties mostly consumed without induction of plant defence. Most of the varieties are found to be near the linear correlation, but Lotos variety for the days studied appears to be significantly above the line with the conclusion that no induction occurs. Wawrzyn variety is found below the line suggesting some degree of resistance induction at vegetation day 59. But for consecutive observation terms, two more varieties Mila and Arkadia, were found to appear below the correlation line. It suggest the presence of herbivore-induced defense in potato plants.

Many volatiles are performed and act in herbivore deterrence (Ferry *et al.*, 2004; Baldwin *et al.*, 2001). The herbivore-induced release of volatiles can be involved in direct or indirect defense. Some volatiles are common to many plant species, including green leaf volatiles, terpenes and indole, whereas others are specific to particular species. The question is what chemical compounds are involved in potato defense. Bolter *et al.*, (1997) demonstrated that the emission levels for sesquiterpenes and other volatiles were greatly increased by feeding by CPB larvae.

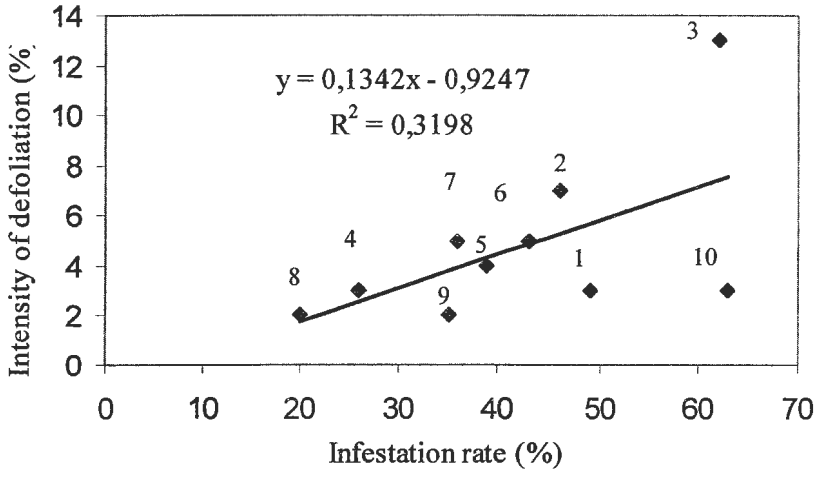

Fig. 1: A correlation between CPB infestation rates and intensities of defoliation of the leaves of different potato varieties (1. Aster; 2. Drop; 3. Lotos; 4. Sumak; 5. Mila; 6. Bryza; 7. Arkadia; 8. Vistula; 9. Wolfram; 10. Wawrzyn) at vegetation day 59.

CPB infestation rates on different potato varieties (vegetation day 59) were correlated to total sesquiterpenes contents (Fig. 2). Inverse straight relation between infestation rate and total sesquiterpene contents is observed with R^2 value of 0.34. It clearly indicates sesquiterpenes involvement in plant-insect interaction. Potato plants producing a large amount of sesquiterpenes are unattractive to CPB. It conformed well with the observations of Dickens (2000a) that blends containing sesquiterpene β-caryophyllene with green leaf volatiles are repellent to CPB adults.

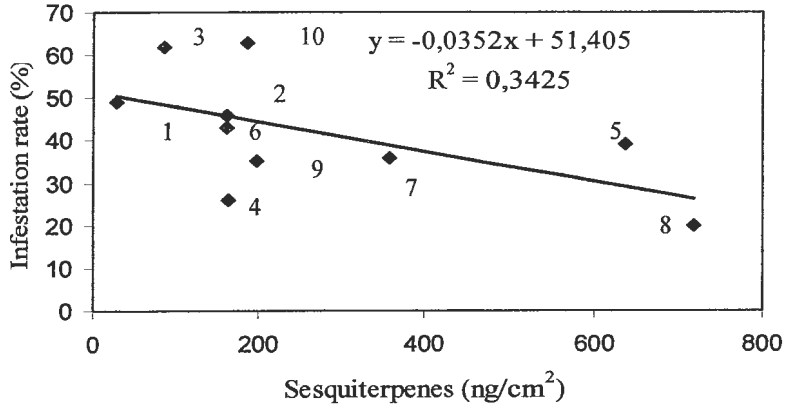

Fig. 2: CPB infestation rates on different potato varieties (1. Aster; 2. Drop; 3. Lotos; 4. Sumak; 5. Mila; 6. Bryza; 7. Arkadia; 8. Vistula; 9. Wolfram; 10. Wawrzyn) in relation to total sesquiterpenes contents at vegetation day 59.

CPB infestation rates on different potato varieties (vegetation day 59) were correlated to the content of every single sesquiterpene. Inverse relation between infestation rate and β-caryophyllene contents is observed with R^2 value of 0.39 and between infestation rate and germacrene D contents (R^2 0.45) (Fig. 3). The conclusion is that β-caryophyllene and germacrene D deter CPB. There was no significant correlation between infestation rate and other main sesquiterpenes and no correlation between sesquiterpene contents and intensity of defoliation.

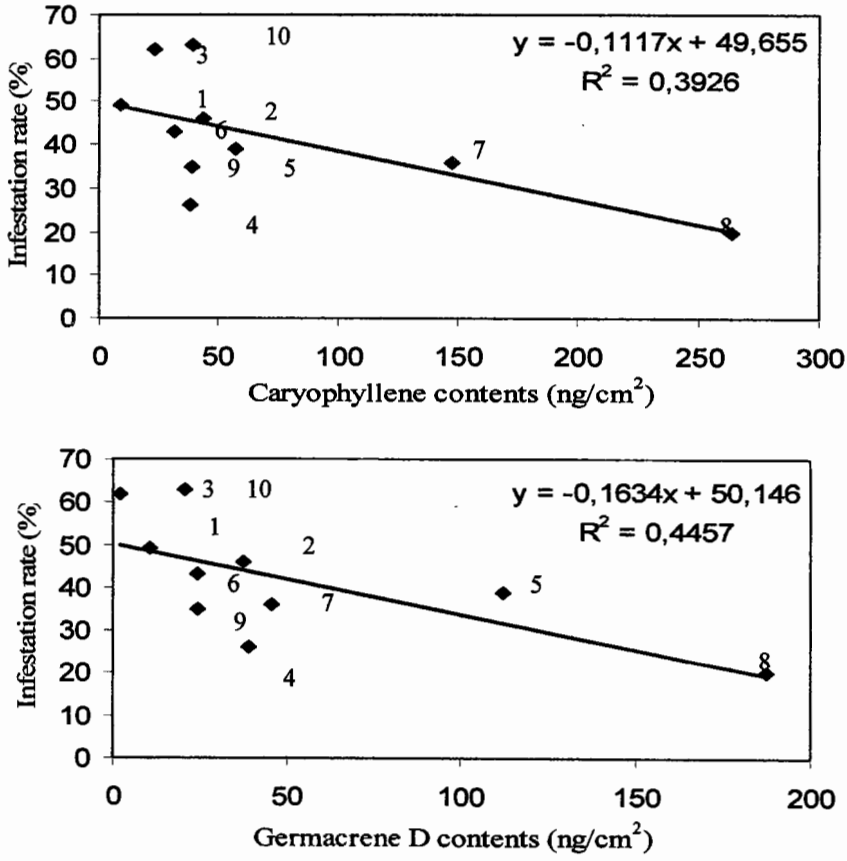

Fig. 3: CPB infestation rates vrs. β-caryophyllene and germacrene D concentrations on the leaves of potato varieties (1. Aster; 2. Drop; 3. Lotos; 4. Sumak; 5. Mila; 6. Bryza; 7. Arkadia; 8. Vistula; 9. Wolfram; 10. Wawrzyn) at vegetation day 59.

Recently, we have used chemometric methods for potato varieties classification (Szafranek et al., 2004). The sesquiterpene compositions of the different potato varieties were subjected to principal component analyses (PCA). PCA with Factor 1 and 2 allowed to distinguish three groups of varieties with an abundant cluster of 8 potato varieties in the centre with full separation of Vistula and Mila cultivars. Factor 2 separates Vistula and Mila varieties from the others. Vistula is unique variety with low infestation and low intensity of

defoliation (Tables 1,2). Mila, in turn, features highest infestation rate with both adults and larvae. It sounds reasonable to correlate infestations with those factors (Fig. 4). The best correlation was obtained for infestation rate and Factor 2 with R^2 value of 0.37. The highest coefficients to Factor 2 are from β-caryophyllene, α-humulene, germacrene D, bicyclogermacrene and germacrene A with negative correlation. It suggest that the mixture of these compounds show deterrent activity against CPB. The second group of the compounds with the positive correlation with the Factor 2 such as α-gurjunene, (*E*)-β-farnesene, *trans*-β-bergamotene, and unidentified sesquiterpene alcohol III are attractant to CPB. Thus, the specific ratios of sesquiterpenes are important in CPB infestation.

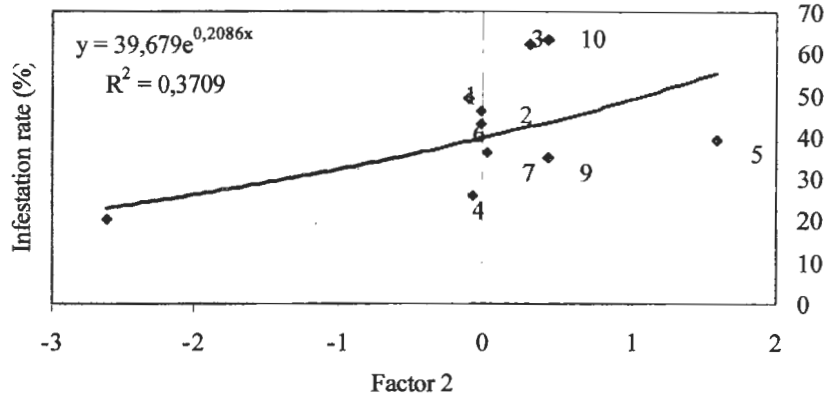

Fig. 4: CPB infestation rates on different potato varieties (1. Aster; 2. Drop; 3. Lotos; 4. Sumak; 5. Mila; 6. Bryza; 7. Arkadia; 8. Vistula; 9. Wolfram; 10. Wawrzyn) in relation to Factor 2 at vegetation day 59.

In summary, total sesquiterpene contents of potato surfaces and individual components, β-caryophyllene and germacrene D, decrease the infestation rates. These field observations provide bases for manipulation of chemically mediated behaviour of CPB. It could be an alternative pest control option to synthetic chemicals use. However, this approach still needs a better knowledge of potato – CPB interactions.

