Post-graduate Student Posters

Session Organiser: Professor Robert Naylor
Trelareg Consultants, Aberdeen, UK

Poster Presentations: PPG-1 to PPG-18

Aphid density influences oviposition behaviour and larval performance in predatory hoverfly

R Almohamad, F Verheggen, F Francis, E Haubruge
Department of Functional & Evolutionary Entomology, Gembloux Agricultural University, Passage des Déportés 2, B-5030 Gembloux, Belgium

Introduction

Most aphidophagous predators, such as syrphids and coccinellids, exploit temporary food resources (aphid colonies) that are patchily distributed and have a short life span (Dixon, 1985). These predatory larvae risk starvation if the aphid colony on which they are feeding get reduced before they complete their development. This could happen when too many eggs are laid in the colony or too late in the development of the colony, i.e., when the aphids are preparing to disperse. Optimal foraging theory predicts that to maximize fitness these predators should lay a few eggs early in the development of an aphid colony (Kindlmann & Dixon, 1993). The present study aims to understand this theory in predatory hoverfly *Episyrphus balteatus* DeGeer (Diptera: *Syrphidae*) and to assess the following question: is a predatory hoverfly female able to evaluate the aphid density and adapt its number of eggs laid in a way that assures their offspring?

Materials and methods

To assess the following question: is a predatory hoverfly female able to evaluate the aphid density and adapt its number of eggs laid in a way that assures their offspring?

Three laboratory experiments were conducted. In the first experiment, foraging and oviposition behaviour of hoverfly were investigated according to different densities of *Myzus persicae* (1, 25, 50, 75, 100 and 125 individuals) using leaf disc bioassay. The behavioural observations were recorded using the Observer v5.0 Noldus, allowing the insect behaviour to be subdivided in different stages. Hoverfly female foraging behavior was then recorded until the first oviposition in net cage 30 x 30 x 60 cm. where we stoped the behavioural observations when the hoverfly female fly after the first oviposition. The number of eggs laid by *E. balteatus* females was also counted during the foraging behaviour on each leaf disc covered with different aphid densities. In the second experiment, we studied the relationship between the survival rates of different densities of *E. balteatus* larvae (1, 3 and 5 larvae) and the evolution of aphid colony size (25, 75 and 125) on broad bean plants. In the final experiment, the effects of different aphid densities (25, 75 and 125 individuals) on the body weight in addition to larval performance were also investigated.

Results and discussion

Our experiments demonstrated that *E. balteatus* females exhibited enhanced behavioural responses according to aphid density in term foraging and oviposition behaviour. Hoverfly female laid more eggs on large aphid colonies. In addition, *E. balteatus* females laid fewer eggs on plants infested with small aphid colony (1 and 25 individuals) compared to plants infested with large aphid colonies (75 and 125 individuals) during the foraging behaviour. Larval performance, including survival rate and development time, was higher for small aphid colony and vice versa. Whereas, the survival rates of *E. balteatus* larvae tended to increase on plants infested with smaller aphid colonies (25, 75) than on the plants infested with larger aphid colonies (125) because the number of offspring (larvae) increased

according to aphid colony size. Nevertheless, there were not significant differences in larvae survival rate according to aphid colony size. Indeed, aphid colony size had a significant impact on the pupal and adult weight of *E. balteatus*. Hoverfly gained more body weight when fed on large aphid colonies. In previous field and laboratory studies, the syrphid females would not lay such high number of eggs in a single aphid colony, as usually only one egg laid by hoverfly female per landing (Chandler, 1968b; Chambers, 1991; Scholz & Poehling, 2000). In our experiments, where syrphid females were unable to disperse, they increase their eggs laying per landing and their offspring performance in response to aphid colony size. Therefore, *E. balteatus* females manifest evolved behavioral mechanisms in response to aphid colony size that enable them to forage for an oviposition site that support the development of their offspring.

Acknowledgements

This work has been funded by a FNRS (Fonds National de la Recherche Scientifique) grant (M 2.4.586.04.F). We thank Dr Yves Brostaux from the FUSAGx for his help with statistical analysis.

References

Chambers, R J (1991). Oviposition by aphidophagous hoverflies (Diptera: Syrphidae) in relation to aphid density and distribution in winter wheat. In: L Polgár, R J Chambers, A F G Dixon & I Hodek (eds), *Behaviour and Impact of Aphidophaga*. SPB Academic Publishing bv, The Hague, pp. 115–121.

Chandler, A E F (1968b). Some factors influencing the occurrence and site of oviposition by aphidophagous Syrphidae (Diptera). *Annals of Applied Biology*, **61**, 435-446.

Dixon, A F G (1985). *Aphid ecology*. Blackie, Glasgow, and London, 157 pp.

Kindlmann, P & Dixon AF G (1993). Optimal foraging in ladybird beetles (Coleoptera: Coccinellidae) and its consequences for their use in biological control. *European Journal of Entomology*, **90**, 443-450.

Scholz, D & Poehling, H M (2000). Oviposition site selection of *Episyrphus balteatus*. *Entomologia Experimentalis et Applicata*, **94**, 149-158

Oviposition preference of oriental fruit moth (*Grapholita molesta* (Busck), Lepidoptera; Tortricidae) for apple cultivars

N K Joshi
Pennsylvania State University, Department of Entomology, 501 ASI Building, University Park, PA 16802, USA
Email: nkj105@psu.edu

L A Hull
Pennsylvania State University - Fruit Research & Extension Center, Entomology, 290 University Drive, Biglerville, PA 17307, USA

C T Myers
USDA-ARS, Appalachian Fruit RS, 2217 Wiltshire Road, Kearneysville, WV 25430, USA

G Krawczyk
Pennsylvania State University - Fruit Research & Extension Center, Entomology, 290 University Drive, Biglerville, PA 17307, USA

E G Rajotte
Pennsylvania State University, Department of Entomology, 501 ASI Building, University Park, PA 16802, USA

Introduction

Oriental fruit moth (*Grapholita molesta* [Busck], Lepidoptera: Tortricidae) is one of the major pest problems in Pennsylvania apple orchards (Hull *et al.*, 2001). Currently, apple growers are using different techniques of pest management, with special emphasis on mating disruption and application of new pesticide chemicals with low mammalian toxicity. This approach is very expensive, costing $200 to $300 /acre, annually (Krawczyk, 2004). With the annual cost of pesticides it is mandatory to explore other control methods. In the past, few scientists have studied host-plant resistance in tree fruits (Goonewardene *et al.*, 1975; Hogmire & Miller, 2005; Myers *et al.,* 2006). However, some of the new (i.e., originating from the NE-183 Project) as well as traditional cultivars still need to be evaluated for their relative resistance to the oriental fruit moth. In this context, we investigated the oviposition preferences of oriental fruit moth for different apple cultivars in the laboratory.

Materials and methods

Laboratory experiments were conducted to study oviposition preferences of oriental fruit moth for 10 commercially important apple cultivars, viz., Stayman, York Imperial, Golden Delicious, Fuji, Red Delicious, Gala, Pristine, Honeycrisp, Arlet and Sunrise. Two sets of experiments were conducted with the fruits harvested during the second week of July and the first week of August. In the first set of experiments (second week of July), the following nine treatments were used: (1) Stayman, (2) York Imperial, (3) Golden Delicious, (4) Fuji, (5) Red Delicious, (6) Pristine, (7) Honeycrisp, (8) Arlet, and (9) Sunrise. In the second set of experiments (first week of August), all treatments were same as the first set of experiments, except Pristine (treatment no. 6), as it was replaced by Gala. Each treatment was replicated eight times in the multiple-choice tests and 10 times in the no-choice tests. Prior to use in experiments, all fruits were washed three times with clean water without damaging the fruit skin and were inspected via a head lens for field oviposition/infestation

of oriental fruit moth. In the no-choice tests, transparent plastic cups (1.0 L) internally covered with fiber screen were used. Sexed pupae of the oriental fruit moth were placed into the oviposition chambers, where adults were left to emerge, mate, and freely oviposit on fruits. In the first set of no-choice tests, two pairs of pupae were released, and observations on oviposition were recorded after 15 days by counting the total number of eggs oviposited on the fruit surface. In the second set of no-choice tests, one pair of pupae was released and observations were recorded after nine days. In the multiple-choice tests, a cylindrical chamber (length= 0.81 m, diameter = 0.17 m) made of transparent fiberglass internally covered with fine aluminum mesh screening was used. In the first as well as the second set of multiple-choice tests seven pairs of pupae were released, and observations were recorded after 14 days.