Acknowledgments

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Prospects of native entomogenous fungus *Metarhizium anisopliae* var *major* for integrated control of termite pests of tea in north east India

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Abstract: Termites are serious pests of tea which have major economic impact in tea industry of North East India. Adverse environmental impact due to rising trend of pesticide use is a cause of concern for tea planters. Prospects of native entomogenous fungi *Metarhizium anisopliae* has been studied *in vitro* against scavenging termite pests, *Odontotermes* spp of tea .Mortality of termite increased with increasing period of incubation and total mortality was observed in 12 days under prevailing laboratory condition. *M. anisopliae* was sensitive to commonly used agrochemicals at field dose in tea ecosystem which reduced conidial germination and fungal biomass. The study indicated that field application of bioagents should be initiated where risk of pesticides residues is at minimum.

Effects of host plant on infection of aphids by the fungus *Pandora neoaphidis*

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Abstract: The performance of the aphid-specific fungal pathogen *Pandora neoaphidis* was studied in relation to changes in herbivore resources at two levels, either for differences between host plant species, or within host plant species (varieties). The aphids examined were the pea aphid, *Acyrthosiphon pisum*, the cereal aphid, *Metopolophium dirhodum* and the peach potato aphid, *Myzus persicae*.

Dose-response bioassays were conducted with *A. pisum* which had been reared on dwarf bean then transferred to dwarf bean, field bean, pea or lucerne after treatment with *P. neoaphidis*. The lowest median lethal dose (LD_{50}) was 7.7 (95% confidence limits 5.6-11.0) conidia mm^{-2} from aphids maintained on dwarf bean, but LD_{50} was 1.5- to 2.2-times larger on dwarf bean and pea, respectively, and 382-times greater when aphids were transferred to lucerne. In a subsequent experiment, *A. pisum* were reared on pea as well as dwarf bean before bioassays. The LD_{50} was 6.5 (3.7-11.6) conidia mm^{-2} for aphids reared and then incubated on dwarf bean which was 1.4- to 1.6-times smaller than values determined if aphids were transferred between dwarf bean or pea, and LD_{50} from aphids reared then incubated on pea plants was 3.7-times greater than for dwarf bean. Hence, the virulence of *P. neoaphidis*, measured by LD_{50}, was highest when *A. pisum* was reared and maintained on dwarf bean, the plant used for long-term routine culturing of the aphid in our facilities.

Experiments were also conducted to compare differences in host plant between *A. pisum*, *M. dirhodum* and *M. persicae*. There was a trend for greater infection by *P. neoaphidis* when aphids were kept on original host plant species compared with alternative plant species (15.2 and 10.0%, respectively). Infection also varied between aphid species, from 21.4% (SE = 4.6) for *A. pisum*, 12.2% (SE = 3.6) for *M. dirhodum*, and 5.1% (SE = 2.6) for *M. persicae*. In plant variety bioassays, there were no differences between amount of infection or time to infection for aphids on original or alternative plant varieties. Mean infection was 80-95% for *A. pisum*, 13-14% for *M. dirhodum* and 45-61% for *M. persicae*. Time to infection was shortest for *A. pisum* (approx. 4 days) compared with *M. dirhodum* and *M. persicae* (both approx. 6 days). Nymphal production rate was significantly faster for *A. pisum* on original compared with alternative plant varieties (10.0 and 6.0 nymphs $aptera^{-1}$; respectively).

In conclusion, virulence and infection of *P. neoaphidis* was greater on plant species to which *A. pisum*, *M. dirhodum* or *M. persicae* had become adapted during long-term culturing in laboratory rearing. Hence, plant resources can affect *P. neoaphidis*, and the fungal entomopathogen will have a greater impact on aphid herbivores which are not suffering physiological stress.

Pest resistant GM crops: A chemical ecology viewpoint

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Abstract: Both GM and conventionally bred crops can be unexpectedly and unintentionally changed during the sequential development to a commercial variety. Underlying causes of changes in plant primary and secondary metabolism will de outlined. To date, the focus on GM biosafety has been largely on the non-target impacts of the GM new trait or gene product(s), despite the fact that in conventional breeding and in GM crop research many unusual alterations in plant metabolism (primary and secondary) can be detected. Examples will be given for both types of crop (GM, conventional). Many plant chemicals are known to have an ecological role in plant interactions (e.g. in defence against pests and diseases and as host recognition semiochemicals at several trophic levels). These plant chemicals can also be influenced in unpredictable ways by the environment or by plant stress factors. I will discuss potential impacts of some unexpected changes in plant metabolism on crop protection and on environmental impacts, using insect-resistant GM potatoes as an example studied at SCRI. I will also outline new guidelines under development for IOBC (Global group) for assessing potential ecological impacts of GM crops in developing countries (based on workshops and case studies in Kenya, Brazil and Vietnam).

Breeding for resistance: An option not only for growers and industry, but also for policy makers?

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Abstract: Minor crops are of major importance, not only to feed ourselves as part of a healthy diet, but also to keep food and fodder diverse. Subsequently, the economic value of minor crops can be substantial. Crop protection problems are as common in so-called 'minor crops' as in larger crops. Due to politics and economic situation 'minor use' pesticide authorizations are disappearing from the European Union (EU) and this will influence the production of minor crops. EU and its member states have recognized the problem and the need for international co-operation to solve the plant protection problems. EU has established a Steering Group to attend to the problem of 'Minor Uses'. This policy group has appointed a Technical Group, consisting of member state co-ordinators. They are specialists in the plant protection (authorization) field, originating from all 25 member states of the EU. They should obtain plant protection solutions for shared problems. That can be achieved by seeking extensions of authorizations or voluntary mutual recognitions of authorized chemical substances. However, non-chemical solutions are also stated and are in high demand by growers, consumers and food retailers. Breeding for resistance against pests and diseases could be a way to solve plant protection problems in 'minor crops'. Via the national co-ordinators of the Technical Group, and co-operation between EU member states, the Steering Group will consider research proposals that lead to solving the plant protection problems for minor crops which will soon lack approved pesticides to control key their pests.

Manuscripts from Posters

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IOBC wprs Bulletin Vol. 28(10) 2005
pp.101 - 106

The level of antitrypsin activity in winter triticale infested by grain aphid (*Sitobion avenae* F.)

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Abstract: Trypsin inhibitor accumulation in ears of winter triticale was studied when the plants were infested by grain aphid. The non- infested ears of Fidelio, a cultivar which was more resistant to *Sitobion avenae,* showed considerable higher antitrypsin activity comparing to the susceptible Lamberto cultivar. However, it was observed, that *S. avenae* feeding on winter triticale resulted in an increase of antitrypsin activity in both cultivars, more pronounced in Fidelio comparing to Lamberto.

Key words: trypsin inhibitor activity, triticale, grain aphid, resistance

Introduction

Plants and insects have been co-evolving for thousands of years, and as e result, plants have defence mechanisms that offer protection against many insects. One such method of plant defence involves the production of protease inhibitors (PIs) (Jongsma & Bolter, 1997).

Protease inhibitors are polipeptides present in many plants when they have different important physiological functions. They regulate the protease enzymes and hence control protein turnover and metabolism. These inhibitors may be found constitutively in various parts of the plant, or may be induced in response herbivore insects (Pearce *et al.*, 1991).

A role in defence against phytophagous is likely considering that unfavorable influence of proteolytic enzyme inhibitors on insects body result mainly from their limiting of insect's digestive enzyme (trypsin and chymotrypsin) activity. Then, the lowering of digestion and worsened usage of nutrients, especially proteins, which leads to a decrease of insect' body weight (Buraczewska, 1991). It suggested that protease inhibitors are anti-nutritional factors that negatively affect the growth of insects (Orozco-Cardenas *et al.*, 1993). Xu *et al.*, (1996) found that transgenic plants with suitable trypsin inhibitor gene can resist against insect damage. It also recorded a sharp increase of concentration of these compounds in plant tissues because of the damage done by aphids, which was a reply to their attack (Casaretto & Corcurea 1998, Ciepiela & Sprawka 2001). Proteolytic enzyme inhibitors are important in mechanisms of cereal plants induced resistance to aphids (Leszczynski 1996). The inducibility of protease inhibitors and their protective role has been demonstrated mainly in dicots (Ryan 1990, Johnson *et al.*, 1989) but in cereals is less documented.

The aim of taken research was to study the changes in antitrypsin activity in winter triticale ears induced by grain aphid feeding.

Materials and methods

Entomological experiments

Entomological research was carried out on small experimental plots of 2 x 3 m size at University of Podlasie, Department of Molecular Biology and Biophysics. Fidelio and

Lamberto cultivars were used for experiment. In order to study the level of their resistance to grain aphid, population number of the pest was assessed on the studied cultivars. Population number was investigated in natural infestation conditions according to Wratten *et al.* (1979) and Lykouressis (1984) methods. Therefore, from the 6th leaf blade stage, until the late milk ripe stage (G.S. 44-78 in Tottman & Broad (1987) scale), the number of *S. avenae* was being assessed on the studied cultivars. The technique of aphid counting across field diagonal, 6 times in a season (G.S.44-6th leaf blade; G.S.52-early earing; G.S.63- mid flowering; G.S.69-late flowering; G.S.75-mid milk ripe; G.S.- late milk ripe), choosing 25 ears at random, was applied. On the basis entomological observation, the indicators of grain aphid population developing on the tested cultivars were calculated. They were: the average percent of infested plants and cumulative aphid index.

Entomological research concerning *S. avenae* infestation impact on antitrypsin activity in winter triticale ears was carried out at the stage of mid milk ripe (G.S. 75) of the plants. Then 10 wingless females of grain aphid were put on the 10 ears of each of studied cultivars. Next, ears were isolated by means of bloting- cloth isolators. Meanwhile, the same number of ears with no aphids (the control) was prepared in the same way. After 7 days, since the moment when the studied cultivars were infested by aphids, plant material was collected and it was used to obtain acetone preparation.

Chemical analyses

To isolate trypsin inhibitor, 10g of acetone preparation was shaken with 40 cm^3 of 0.1 M of citrate buffer of pH 4.4 for 15 minutes and then it centrifuged for 15 minutes at 9000 x g. The obtained supernatant was an extract of trypsin inhibitor. The identification of antitrypsin activity was carried to the method of Stasińska *et al.* (1995). The activity was expressed in antiproteolytic units (JAantyP), using the following equation:

$$JAantyP = \frac{(T - P) \cdot v}{E \cdot t}$$

where:

T- extinction for trypsin alone,
P- sample extinction,
v- volume of sample,
E- 1-mill-equivalent of tyrosine extinction which equals to 1620,
t- time of reaction.

Chemical analyses were repeated three times. To assess inter- cultivar differences on the antitrypsin activity level, under of grain aphid feeding, the results went under deviation analysis, using t-Student test of independent variable at the significance level $P \leq 0.01$ and $P \leq 0.05$.

Results and discussion

The results of resistance tests concerning grain aphid population density on the studied cultivars are displayed in Figure 1. The entomological observations showed there are inter-cultivar differences in the number of *S. avenae* population on the tested triticale cultivars. They prove that on the Lamberto cultivar was a greater cumulative aphid index and higher percent of plants infested by grain aphid in comparison with Fidelio cultivar. Thus, cultivar classification, with reference to grain aphid resistance was made. The Fidelio cultivar was relatively resistant to *S. avenae*, whereas the Lamberto one was more susceptible.