Results and discussion

In the no-choice tests of the first set of experiments, a strong ovipositional preference for Golden Delicious by oriental fruit moth females was recorded, while the least preference was recorded for Sunrise. Significantly higher numbers of eggs were also found on Stayman fruits. Comparatively, very few eggs were found on the cultivars Pristine, Honeycrisp and Arlet. In the second set of no-choice tests, more eggs were recorded on the cultivars Fuji, Golden Delicious, Stayman, and Red Delicious. As compared to the first set of no-choice tests, higher numbers of eggs were found on the cultivars Pristine, Sunrise, Honeycrisp and Arlet. This increased preference could be related to the increased maturity level of the fruits. In the second set of experiments these cultivars were near harvest, and this increased level of fruit-maturity might have influenced the ovipositional preference of oriental fruit moth females. In the multiple-choice tests, there was greater preference for Fuji than other varieties. However in the second set of multiple-choice tests, equal preference was recorded for all varieties. This finding could be due to the maturity of fruits, as at the time of the experiment these varieties were also near to harvest. In all experiments oriental fruit moth strongly preferred Golden Delicious and Fuji and least preferred Sunrise for oviposition. In addition, more eggs were recorded on Stayman than Pristine, Honeycrisp and Arlet. Results of this study further suggest other possibilities of cultivar preferences by fruit moths.

Acknowledgments

The authors would like to thank the SHAP for providing funding, and Teresa Krawczyk, FREC, Penn State for providing the insect colony.

References

Goonewardene, H F; Kwolek, W F; Dolphin, R E; Williams, E B (1975). Evaluating resistance of apple fruits to four insect pests. *HortScience.* **10**, 393-394.

Hogmire, H W; Miller, S S (2005). Relative susceptibility of new apple cultivars to arthropod pests. *HortScience.* **40**, 2071-2075.

Hull, L A; Krawczyk, G; Ellis, N (2001). Management tactics for the oriental fruit moth (*Grapholita molesta*) in Pennsylvania apple orchards. *Penn Fruit News.* **81**, 23-26

Krawczyk, G (ed.) (2004). *Pennsylvania tree fruit production guide.* College of Agricultural Sciences Publication, The Pennsylvania State University. 135-216.

Myers, C T; Hull, L A; Krawczyk, G (2006). Seasonal and Cultivar Associated Variation in the Oviposition Behavior of Oriental Fruit Moth, (Lepidoptera: Tortricidae) Adults and Feeding Behavior of Neonate Larvae in Apples. *Journal of Economic Entomology.* **99**, 349–358.

Population dynamics of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), and its natural enemies in the south and north of Iran

S Mohammadi, A A Seraj
Plant Protection Dept., Shahid Chamran University, Ahvaz, Iran
Email: seraj_a@scu.ac.ir