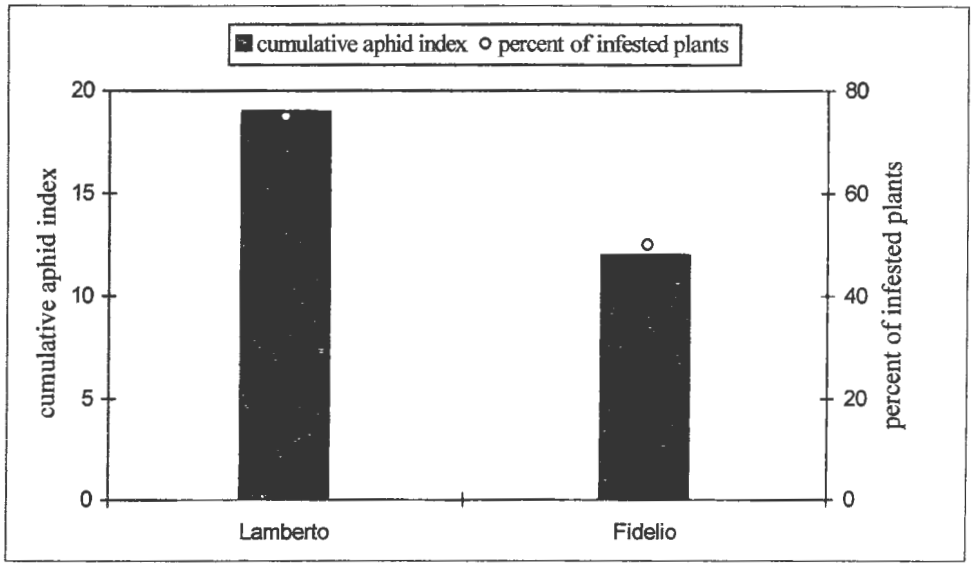

Fig. 1: Population density of grain aphid on the studied winter triticale cultivars.

The chemical analyses showed that in the relatively resistant Fidelio ears which not infested by aphids, antitrypsin activity was higher than in the susceptible Lamberto cultivar (Table 1). This may have resulted from a higher level of its natural resistance to grain aphid, than was found in the Lamberto cultivar. The obtained results are close to those of Casaretto & Corcurea 1998. These authors showed that resistant barley cultivar Frontera which not infested by aphids *Schizaphis graminum* and *Rhopalosiphum padi* had higher antitrypsin activity in comparison with the susceptible barley cultivar Aramir.

Table1. Antitrypsin activity (JAantyP) in ears of studied winter triticale cultivars

Cultivars	**Non-infested ears**	**Infested ears**
Lamberto	0.64	0.75*
Fidelio	1.75	2.52**

* - values in the same rows are significantly different at $P \leq 0.05$ (t-Student's test).

** - values in the same rows are significantly different at $P \leq 0.01$ (t-Student's test).

Grain aphid infestation causes a great antitrypsin activity increase in the studied cultivars, in comparison to the control plants (Fig. 2). In the relatively resistant Fidelio ears

this activity level was higher than in the Lamberto cultivar. Statistical analysis proved that the increase of antitrypsin activity in ears of both analysed triticale cultivars, induced by aphid were statisticaly significant in comparison with the control plants (Table 1).

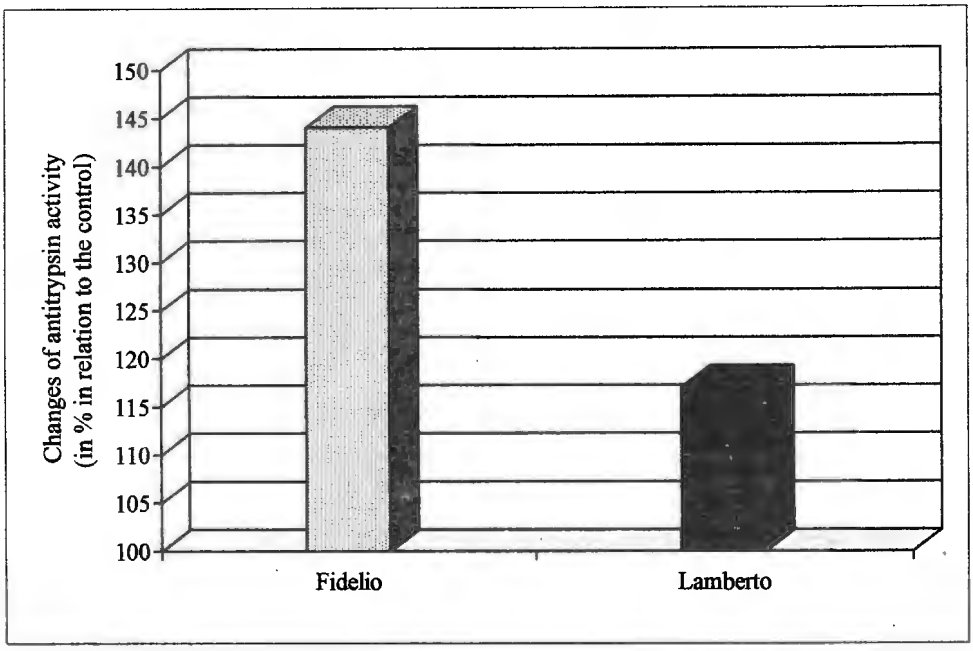

Fig. 2: The changes of antitrypsin activity in ears of studied winter triticale cultivars caused by grain aphid feeding.

Casaretto & Corcurea (1998) observed a similar tendency, *S. graminum* infestation resulted increase of antitrypsin activity in both tested barley cultivars, but Frontera, culivar which was more resistant to aphids, accumulated more inhibitor than most susceptible cultivar Aramir.

It may be concluded that an increase concentration of trypsin inhibitor in ears infested by *S. avenae* is a result of harm done to plants by this pest. It has been reported earlier that PIs were actively induced to high levels in many plants when they attackted by insects or mechanically damaged (Green & Ryan 1972, Peña-Cortés *et al.*, 1988 Nelson *et al.*,1983, Alarcon & Malone 1995). In addition to a local synthesis of PIs it was found that signals from the wound site were transported through the phloem and stimulated the syntesis of PIs throughout the plant (Nelson *et al.*,1983, Peña-Cortés *et al.*, 1988, Pearce *et al.*, 1993, Jongsma *et al.*,1994). Quick grouping of PIs takes place by means of defence reaction. One may suppose that the level of trypsin inhibitor of plants infested by grain aphid decides about intracellular proteolysis. They may have an impact on induced resistance level of winter triticale to *S. avenae.* The accumulation of PIs induced by aphids may be of importance as a barrier to another pathogen or pest that attacks triticale. These observation suggested that plants possess active mechanisms that were intended to protect them against insect attack.

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Breeding for Plant Resistance to Pests and Diseases
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pp.107 - 112

The participation of polyamines in mechanisms of winter triticale resistance to grain aphid (*Sitobion avenae* F.)

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Abstract: The aim of presented research was to quantify changes in content of generally occurring plants polyamines: putrescine, spermidine and spermine in winter triticale attacked by grain aphid (*Sitobion avenae* F.). Material for studies included the ears, shoots and roots of two winter triticale cultivars: Lamberto (more resistant to attack by grain aphid) and Fidelio (more resistant). On the basis of our results we demonstrated that in case of the control plants(no aphids), the higher content of studied compounds (except putrescine) characterized all the analyzed organs of cultivar Fidelio (R) in comparison with triticale Lamberto (S). The feeding of grain aphid caused a decrease in the amount of the polyamine content in ears of both studied cultivars, with the exception of putrescine in cultivar Lamberto. However the increase of the analyzed compounds concentration was noted in the vegetative parts of plants attacked by *S. avenae*, with the exception of shoots of cultivar Fidelio, where the polyamines quantity decreased in relation to control plants. The presented results indicate that analyzed polyamines play an important role in mechanisms of constitutive and induced resistance of winter triticale to grain aphid. Moreover, the changes of polyamines content, caused by grain aphid feeding in ears of winter triticale, is systemic.

Keywords: polyamines, putrescine, spermidine, spermine, winter triticale, grain aphid, constitutive resistance, induced resistance

Introduction

Polyamines are substances occurring generally in plants. These compounds are products of decarboxylation of amino acids, especially ornithine, arginine and S-adenosylomethionine. Apart from many different metabolic functions, polyamines participate in mechanisms of the resistance of plants on stressors, such as the growth of salinity, high concentration of acids, the deficiency of the macro - and the microelements, unfavorable temperature, drought and others (Erdei *et al.*, 1990; Basu & Gosh, 1991; Hauschild, 1993, Aziz *et al.*, 1999).

Polyamines play an important role in defensive plants reactions against pathogenic bacteria's, viruses and fungi Walter *et al.* (1985) stated the increase of the putrescine and cadaverine content as well as the decrease of spermine concentration in barley seeds infected by *Erysiphe graminis* sp. However, in barley infected by *Bluereria graminis* content of putrescine, spermidine, and spermine increased (Walters, 2000). Bharti (1995) announce that inhibition of ornithine decarboxylase (a key enzyme of polyamine biosynthesis) by DL-α-difloromythylornithine (DFMO) limited the growth of photogenic fungi to winter wheat. DFMO is completely able to stop the growth of *Puccinia recondita* in *in vivo* and *in vitro* conditions (Bharti & Sawhney, 1996).

Information about the significance of polyamines in interactions between plants and herbivorous insects are not many in the literature. Ciepiela & Sempruch (2002) observed induction of ornithine decarboxylase activity in spring forms of triticale and wheat attacked

by *S. avenae*. It was stated that activity of this enzyme in winter triticale was modulated activitely by grain aphid too, and the pest attack had systemic character (Sempruch *et al.*, 2004).

The aim of presented study was the qualifing of changes in content of generally occurring plants polyamines: putrescine, spermidine and spermine in winter triticale attacked by grain aphid (*Sitobion avenae* F.).

Materials and methods

The cultivation of winter triticale as well as entomological observations was conducted in the natural field conditions in Agricultural Experimental Station in Zawady near Siedlce in the years 2002-2004. Material used for studies stated the ears, shoots and roots of two winter triticale cultivars: Lamberto – in large numbers settled by grain aphid and Fidelio – settled in not large numbers.The shown data stated the means of the obtained results from the studied years.

The density of *S. avenae* population estimated on tested plants with the method of Wratten *et al.*, (1979) and Lykouressis (1984). Observation was conducted six times per season (in one week intervals) on the fifty random selected plants. The experiments were performed on experimental plots 2 x 9 m in six replications for each studied cultivar. The basis of obtained results number of aphids/blade and percent of attacked plants were calculated and the dynamic of the pest's population was defined.