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was unreported in the North of Iran until 1996 when it was discovered in citrus nurseries in Sari. In South, *P. citrella* was reported in 1999 in the Khuzestan province (Safi-Abad). Because of the potential threat to the citrus industries in both districts, a bi-province collaborative research project was initiated in 2000 to define and compare the status of *P. citrella* and levels of biological control between the two districts. In general, *P. citrella* populations increased in Sari over the three year period from 2002 to 2004. In 2002, *P. citrella* peaked at ≈0.4 immatures/leaf in mid-October. In 2004, the pest began to increase in April, reaching peaks of ≈1.0 larva/leaf in early July. In both years, overall parasitism averaged ≈18%. The dominant parasitoids in North of Iran were *Ageniaspis citricola* (Hymenoptera: Encyrtidae) and *Pnigalio pectinicornis* L., which constituted >34% of the parasitoid complex. Predators recovered were *Chrysoperla spp.* (Neuroptera: Chrysopidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), and *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae). In contrast, *P. citrella* generally declined in Khuzestan over the same period. In 2005, the pest peaked at 0.7 immatures/leaf in August and did not exceed 0.35 immatures/leaf in 2004. The decline of *P. citrella* in Khuzestan is more evident when compared to a 2003 survey when pest densities exceeded 5.5 immatures/leaf. The monthly percentage parasitism of *P. citrella* in Khuzestan increased from <1% in May to ≈12% in November 2003. In contrast, parasitism in 2004 fluctuated from 0 to 18%. Numbers of parasites were always <0.05 individuals/leaf and often zero. Similar to North, the dominant parasitoids were *A. citricola* and *P. pectinicornis*, which constituted 39.9% (115 of 265) of the parasitoids sampled. Differences in *P. citrella* populations and those of its parasitoid complexes may be partially attributed to a hotter, drier climate in South than in North.

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), originated from southeast Asia and established itself as a major pest of citrus throughout Australia, the Middle East, and parts of Africa. The known geographical range of *P. citrella* in Iran currently includes North, East and most recently, South. Control measures include insecticides such as chlorfenapyr and tebufenozide (French *et al.*, 1997) and cultural controls to limit growth of the susceptible young flushes. Recent records indicate that ≈80 species of parasitoids have been reared from the leafminer worldwide. These parasitoids are predominantly in the family Eulophidae. Efforts at classical biological control of *P. citrella* have focused on the introduction of *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) in North and South of Iran. Between May 1998 and September 2000, *A. citricola* was imported from Australia and released into 22 sites in Southwest Sari. *Ageniaspis citricola* has apparently established, attaining parasitism levels of 86% in October 2000 and surviving winter conditions. A similar program was undertaken in South (Safi-Abad), but *A. citricola* has not established to date (Seraj, 2003). In this paper, we report the results of this project, i.e., the population dynamics of *P. citrella* and its natural enemies from 1998 to 2003 in South and North.

Material and methods

Sampling for *P. citrella* and its natural enemies was conducted in North and South from 2002. Comparisons were made of historical temperature and rainfall data between citrus-growing areas of South and North to assess possible effects of climate on differences in population dynamics between the two areas. At about two week intervals, leaf samples were collected from selected citrus orchards in North and South.

Populations of *P. citrella* began to increase during the summer months of 2002, peaking at ≈0.4 immatures/leaf in mid-October. In 2003, *P. citrella* populations began to increase earlier (in April) and numbers per leaf were generally higher, reaching peaks of ≈1.0/leaf in early July and ≈0.5/leaf in late September. In both years, overall parasitism averaged ≈18%. However, parasites were recovered earlier in the year in 2003 (early May) compared to 2002 (mid-June). The parasitoid *Z. multilineatum* (Ashmead) (Eulophidae) increased in dominance from 2002 to 2003. Predators recovered on citrus leaves were larvae of *Chrysoperla* spp. (Neuroptera: Chrysopidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). Over the two years from 2002 to 2003, *P. citrella* populations generally increased in the sample sites in North, with *Z. multilineatum* being the dominant parasitoid.

When sampling was initiated in May 2002, numbers of *P. citrella* per leaf were already quite high, at about 0.6 total insects/leaf. The peak of 0.7/leaf was reached in August, after which numbers declined in the fall. Populations of *P. citrella* were lower in the following year, never exceeding 0.35/leaf. The monthly percentage parasitism of *P. citrella* in South increased from <1% in May 1997 to ≈12% in November. In contrast, parasitism in 2003 fluctuated from 0 to 18% with no definite trend apparent. Numbers of parasites were low for both 2002 and 2003, always <0.05/leaf and often zero. A total of 277 parasitoids were recorded in 2002, of which 115 (39.9%) were *Z. multilineatum*. In 2003, only 41 parasitoids were recorded, of which *Z. multilineatum* was most abundant (13; 31.7%). A comparison with the surveys conducted in South in 2002 and 2003 indicates declines in the native parasitoid complex attacking *P. citrella*. The maximum percentage parasitism in 2002 was almost 40%. The maximum level of 18% found in 2003 is misleading because of the low host densities available. Corresponding counts in 2002 through 2003 were 8.6 (314 parasitoids from 3658 leaves), 9.4 (605/6426), and 0.81 parasitoids/100 leaves (42/5160), respectively.