In the aim of defining of influence of the grain aphid on polyamine content thirty ears of each studied cultivars was artificially settled by five wingless females of *S. avenae*. The settled ears were isolated with the blotting-cloth isolators (25 x 15 cm). The control ears (without aphids) were prepared similarly. Experiments were conducted one week at early milk (G.S.73, according to Tottman & Broad (1987) scale) to late milk of both cultivars (G.S.79). During experiments, the number of aphids was controlled every day and stated on constant level 5 individuals/blade.

Polyamines were extracted from fresh ears, shoots and roots of plants settled by aphids and control ones by homogenization with 5% v/v $HClO_4$ solution. The obtained suspensions were centrifuged at 25 000 x g and supernatants were collected and used to further analyses. The quality and quantity of polyamines were analyzed according to method of Villanueva *et al.*, (1977) with the usage of amino acids analyzer type T -339 (Microtechna Praha).

Results and discussion

The results of entomological observations showed the winter triticale cultivars used to experiments differed in the degree of susceptibility to *S. avenae* (Figure 1). Lamberto cultivar was in large number settled by the pest in comparison with Fidelio cv. during majority of studied developmental phases.

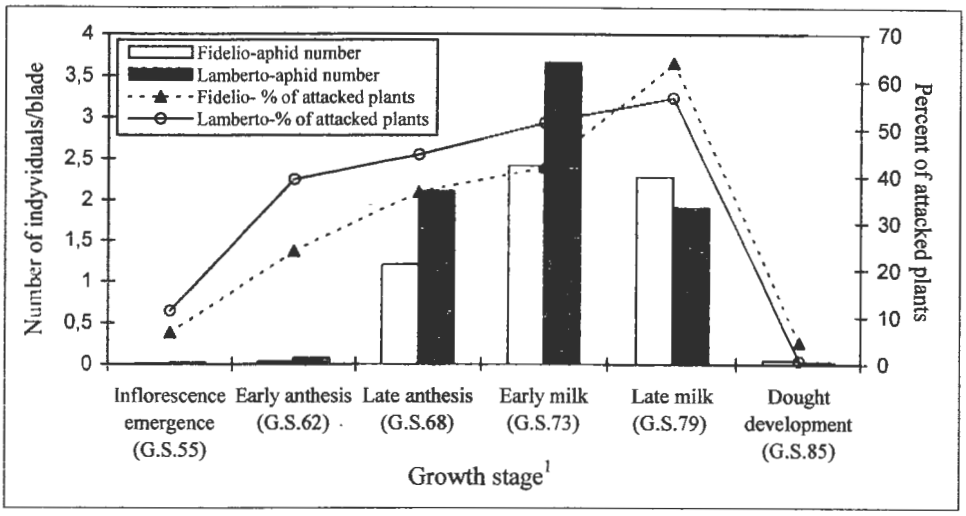

Fig. 1: Dynamics of grain aphid population on the studied winter triticale cultivars.
[1]Growth stage was showed in Tottman & Broad (1987) scale.

The maximum density of *S. avenae* population on both studied cultivars was achieved in early milk stage (Fig. 1). The received results are consistent with observations of Kąkol & Miętkiewski (2001), who showed that the highest density of the grain aphid population on winter wheat occurred during milk maturity. The differences stated in density of *S. avenae* population during vegetation of studied cultivars may be connected with biochemical - physiological changes accompanying their maturation. Honék (1987) found that the rate of natural increase of grain aphid population depended on the content of water in ear which fell below 50% caused abandonment of crops by this pest.

Table 1. The content of analyzed polyamines (in µg/g dry wt) in analyzed organs of studied winter triticale cultivars not attacked by grain aphid.

Cultivars	*Organs*	*Polyamines*		
		putrescine	spermidine	spermine
Fidelio	ears	33.6 b	61.5 a	152.5 a
	shoots	33.5 b	36.2 c	38.7 c
	roots	10.6 c	19.5 d	16.2 d
Lamberto	ears	43.3 a	49.1 b	85.1 b
	shoots	10.5 c	5.2 f	0.1 e
	roots	0.1 d	14.7 e	0.1 e

Values in the same columns signed by another letters are statistically different at $P \leq 0.05$ (Duncan's test).

On the basis of obtained results it was affirmed, that in case of the control plants the higher content of studied compounds characterized all analyzed organs of cultivar Fidelio

(commonly attacked by the pest) in comparison with triticale Lamberto (Table 1). The exception to this rule stated the putrescine, which higher concentration was noted in vegetative parts of Fidelio cultivar and ears of Lamberto cv. The results may prove polyamines especially spermidine and spermine play significant role in mechanisms of constitutive resistance of winter triticale to grain aphid.

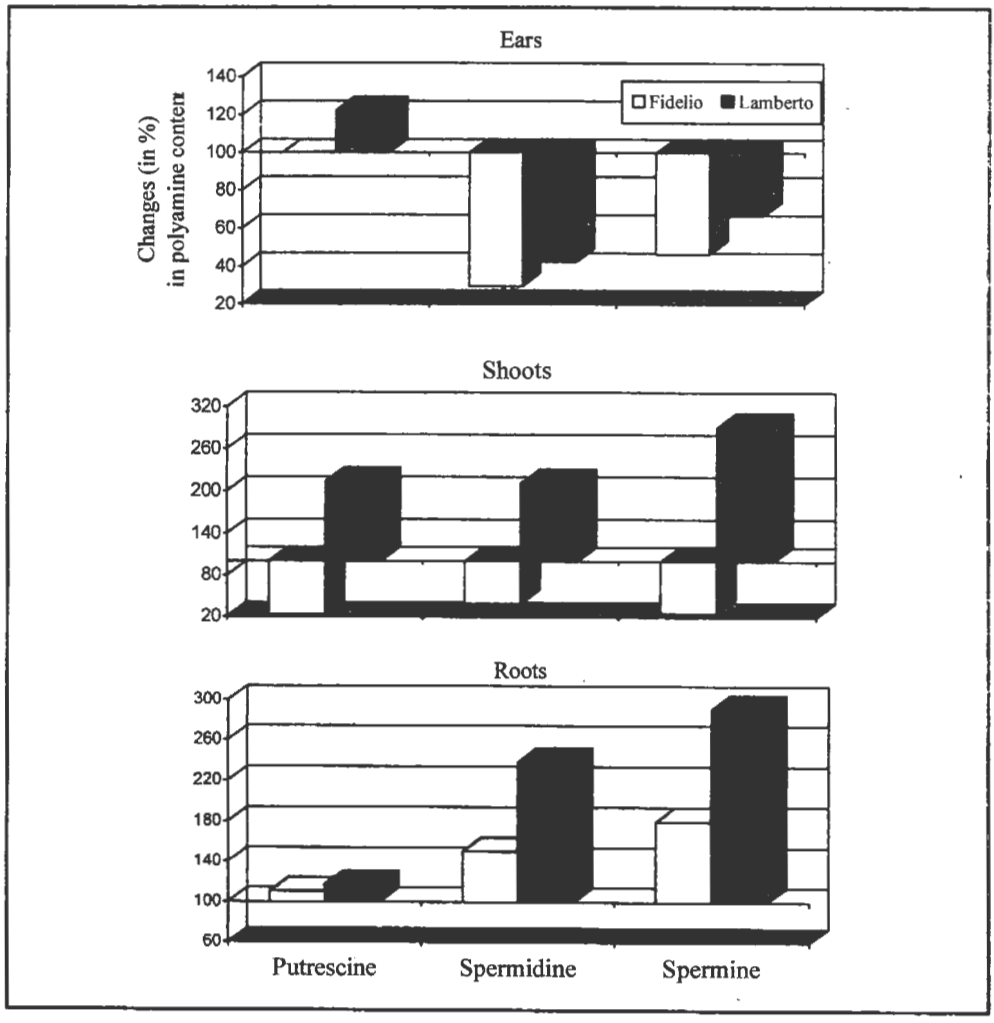

Fig. 2: Changes (plants were not settled by aphids = 100%) in content of studied polyamines.

It was proved, that feeding of wingless females of grain aphid caused the decrease of polyamine content in the ears both of studied winter triticale cultivars, with the exception of of putrescine in Lamberto cv. (Fig. 2). The concentration of putrescine in generative organs of Lamberto cv. settled by the pest increased during milk maturity (date of plant collection), while Fidelio cv. was more preferable by *S. avenae*. These results confirmed earlier observation (Sempruch *et al.*, 2004) that proved the possibility of existing in winter triticale mechanism of induced resistance, being the consequence of changes in equilibrium between content of amino acid ornithine and product of it decarboxylation - polyamine putrescine. On the other hand, endogenous polyamines and especially spermidine and spermine participated in control of insect development (Carye *et al.*, 1995; Kogan & Hagendorn, 2000). Nakabachi & Ishikawa (2001) stated that spermidine occured in bacteriocytes which existed in *Acyrthosphon pisum* /Haris/ and its content altered with development and senescence of hosts. Thus the presence mRNA of S-adenosylomethionine decarboxylase in Procaryota living in young aphids organisms may suggest that this enzyme regulates function of intercellular symbiotic system of these insects.

Moreover, the increase of content of analyzed compounds was noted in the vegetative parts of attacked by *S.avenae* plants, with the exception of the shoots of cultivar Fidelio, where the polyamines quantity decreased in relation to control plants (Fig. 2). The presented results permit to conclude, that the changes of polyamine level, caused by grain aphid feeding in ears of winter triticale, have the systemic character. But the trend and intensity of the changes depend on organs of plants and cultivar to same extent.

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.113 - 119

Polyphenol oxidase activity and its participation in spring triticale resistance to grain aphid (*Sitobion avenae*)

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Abstract: Polyphenol oxidase (PPO) [EC 1.10.3.1] is copper containing enzyme responsible for oxidation of *o*-diphenols to *o*-diquinones and also hydroxylation of monophenols to o-diphenols. These two reactions are closely related probably. This enzyme was involved in three physiologically important processes viz., cuticular hardening, plant defense reactions and detoxification of phenols in insects. In this work PPO was isolated from the ears of four spring triticale cultivars (Gabo, Migo, Wanad, Kargo). PPO activity was determined after 12, 24 and 168 hours *S. avenae* feeding on each cultivar. In this report was also examined the antibiosis and the tolerance of the spring triticale to grain aphid. To evaluate the level of antibiosis the intrinsic rate (r_m) and average generation development time (T) were estimated on the base pre-reproductive period (PRP) and daily fecundity (DF). The tolerance was defined with use weight index (WI), tolerance index (TI) and relative plant mass change (DWT). Calculated tolerance coefficient indicates that highest level of this resistance possessed Gabo cultivar and lowest – Kargo. Analysis of the antibiosis tests results showed that cultivar Migo and Kargo had higher level of this resistance mechanism than Wanad and Gabo. On the basis chemical analysis was showed that increase of PPO activity in Kargo and Wanad plants (after 12 hs), Wanad (after 24 hs) and Gabo (after 168 hs) was observed.

Keywords: oxidizing enzymes, cereals, aphids

Introduction

Polyphenol oxidase is a copper containing protein also known as tyrosinase. It is bifunctional enzyme possesing both monophenol monooxygenase activity for hydroxylation of monophenols to *o*-diphenols and *o*-diphenoloxidase activity for oxidation of *o*-diphenols to *o*-quinones (Duran & Esposito, 2000). PPO is widely distributed in higher plants but it still has no defined biological functions, although many possible roles have been proposed (Hind *et al.*, 1995, Lax & Vaughn, 1991, Steffens *et al.*, 1994, Trebst & Depka, 1995). The most likely functions for PPO are its involvement in plant resistance against diseases (Goy *et al.*, 1992, Ray & Hammerschmidt, 1998), positive role of phenolic compounds oxidised in plants was also showed (Boss *et al.*, 1995). PPO participates in resistance of plants to insect herbivores (Felton *et al.*, 1992) and possibly in photosynthetic regulation (Vaught *et al.*, 1988).

The phenolic compounds produced in the oxidation processes are highly toxic (Pillinger *et al.*, 1994). The enzymatic activity for phenolic oxidation is regulated by many factors, both biotic and abiotic (Smith-Becker *et al.*, 1998) and also different types of stress (Dixon & Paiva, 1995).

In this study was examined the effect of different time feeding of the grain aphid (12, 24 and 168 hours) on the PPO activity in the ears of spring triticale cultivars. The antibiotic

properties and tolerance of some triticale cultivars to grain aphid (*Sitobion avenae* /F./) were also tested.

Materials and methods

Entomological experiments

The all entomological experiments were carried out in years 2002-2003. The tolerance was tested in field conditions, whereas antibiosis in laboratory tests.

To evaluate the tolerance of spring triticale to grain aphid, 10 ears infested by *S. avenae* and 10 control plants (without aphids) were selected and isolated with the fine-mesh gauze. This test was begun at the flowering stage of triticale development (G.S. 63, according to Tottman & Broad, 1987). The aphids were counted on the infested ears at the medium milk development stage (G.S. 75). Test was terminated at the stage of technical maturation of triticale and infested and control plants were harvested. The dry ears were weighed and the following tolerance indices were calculated: weight index /WI/ according to Bramel-Cox *et al.*, (1986); tolerance index /TI/ (Dixon *et al.*, 1990); relative plant mass change /DWT/ (Reese *et al.*, 1994).

Antibiosis was measured by several components of population growth. Test was carried out on the 7-day old seedlings of spring triticale cultivars. 10 seedlings of each cultivar were inoculated with one wingless female. The each plant was isolated with plexiglass tube. When females gave birth to their offspring, one first-instar nymph was left in the cage to maturity, other were removed. The following metrics were recordered for each plant: pre-reproductive period (PRP) of the ensuing adults (expressed as day degrees); daily fecundity (DF) of the adults over a 10-day period. These data were used for calculation of the intrinsic rate of natural increase (r_m) and average generation development time (T) according to Wyatt and White, (1977).

In order to asses the influence of grain aphid infestation on PPO activity 5 wingless of *S. avenae* were put on the ear of tested plant. Experiment was started at the flowering stage (G.S. 63). 30 ears with aphids and the same number without aphids were isolated with fine-mesh gauze separators. After 12, 24 and 168 hours the plant material was harvested (10 ears for each time), placed in dry ice and transferred to laboratory, where were subjected to chemical analyses.

The significance of differences in antibiosis and tolerance among the cultivars was evaluated using Duncan's test at $P \leqslant 0.05$.

Chemical analyses

A 150 mg sample of acetone powder was ground with 15 cm^3 of 0.05M phosphoric buffer at pH 7.4. After centrifugation (12000g, 20 min., 4°C) the supernatants were recovered and desalted. The protein concentration in the extracts was examined using Lowry et al., (1951) method.

Polyphenol oxidase activity was determined using modified method described by Chrzanowski *et al.*, (2003). PPO activity was assayed by incubation 1 cm^3 extract, 0.5 cm^3 0.05M phosphoric buffer pH 7.4 and 0.5 cm^3 0.4% catechol for 30 min. Unit of activity (U) was defined as increase of absorbance at 460 nm per 30 min ($\Delta A \cdot 30\ min^{-1}$).

The all chemical analyses were carried out in three replicates. Differences in the activity of PPO between examined cultivars were calculated using Duncan's test at $P \leq 0.05$.

Results and discussion

The entomological observation to evaluate of the tolerance in a field conditions showed that Gabo cultivar possessed lowest values of the all parameters while cultivar Kargo had highest values of tolerance index (TI) and relative mass change (DWT). Migo cultivar had a low tolerance index, too (Table 1).

Moreover, the results of this work (Table 2) suggest that Kargo and Migo cultivars strongly reduced daily reproduction (DF) and population growth (r_m). These parameters were higher for insects feeding on Wanad and Gabo ears.

Table 1. The tolerance indices of spring triticale cultivars in relation to grain aphid

Cultivar	Tolerance indices		
	WI	DWT	TI
Kargo	21.49b	32.74a	8.51a
Migo	23.92a	27.91b	3.77c
Wanad	23.42a	27.76b	4.91b
Gabo	10.45c	25.47c	3.53c

The values in the same row followed by other letters are significantly different at $P \leq 0.05$ (Duncan's test).

Table 2. Antibiotic effect of spring triticale cultivars on growth and fecundity of wingless parthenogenetic females of grain aphid

Cultivar	Antibiosis indices			
	PRP	DF	T	r_m
Kargo	8.25a	0.40b	11.18a	0.10b
Migo	8.00a	0.22c	10.84a	0.07b
Wanad	5.50b	0.68a	7.45b	0.16a
Gabo	5.75b	0.72a	8.13b	0.18a

The values in the same row followed by other letters are significantly different at $P \leq 0.05$ (Duncan's test).

Obtained results are consistent with our observation on grain aphid population density (data not published). It was showed that cultivars with higher number of aphids possessed higher level of tolerance. Moreover, Ciepiela *et al.*, (1999b) proved that cultivars of winter tritjcale of higher antibiosis mechanism were less tolerant to grain aphid.

Considering all cultivars, activity of PPO in healthy ears (Table 3) fluctuated between 0.196 and 0.388 $U \cdot mg^{-1}$ of protein in flowering developmental stage (G.S. 63). Activity of analysed enzyme varied between 0.313 and 0.731 $U \cdot mg^{-1}$ of protein in water-ripe stage (G.S. 72). These results are similar as Chrzanowski *et al.*, (2003) received. They showed that cultivar Migo had higher activity of polyphenol oxidase in relation to Wanad in G.S. 63.

Significant differences ($P \leq 0.05$) were showed between cultivars in both analysed time period. Only in flowering stage (G.S. 63) the PPO activity for Gabo and Wanad was similar and without statistical difference.

Table 3. Activity of polyphenol oxidase (in $U \cdot mg^{-1}$ protein) in the ears of spring triticale during the time period of infestation experiment

	Developmental stage	
Cultivar	**G.S. 63**	**G.S. 72**
Kargo	0.259b	0.639b
Migo	0.388a	0.605c
Wanad	0.210c	0.313d
Gabo	0.196c	0.731a

The values in the same row followed by other letters are significantly different at $P \leq 0.05$ (Duncan's test).

During infestation experiment, increase of PPO activity was observed after 12 and 24 hs for Wanad cultivar; after 12hs for Kargo; after 24hs for Migo in relation to the earlier time, but this level was similar to the control. In the ears of Gabo cultivar infested with *S. avenae* activity of PPO was lower than in control plants, but after 168 hs of grain aphid feeding was observed increase (Fig. 1). In cultivars of high antibiosis the activity not exceeded 30% over control plants. It is interested that cultivar Wanad of high tolerance and low antibiosis had a very high PPO activity in infested ears over 100% to control, and after 24 hs of infestation over 300% to control.

This result was compared with Miles & Oertli, (1993), they showed that too high oxidation of phenols can induce an excessively rapid loss of toxicity by further transformation into harmless polymers. Insertion of oxidases with aphid's saliva into tissues of the host plants cause changes of phenol oxidase activity in infested plants (Jiang & Miles, 1993).

Among constutively and inducible secondary compounds (Chrzanowski *et al.*, 2002), protein and amino acids (Ciepiela *et al.*, 1999a), PPO and other enzymes (Tyagi *et al.*, 2000) and proteinase inhibitor (Casaretto & Corcuera, 1998) have been shown to be resistance factor.

According to Casaretto & Corcuera, (1998) experiment the maximum increment proteinase inhibitor activity in the leaves of barley was observed after 48 hours infestation with aphids. Moreover, a decrease in the content of protein in resistant wheat cultivar was noted after 24hs (Sempruch & Ciepiela, 1998). The results of this work are according to Constabel *et al.*, (1995) and Thaler *et al.*, (1996) they were found that jasmonic acid – resistance signalling molecule is produced by the plants after increase production of resistance marker as polyphenol oxidase.

Because cultivars Kargo and Migo had a high antibiosis, it was possible that this mechanism of resistance was running after 12 hours aphid infestation in Kargo plants and after 24 hours in Migo

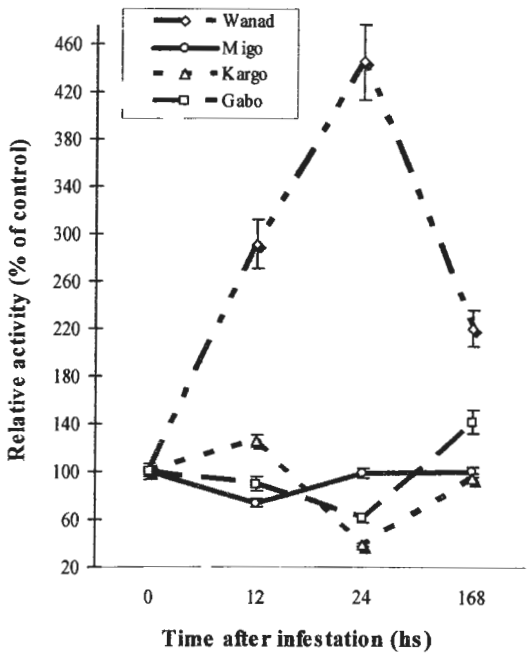

Fig. 1: The changes of polyphenol oxidase activity caused with grain aphid feeding of different time.

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Does red pepper contain an insecticidal compound for Colorado beetle?