Conclusion

In general, *P. citrella* and its parasitoids increased in North from 2002 to 2003 and decreased in South from 2002 to 2003. The decline becomes more apparent when compared with surveys taken in 2000. Many factors probably contributed to these differences, and one likely important determinant is climate. Mean monthly temperatures were higher in South (Safi-Abad) than in North (Sari) (paired $t = 2.74$; $df = 11$; $P < 0.05$). Although not conclusive, differences in *P. citrella* populations and, consequently, those of its parasitoid complexes may be at least partially attributed to a hotter, drier climate in South than in North. Overall percentage parasitism was higher in North (17.2%, SE= 1.8; $n = 25$) than in South (9.6%; SE = 1.5; $n = 39$) ($t = 2.7$; $df = 23$; $P< 0.05$). The numbers of *P. citrella* hosts per leaf and percentage parasitism yielded a significant regression in North ($F =11.4$; $df = 1, 23$; $P< 0.01$; $R^2 = 0.33$) but not in South ($F = 1.03$; $df = 1, 36$; $P = 0.32$; $R^2 < 0.01$). While this result is interesting, it is difficult to draw strong conclusions regarding the apparent density-dependent parasitism found in North and its absence in South.

Predacious mites for control of citrus thrips, *Scirtothrips citri* (Thysanoptera: Thripidae) in nursery citrus

L Akbari, A A Seraj
Plant Protection Department, Shahid Chamran University, Ahvaz, Iran
Email: seraj_a@scu.ac.ir

Predation on citrus thrips, *Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae), by four species of predacious mites, *Anystis baccarum* (Linnaeus), *Euseius libanesi* (Dosse), *Amhlydramella kettanehi* (Dosse), and *Neosieulus barkeri* (Hughes) was studied. In the laboratory, *A. baccarum* and *E. libanesi* exhibited a consistently high consumption rate of second-instar citrus thrips (four to five thrips per mite per day), 68 - 96% survival, and reproduction of 0.9–2.5 eggs per day during the five day experiment. Initially, *A. kettanehi* exhibited a lower consumption rate as well as lower survival and egg production compared with *A. baccarum* and *E. libanesi;* however, these rates increased as the experiment progressed. In experiments using potted citrus in a greenhouse, immature stages of citrus thrips were not always available for predation. Under these circumstances, *A. baccarum* and *E. libanesi* survived well and reduced immature citrus thrips to undetectable levels for the four week period after release. In contrast, *A. kettanehi* and *N. barkeri* exhibited poor survival and did not fully control citrus thrips. When *A. baccarum* and *E. libanesi* were released onto potted citrus trees in a commercial citrus nursery, releases of *A. baccarum* reduced citrus thrips for two sampling periods and improved tree height and leaf number comparable with an abamectin insecticide treatment. In contrast, *E. libanesi* releases did not control citrus thrips, resulting in less tree growth. These data suggest that *A. baccarum* shows promise for commercial releases and control of citrus thrips in citrus nurseries.