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Abstract: Capsaicin is synthesized naturally by *Capsicum* plant - red pepper (Solanace). Accumulation of this alkaloid in plants may serve as part of a defence mechanism protecting plants from being eaten by mammals. However, interestingly, it has been noticed that capsaicin-producing plants, do not attract also for insects, which normally feed on Solanace. Larvae of Colorado beetle (*Leptinotarsa decemlinneata*) for example shows a clear preference for potato and tomato diet. We think, that is highly possible, that one of the reasons that red pepper does not attract Colorado beetle is the fact that these insects are sensitive to capsaicin and that capsaicin may affect their thermoregulation and may cause similar overheating effects as observed in mammals. The aim of this study was to examine the effect of capsaicin on thermal behaviour and survivability (in different temperature) of last larval instar of Colorado beetle. Animals fed on red pepper in comparison to animals fed on potatoes, preferred an environment warmer by 10^0C.We show for the first time here, that capsaicin may be a part of defence mechanism of *Capsicum* plant not only against rabbits or squirrels but against Colorado beetles either.

Keywords: capsaicin, red pepper, Colorado beetle, thermal behaviour

Introduction

Capsaicin, the pungent ingredient of hot pepper, activates vanilloid receptors located primarily on C and Aδ fibre (mechano-heat) sensory nociceptors (Marsh *et al.*,1987) and initiates a complex cascade of events, including release of proinflammatory mediators and neuronal excitation, by activating cation-specific channels. Repeated applications of capsaicin causes refractoriness to itself and to other stimuli, while sparing motor function (Liu *et al.*, 2001). Such refractoriness to thermal stimulation may cause impairment of thermoregulation which was observed in homeotherms (Jancsó-Gábor *et al.*,1970; Cabanac *et al.*,1976; Cormarèche-Leydier *et al.*,1985) and also in heterotherms (Cormarèche-Leydier, 1986).

The first application of capsaicin induces temporary hypothermic reactions in these animals, however, repeated application causes permanent phenomenon of desensitisation along with impairment of thermoregulatory reactions. Capsaicin-desensitised mammals are no longer able to protect themselves against heat and respond with pronounced hyperthermia when exposed to high ambient temperatures (Jancsó-Gábor *et al.*,1970).

To the best of our knowledge there is no data describing the influence of capsaicin on thermoregulation in insects. However, the effects of insecticides modulating sodium channels' activity on thermoregulation in insects and frogs has been shown (Tęgowska *et al.*, 2001, Tęgowska, 2003; Grajpel *et al.*, 2002; Grajpel & Tęgowska 2004). Capsaicin exerts its influence via vanilloid receptors 1 (VR1) which are temperature-sensitive cation channels (Choi & Kim,1999), therefore it is possible that it changes thermoregulation in insects as well. If capsaicin causes an impairment of thermoregulation in insects exposed to hot environment,

this alkaloid could make up the defence mechanism in red pepper. The Colorado potato beetle is one of the insects that inhabit the same climate zone as red pepper. It is know to feed on solanaceous plants, which include red pepper, potatoes and tomatoes. But the last two plants are its preference, when the animal is given a choice under experimental conditions and when it has free access to them in its natural environment. These preferences can be caused by various factors, e.g. mellowness or succulence of plants, but they can also be the effect of alkaloids present in these plants. The content of these secondary products of plants' metabolism is different in individual plant species and it can be also the cause of various feeding preferences of Colorado potato beetle and in extreme cases it is even a plant-protective factor.

This made us test the effect of capsaicin on survivability and thermopreferences in Colorado potato beetle feeding on potato or red pepper.

Materials and methods

Adult and larval last instar individuals of Colorado potato beetle were collected from the farm located 30 km from Bialystok, where no insecticides spraying was done current year. Before experiments started Colorado potato beetle were fed on potato leaves and tubers. During the experiments, lasting 48 hours (concerning both thermal preferences and survivability) animals in gradients were fed on potatoes or red pepper, exchanged every 4 hours (to secure succulence), water was delivered at the same time. Larvae (n=20) were weighted before and after the experiment. In order to study thermal preferences, insects were placed in thermal gradient made of 100 cm long aluminium runway. The temperature varied from 10^0C in the coolest to 42^0C in the warmest part of the gradient. The thermal preferences of larvae and adult individuals fed exclusively on potatoes and of larvae fed on potatoes or red pepper were compared. Animals' behaviour was recorded with a video camera and data were elaborated with the use of Pinnacle DC Plus. Programme Tukeya (HSD) test was used to assess the effect of capsaicin on insects' thermoregulation.

In one of thermal-gradient insects were free to move along its length, whereas in the second and third animals were distributed into eight compartments, marked with numbers 1-8. The temperatures in individual compartments were: 10-12, 14-16, 18-20, 22-24, 26-28, 28-30, 32-34, 36-38^{0}C respectively. Ten insects were placed in each compartment and were supplied with potatoes or red pepper and water. Their survivability was assessed.

Results

Both adult and larval Colorado beetle individuals, fed exclusively on potatoes, actively chose ambient temperature. However, adult chose higher temperature and this choice showed a clear circadian rhythm (Fig.1). They chose the warmest environment at noon and the coolest at night. The rhythm in larvae was only poorly expressed and its extremes occurred in reverse order to adult individuals. The possibility cannot be excluded that the choice of preferred temperature during this period was affected by the fact that experiments were performed on last larval instar of the beetle. Some of one larvae pupated during the second day of experiment and change in their thermal preference could be a part of a developmental adaptation, as larvae enter the soil before pupation, i.e. a cooler environment.

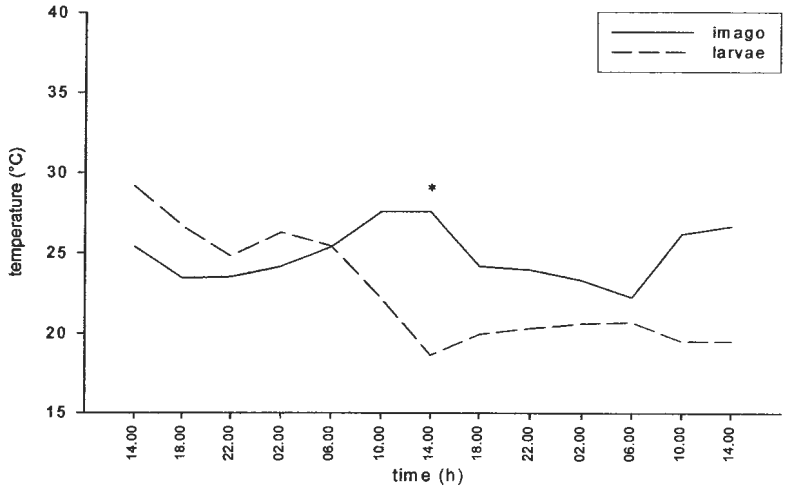

Fig. 1: Temperatures preferred by Colorado beetles feeding on potatoes only – imago and larva. Each point represents the mean for 30 min (from every 10 s period) for 20 insects.
*– The difference between the temperatures preferred by imagoes and larvae: $p<0.05$.

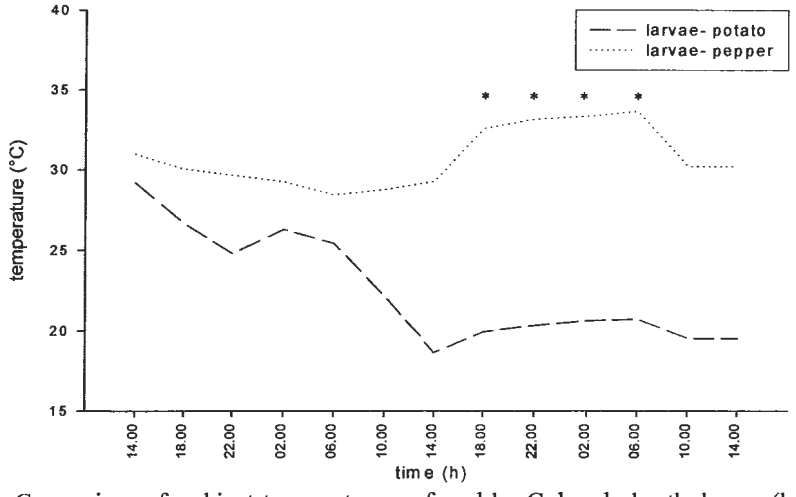

Fig. 2: Comparison of ambient temperature preferred by Colorado beetle larvae (last instar) feeding on red pepper and on potato. Each point represents the mean for 30 min (from every 10 s period) for 20 insects.*- Shows a statistical difference $p< 0.05$.

Feeding on red pepper caused larvae to choose a warmer environment with simultaneous complete suppression of circadian rhythmicity of thermal preferences. It is worth to note that

larvae feeding on red pepper chose even higher temperatures than those chosen by adult individuals, despite the fact that few of them pupated (it preferred colder environment).We compared body masses of larvae kept on diets with and without red pepper. Those animals that were fed on red pepper had lower body mass by about ~10% (individuals that pupated during the experiment were not included in this assessment).

The design of the next part of an experimental series allowed us to monitor the effect of an ambient temperature on the rate of survival of animals. The animals were placed in closed compartments with temperatures stabilized at different levels as described in Methods. After 48 h the survival of Colorado potato beetles fed on potatoes was 100% in compartments 1-6, however in 5 and 6 compartments animals were lethargic but alive. Whereas in the two warmest compartments 7-8 the survival rate was dramatically lower, did n't exceed 50%.

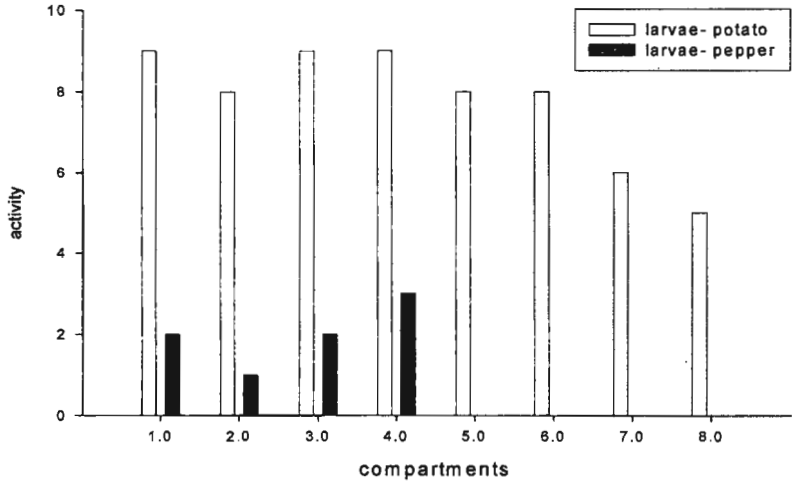

Fig. 3: Motor activity of Colorado beetles larvae (n=10/compartment) fed on red pepper or potatoes. Lack of motor activity = lethargic or dead

Red pepper remarkably limited Colorado beetle's mobility – especially in warmer compartments. During light phase of the day almost all control larvae showed activity (eating and translocation). Larvae which feed pepper were sedative (only eating). Larvae which permanently (without possibility a choice) stay in warmer compartments (Fig. 3) were lethargic in spite that it was a preferred ambient temperature of free moving larvae (Fig. 2).

Discussion

Capsaicin belongs to alkaloids deriving from tyrosine - it is a secondary metabolite, probably redundant in basic cellular life processes but energetically expensive. Therefore, it is considered that there are necessaries of life and that its natural function is to protect the plants from being eaten by scaring off squirrels and rabbits.

Alkaloid-like substances, because of their bitter and hot taste, can be a protective factor against animal pests attacking the plants in the field or in the storage. However, capsaicin

additionally affects thermoregulatory processes, especially related to stress of heat (Jancsó-Gábor *et al.*, 1970; Cabanac *et al.*, 1976; Cormarèche-Leydier *et al.*, 1985), what is of great importance because capsaicin is present in the plant originally growing in hot climates. The Colorado potato beetle, a pest of solanaceous plants, primarily potato, tomato but also, under extreme conditions, red pepper, comes also from the same areas. Rearing Colorado potato beetle on red pepper caused growth limitation, the difference in body mass between the control group and the group supplied with capsaicin reached 10% after 48 h. A similar reaction was observed in rats (Cormarèche-Leydier, 1981, 1984). Capsaicin did not prevent, at least in the case of some insects, development of larvae to pupation, therefore it is not a deadly poison for Colorado potato beetle. However, it did influence thermoregulation of the insect and when the experiment continued further, animals suffered death. Avoiding the heat in hot climate protects not only from lethal hyperthermia but also from loss of body fluids. Choosing warmer environment (almost 10°C difference in preferred temperatures) by Colorado potato beetle larvae feeding on red pepper in comparison to control larvae, despite the fact that it is not a temperature threatening with overheating (larvae that died the 3rd day did not occupy the warmest places in the gradient), can be a significant danger to life because the loss of water through poor isolation of larvae's integument. The quantity of water drank by larvae during the experiment was not measured (water refilling was supposed to maintain mainly the humidity in the gradient). However, in rats that were supplied capsaicin much lower water intake was observed in comparison to control ones. (Cormarèche-Leydier, 1984) If this type of phenomenon occurs in insects (especially in larvae) living in the field, even such a 10-degree increase of temperature preference, along with the lack of water, could limit Colorado potato beetle's survivability.

There is an additional possibility of increasing the hyperthermic effect of capsaicin applied to larvae and taking effect on adult individuals. For it has been shown in newborn rats that short-term exposure to capsaicin caused the danger of hyperthermia in adult individuals exposed to heat (Hori & Tsuzuki, 1981), because these adult individuals, despite the lack of recent contact with capsaicin, had impaired heat-protecting mechanisms. In current study we did not breed Colorado potato beetle pupae of larvae that were fed on red pepper, therefore we do not know whether the effect of choosing a warmer environment lasted in imago forms of Colorado potato beetles.Obviously mammals' defence against the heat stress comprising of vasodilatation or salivation that is impaired by capsaicin does not exist in insects, but the fact of changing the onset of thermolytic reactions, proven in mammals (delay), can be expressed as a change of preferred temperatures in insects (Obal *et al.*, 1979). Although the existence of thermoregulatory centre in insects is proven (Janiszewski 1986; Janiszewski & Otto, 1988), there have been no studies showing the change in electrophysiology of brain neurons or peripheral thermoreceptors in insects that were supplied with capsaicin. It has been, however, shown in mammals (Obal *et al.*, 1979; Rabe *et al.*, 1980; Obal *et al.*, 1983; Shimada *et al.*, 1990). The change in activity of membrane channels can be grounds for these electrophysiological changes. Capsaicin acts through vanilloid receptors, which are temperature-sensitive cation channels (Choi & Kim, 1999). Initially, capsaicin excites neurons by activating a cation-specific ion channel that is permeable to cations e.g. Ca^{+2}. Application of capsaicin for prolonged periods of time can cause an increase in calcium concentration which induces irreversible toxic effects leading to the loss of pain and hot-sensing neurons (Chard, 1985) and so an insect could have not felt the danger of the heat.

Additionally, it has been also shown that capsaicin inhibited action potentials and modulated activity of voltage-gated sodium channels (Liu *et al.*, 2001). Such major impairment of neuronal activity in mammals caused lack of integrative control of body temperature (Lee *et al.*, 2000; Osaka *et al.*, 2000).

The results of our study and previous studies on mammals show that it is worth to broadening the investigations on capsaicin effect on insects because its effect would be expressed exactly when some insecticides, e.g. pyrethroids loose their efficacy during exposure to high temperatures. Therefore, it seems we can give a positive answer to the question raised in the title of the paper – at least it can be an agent broadening the efficacy of pyrethroids also to high ambient temperatures.

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Breeding for Plant Resistance to Pests and Diseases
IOBC wprs Bulletin Vol. 28(10) 2005
pp.129 - 132

Combining ability of resistance of yellow rust in some wheat varities

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Abstract: In 2000 five wheat varities, one susceptible and four resistant to yellow rust were inter-crossed using a half diallel fashion in Ardabil university. In 2001 parents and f1 progenies were planted in a randomized complete block design with three replications. Then spors of different races of rust were shed on all genotypes and the latent period were evaluated. The results showed significant genetic difference between genotypes. Combining ability analysis showed significant Msgca / Mssca ratio for this character, indicating additive variance to be more important than non-additive variance. Broad and narrow sense heritability estimates were 58% and 79% respectively.

Keywords: wheat, rust yellow, diallel, resistance

Introduction

Yellow rust caused by(P*uccinia striiformis)* in wheat (*Triticum aestivum*) is a serious problem in Iran. The most effective way of controlling this disease is to develop resistant cultivars. Currently there is much interest in the mature plant resistance, as opposed to seedling resistance (Kuhn *et al.,* 1980; Knott 1982; Yadav 1993; Broers *et al.,* 1996). This form of resistance usually is longer lasting and quantative in inheritance. The importance of this disease led plant breeders to attempt new breeding approaches for developing resistant genotypes. The use of transgressive or heterosis to breed new varieties which which surpass the best parent has been considered a valuable approach for developing resistant genotype (Smith 1996; Waliwork & Johnson 1984; Broers & Jacob 1989; Yadav *et al.,* 1992). Quantative genetic theory provides models for predecting frequencies of transgressive segregants. Vanderplank (1963) cited two models, for horizontal and vertical resistance.

In Iran, damage of yellow rust approached to 1.5 million t/ha (Kashani.1995). The aim of this experiment was the study of resistance in parents and the use of better hybrids in crosses.

Materials and methods

In 2000, five wheat genotypes (Bolani,Tajan, M-73-7,Chamran and Shiroodi) were crossed using a half diallel design at the University of Azad. These parents and progenies were transferred to Lahijan City in the north of Iran, because of suitable weather from the aspect of humidity and temperature. They were planted in greenhouse then rust spores were distributed on the total plant at the stem elongated stage. Fifteen genotypes were planted in a randomized block design with three replications. In this experiment the row length for every treatment was 2m, spaced 0.5m apart. The plant to plant distance within rows was 10cm. T to ensure yellow rust development, the susceptible genotype Bolani was planted in spreader rows around each replication. This variety was inoculated artificially with a mixture of races of yellow rust of wheat. Analysis of variance for randomized block design was carried out and combining ability analysis was carried out follow method 2, model 1 of Griffing (1956).

Result and discussion

Table1 shows of analysis of variance for all wheat genotypes. The results showed significant genetic differences among genotypes. This difference was caused by additive and non-additive gene effects.

Table 1. Analysis of variance for latent period to yellow rust of wheat

Source of variation	DF	Mean Square
Replication	2	0.8
Treatment	14	109.28**
Error	28	0.48

** significant at 1% level of probability

Results also indicated additive gene effects controlling traits influencing latent period control of the pathogen (Ghanadha et al 2000), agreeing with Macintosh (1988)

Table 2. Means comparison latent period for tested wheat genotypes.

Genotype	Latent period
Bolani .	8 h
Bolani . Tajan	17.66 b
Bolani . M-73-7	19.98 a
Bolani . Chamran	16 cd
Bolani . Shiroodi	16.1 cd
Tajan	17.3 b
Tajan . M-73-7	11.24 f
Tajan . Chamran	14.53 e
Tajan . Shiroodi	10.85 g
M-73-7	15.98 cd
M-73-7 . Chamran	16.21 cd
M- 73-7 . Shiroodi	19.32 a
Chamran	10.93 g
Chamran . Shiroodi	13.76 e
Shiroodi	16.90 b

Non-overlapping letters = significant difference at 5% probability level.

Table 2. contains result comparing means and their difference among wheat genotypes. Hybrid Bolani. M-73-7 was found to be the most resistant cross. In Table 3, the variance of gca is more than sca, which indicates that additive effects are more important than dominant and epistatic effects in enhancing the pathogen's latent period (i.e. more resistance), as also found by Lewellen et al., 1967.

Table 3. Mean square from combining ability analysis.

Source of variation	DF	Mean squares
Gca	4	68.25**
Sca	10	5.32
Error	28	0.68
Msgca/ Mssca		12.82**
h2B.S		0.58
h2 N.S		0.79

** significant at %1 level of probability

From the results of Table 4 one can conlude that the effect of gca and sca depends on the parents used. Some hybrids increased the pathogen's latent period. Using the parameter gca, the parental line Shiroodi was found to be a good parent for enhancing the latent period (more resistance). M-73-7 hybrid and this condition define existence of dominance in increase direct of latent period. For this trait can use for increase of resistance.

Table 4. Estimates of gca (diameter) and sca (out of diameter)

Latent period	Bolani	Tajan	M-73-7	Chamran	Shiroodi
Bolani	-3.8**	1.98**	3.29**	0.58	0.23
Tajan		-2.3**	-1.23**	0.05	-2.31**
M-73-7			-0.78**	-0.59	3.44**
Chamran				-2.33**	0.05
Shiroodi					1.7**

**significant at 1% level of probability

Conclusions

For this trait i.e. to increase resistance to rust, we recommend for screening and selection that latent period is a suitable parameter because this method is simple and reliable for identifying rust resistant genotypes in our breeding project.

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IOBC wprs Bulletin Vol. 28(10) 2005
pp.133 - 137

Exploitation of phenotypic expression of developmental and quantitative trait(s) towards seedlessness as major genetic potential for bollworm avoidance in cotton (*Gossypium* spp.)

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Abstract: Cotton (*Gossypium* spp.) (2n=26 or 56) is the most important fiber crop for textile industry use. Among many insect pest attacking cotton, the bollworm complex alone can cause from approx. 50% total damage to the crop. Number of seeds per boll, mean weight of one seed (g), seed volume per boll (cc), mean density of one seed (g/cc), initial boll weight (g), final boll weight less seed (g), lint volume per boll(cc), and lint density per boll(g/cc) were proved to be important parameters for improving the seedlessness in cotton. Three diploid and two tetraploid genotypes were considered under a completely randomized block design, with five replications for evaluating their performance for bollworm avoidance in relation to increased lint yield potential. The lack of relationship between fiber and seed parameters indicated involvement of separate biosynthetic pathways in their development. Number of seeds per boll in decreasing order over the genotypes follows similar trend for bollworm pressure (on 1-10 scale) in decreasing order over these genotypes. Deviation from the trend for bollworm pressure in DLSA-17 indicated involvement of pleiotropic effect of genes. These observations lead to the hypothesis that a high auxin level, necessary for the unfertilized ovule growth, is modulated through gibberellic acid pathway lignin content in endocarp of the fruit and provides resistance against cotton bollworm pressure.