Introduction

Citrus thrips, *Scirtothrips citri* (Moulton), are frequently severe pests of flush scion growth on nursery citrus trees. Citrus nurserymen need cultural and biological control alternatives to pesticides for citrus thrips. Predacious mites, especially *Neosieulus barkeri* (Hughes) and *N.* (=*Amblyseius*) *kettanehi* (Dosse), have been used extensively in greenhouses. These species may have a potential use in citrus nursery situations against citrus thrips. The predacious mite *Euseius libanesi* L. is commonly found in Safi-Abad citrus orchards and is known to be an important predator of citrus thrips. Densities of *E. libanesi* have been manipulated through pruning and augmentation in mature orchards to reduce citrus thrips scarring of fruit; however, releases in citrus nurseries to protect foliage had not previously been evaluated. *A. baccarum* and *E. hibisci* were shown to be more promising candidates for western flower thrips predation under conditions of low humidity and short day length than *N. barkeri* or *A. kettanehi.* Safi-Abad, Khuzestan, citrus nurseries have partial to fully enclosed greenhouses for seedling production. Once >30 cm in size the seedlings are repotted and placed outdoors or planted in the ground where they are exposed to full sun, rain, and ambient temperatures. Grafting is completed outdoors in moderate temperatures.

Materials and methods

A. baccarum and *E. libanesi* colonies were maintained in the laboratory on citrus leaves surrounded by water-soaked and fed pollen twice weekly. Vials of *A. kettanehi* and *N. barkeri* shipped in corn cob grits with bran mites (*Acarus* spp.) as a food source were utilized for experiments within eight hours of arrival. Citrus thrips were collected from navel trees at the Safi-Abad. Ten adult female of each mites were placed on five leaves.

Results

Laboratory Experiment: During the first two days, *A. baccarum* and *E. libanesi* consumed nearly all of the citrus thrips provided (50 second-instar thrips per 10 predacious mites) and their consumption rates were significantly higher than those of the other two predacious mite species. During the third through fifth day of the experiment, the consumption rate of *A. kettanehi* increased to a similar level and then surpassed the levels of *A. baccarum* and *E. libanesi.* The prey consumption of *A. kettanehi* was significantly higher on days four and five compared with days one and two ($F = 7.43$; $df = 4, 16$; $P = 0.001$). In addition to poor consumption of citrus thrips, *N. barkeri* exhibited the poorest survival. Less than one third of the mites were alive at the end of the first day. In contrast, *A. baccarum* and *E. libanesi* exhibited 98 - 68% survival during the five days of the experiment. Initially, *A. kettanehi* exhibited relatively high survival; however, during the fourth day survival dropped below 33%. Egg deposition by surviving *N. barkeri* was significantly lower (<0.4 eggs per day) than two or three of the other predacious mite species during the first four days of the experiment. In contrast, *A. baccarum* and *E. libanesi* deposited 0.93 to 2.54 eggs per day per predacious mite throughout the experiment. Females of *A. kettanehi* exhibited a trend of increased egg deposition from 0.41 to 0.64 eggs per day during the first three days to 1.78 to 1.58 eggs per day during the last two days of the experiment; however, this difference was not statistically significant ($F = 2.09$; $df = 4, 16$; $P = 0.13$). Mean total egg production per female over the five days was 1.3, 5.0, 9.2, and 9.8 eggs for *N. barkeri, A. kettanehi, E. libanesi,* and *A. baccarum,* respectively.

Greenhouse Experiment: The number of citrus thrips nymphs per cage (three trees) averaged 34.25 ± 6.64 to 38.75 ± 8.50 and there were no significant differences between treatments. One week after predacious mites were released, citrus thrips densities were very low (0–0.5 nymphs per cage) even in the untreated control. We released primarily late second-instar thrips in the two weeks prior to this date. Low citrus thrips nymph densities suggest that the majority of the populations were in the pupal, adult, or egg stage on that sample date. *A. baccarum* were found in significantly higher numbers (5.25 mites per cage) than the *E. libanesi* (3.25 mites per cage). *A. kettanehi* and *N. barkeri* densities (0.50 and 0.25 predacious mites per cage) were not significantly different from the control.

Commercial Citrus Nursery Experiment: Because of the poor performance of *N. barkeri* and *A. kettanehi* in the laboratory and greenhouse experiments, these species were not included in the commercial citrus nursery experiment. Second-instar thrips feed significantly more than adults and so biological or chemical control measures that reduce nymphs may be more important for reducing leaf damage than control methods that reduce adults. There was a significant difference in tree height ($F = 11.06$, $df = 2, 171$, $P = 0.001$) and in the number of leaves per tree ($F = 6.71$, $df = 2, 171$, $P = 0.002$) between treatments. The trees in which *E. libanesi* was released, on which no reduction in citrus thrips densities was observed, had the shortest height (85.60 cm) and the fewest leaves (31.7 leaves/tree).