Keywords: seedlessness, bollworm complex, bollworm pressure, anthesis, endocarp lignification.

Introduction

Cotton (*Gossypium* spp.) has retained its prominent position in the international arena despite stiff competition from synthetic fiber. Besides one of the major cotton producing countries in the world, occupying the third place (2.6 million MT) and having the largest acreage (8.8 million ha), the average productivity of India (298 kg lint/ ha) is the lowest and less than the world average productivity level of 581 kg lint/ ha (Mayee *et.al.*, 2001). The major impediment to such low productivity is the susceptibility of existing cotton germplasm to major insect pests, particularly bollworm complex predominant in high rainfed areas. Pink bollworm, *Pectinophora gossypiella* saunder and Spotted bollworm, *Earias vittella* Fabricius and *E. insulana* Biosed can cause 44.5% reduction in seed-cotton yield and American bollworm, *Helicoverpa armigera* Hubner may result in total failure of the crop (Dhawan *et. al.*, 2002). The figures indicate that the major genetic potential of the crop remains yet to be fully exploited. Seedlessness may prove to be an essential indirect selection index in cotton for bollworm avoidance.

Materials and methods

A field experiment was carried out in locally adopted three diploid (2n=26) genotypes, namely, Jawahar Tapti, Turabh and DLSA-17 and in two tetraploid (2n=52) genotypes, namely JK-4 and Khandwa-2 in five replications in Randomized Complete Block Design (RCBD) during 2002-03 cropping season at the Regional Agriculture Research Centre, AICCIP, JN Agriculture University, Khandwa (M.P.) India. Major developmental and quantitative traits used as indicators for seedlessness in cotton were number of seeds per boll, mean weight of one seed (g), seed volume per boll (cc), mean density of one seed (g/cc), and initial boll weight (g), final ball weight less seed (g), lint volume per boll (cc) and lint density per boll (g/cc). The weight measurements were recorded on Sartorius Universal balance and volumetric measurements were quantified by volumetric cylinder method. The observations on bollworm avoidance were recorded in percentage incidence of bollworm complex on squares, final bolls and locules and scored on a 1-10 rating scale as bollworm pressure. The data were analyzed for RCBD following Dudley (1997). Compared data points at $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered significantly different.

Results and discussion

Strong relationships among number of seeds per boll, mean weight of one seed (g), seed volume per boll (cc) and mean density of one seed (g/cc) were observed. The presence of no-relationship between seed and fiber parameters indicated an inverse trend between them (Fig.1). The volume of seeds and lint in a single boll averaged 2.6 and 1.1 cc, respectively over the five genotypes and represented 70.27% as the potential volume for lint, occupied by seeds. Significant lint yield increases can be achieved in cotton by making direct selection for the trait under crop improvement programmes.

Changes in the nuclear chromatin content, as revealed by the variation in ploidy levels in various genotypes under study, have significantly affected the component developmental and quantitative traits for seedlessness in cotton. The lack of relationship between fiber and seed parameters, as expressed in significant phenotypic variations for the traits, supports the notion that the enzyme systems operating in the development of fiber and seeds follow separate biosynthetic pathways. Cotton fibers are the result of a terminal differentiation sequence that begins with ovule epidermal cells (Wilkins & Jernstedt, 1999). Developmental cues for fiber are present over a long period of time that provides sufficiently long time over which to manipulate development to increase fiber production (Seagull & Giavalis, 2004). Plants possess integrated signaling network that mediate the nuclear responses such as hormonal responses of plant growth and development (Satpute & Satpute, 2003). *Ex-situ* gibberellin treatment at pre-blooming stage in *Vitis vinifera* was reportedly resulted in a significant reduction in number and size of seed (Striem, 1994).

Hormone treatment experiments (Seagull & Giavalis, 2004), particularly an increased level of IAA, resulted in the increase in fiber production due to an increase in the proportion of epidermal cells that become fibers (Seagull & Giavalis, 2004, Jasdanwala, *et al.,* 1980). Morphogenic pathways for epidermal cells remain flexible during early fiber development (0-5d post anthesis). Changes that result in increases in endogenous IAA levels during the first 5d post-anthesis could result in significant increases in fiber production (Seagull & Giavalis, 2004).

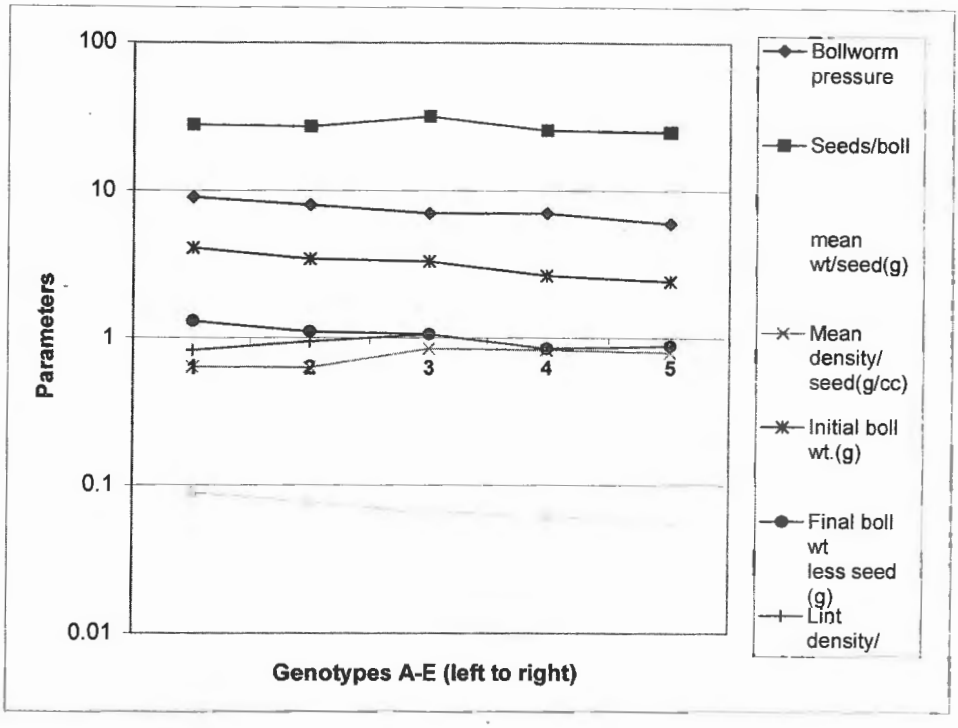

Fig. 1: Trends of various parameters of seed and fibre over five locally adapted genotypes of different ploidy levels. [A=Khandwa-2(2n=52), B= JK-4(2n=52), C= DLSA-17(2n=26), D= J.Tapti(2n=26), E= Turabh(2n=26)]

Studies on the endogenous IAA level in cotton ovules demonstrated a high level near the day of anthesis followed by a rapid decline (Chen *et al.,* 1996, Nayyar *et al.,* 1989, John 1994). Increasing levels of GA at pre-anthesis and low levels in early post-anthesis ovules coincides with fiber initiation (Chen *et al.,* 1996, Seagull & Giavalis, 2004, Nayyar *et al.;* 1989). Seagull & Giavalis, (2004) reported that the most effective treatment for increasing fiber number was the post-anthesis treatment with IAA, which produced an approximate 12% increase over the next highest value, pre-anthesis treatment with GA_3 and an approximate 59% increase above the comparable (post- anthesis treatment) control ovule.

Bollworm pressure (on 1-10 scale) over the genotypes in decreasing order was observed to follow a similar trend to that of the decreasing number of seeds per boll over the genotypes. (Table 1). Plants utilize in their biosynthetic and detoxification pathways a diverse array of Cytochrome P450 proteins, which are heme- containing monoxygenases. Biosynthetic P450s play a paramount role in the synthesis of lignin intermediates (Schuler, 1996). CYP87A3, a new member of the cytochrome P450 (CYP) super-family was reportedly induced by auxin (Chaban *et al.,* 2003). Kim (1998) showed association of increased resistance of boll wall to penetration with lignification of the boll wall endocarp. Approaches for increasing lignification may provide a potential for a decreased use of pesticides in cotton plants with improved pest resistance. Deviations from the trend for bollworm avoidance, in the present study, particularly in case of DLSA 17 have indicated that interplay of pleiotropic effect of

genes involved in bollworm avoidance are important. Based on experimental observations a hypothesis is proposed that a high auxin level, necessary for the growth of unfertilized ovule, modulates through GA pathway the lignin content in the endocarp of the fruit, which adds to the increased resistance against bollworms. In seedless floral mutants of another agriculturally important crop (apple), seediness in fruits after hand pollination was reportedly re-established for its commercial multiplication (Yao *et al.*, 2001).

Table1. Phenotypic variations in seed and fibre parameters at various levels of polyploidy in different genotypes of cotton. SEM± = Standard Error of mean; CV% = Co-efficient of variation; *, **, ***, ns: sig. Slope at P <0.05, P <0.01, P <0.001, P >0.05.

Characters / Local Genotypes (Ploidy levels)	Bollworm pressure (1-10 scale)	No. of seeds per Boll	Mean Weight of One Seed (g)	Seed Volume per Boll (cc)	Mean Density of One Seed (g/cc)	Initial Boll Weight (g)	Final Boll Weight Less Seed (g)	Lint Volume per Boll (cc)	Lint Density per Boll (g/cc)
	1)	2)	3)	4)	5)	6)	7)	8)	9)
Khandwa-2 (2n=52)	9	27.8^{ns}	0.089	3.9	0.628*	4.06	1.30	1.7	0.82*
JK-4 (2n=52)	8	27.0*	0.076^{ns}	3.2*	0.627*	3.42*	1.10^{ns}	1.2***	0.94^{ns}
DLSA-17(2n=26)	7	31.6	0.065**	2.4***	0.840	3.30*	1.06^{ns}	1.0***	1.06
J. Tapti (2n=26)	7	25.6**	0.062**	1.9***	0.824^{ns}	2.64***	0.84**	1.0***	0.84^{ns}
Turabh(2n=26)	6	24.8**	0.055** *	1.7***	0.794^{ns}	2.42***	0.88**	1.0***	0.88^{ns}
Mean	-	27.40	0.069	2.6	0.742	3.16	1.03	1.1	0.90
SEm±	-	1.34	0.005	0.2	0.070	0.21	0.09	0.1	0.07
CV%	-	10.97	17.493	19.2	20.540	14.74	20.67	12.7	18.62

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