Conclusion

The timing of development of immature citrus thrips populations is fairly unpredictable and may occur under environmental conditions that render the predacious mites ineffective. However, our data suggest that even short-term control by predacious mites improves growth of trees and so early spring and fall releases should be useful to reduce the number of insecticides needed for citrus thrips control in nursery trees.

Analysis of plant lectins to protect against different pest insects and identification of receptor(s) in the insect midgut

A Sadeghi, E J M Van Damme, G Smagghe
Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
Email: Amin.Sadeghi@Ugent.be

Introduction

Plant lectins are a heterogeneous group of carbohydrate-binding proteins, some of which play a role in plant defence. During the last two decades, numerous reports have been published dealing with the insecticidal activity of plant lectins against many pest insects belonging to the orders Lepidoptera, Coleoptera, Diptera and Homoptera. In this research we tested the detrimental effects of different mannose-binding plant lectins against a typical biting-chewing pest insect, the cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae), and also against piercing-sucking insects like the aphids *Myzus nicotianae* and *Acyrthosiphon pisum* (Homoptera: Aphidae). The lectins were exposed to the insects via artificial diet and/or via transgenic tobacco plants, expressing the lectin constitutively or in the phloem specifically.

Material and methods

Insect bioassay with transgenic tobacco leaves expressing different lectins

Detached leaves of four transgenic tobacco lines (APA1, APA2, APA3 and APA4) expressing the *Allium porrum* L. (leek) agglutinin (APA) under the control of the 35S constitutive promoter and wild type tobacco (control) were placed in cages and fed to *S. littoralis*. Treatment was started with second-instar larvae. Weight gain, development into pupae, morphological changes and mortality were scored. For each transgenic line and wild type (control) tobacco plants 18 second instar larvae were used.

Six transgenic tobacco lines (T1-T6) expressing the *Allium sativum* L. (garlic) agglutinin from leaf, (ASAL, expression level: 0.02-0.03% of total soluble protein) and from bulb (ASAII, expression level: 1.48-2.21% of total soluble protein) under the control of the phloem-specific sucrose synthase promoter as well as wild type tobacco plants were challenged by first instar aphids of *M. nicotianae*. Developmental stage and nymphal survival were scored at 24h intervals. After completion of the nymphal period, individuals were followed for adult survival and their fecundity was recorded daily. For each transgenic/control plant one nymph per cage and 30 nymphs were used. The experiments were conducted over 30 days.

Insect bioassay with artificial diet

An artificial diet was prepared using a standard liquid diet to which *Galanthus nivalis* L. (snowdrop) agglutinin (GNA) was added in a concentration range of 50–1200 µg GNA/ml diet. PBS buffer is added to the diet in the controls. First instars (0-12 h old) of *A. pisum* obtained from a synchronised population reared on *Vicia faba* plants, were transferred from bean at day 0 to parafilm sachets, containing 230 µl of the artificial diet. We challenged 20 aphid nymphs per cage, and four cages were used per concentration.

Preparation of receptor(s) from *Spodoptera littoralis* midgut

After dissection of midguts of *S. littoralis* caterpillars in ice-cold PBS, soluble protein and and membrane protein fractions were prepared by differential centrifugation and freezing-thawing cycles. The protein samples were subjected to GNA-Sepharose affinity chromatography. Proteins bound to the lectin column have been analyzed by SDS-PAGE.

Results

Insect bioassay with S. littoralis

Feeding of tobacco leaves expressing APA to *S. littoralis* larvae resulted in a reduction of the larval weight. An inhibitory effect of larval growth was observed throughout the experiment, and at day 11 the caterpillars fed on transgenic tobacco leaves showed a fresh weight that was 25-30% lower than that of control caterpillars fed on wild type plants. In addition, the lectin retarded the development of the larvae and metamorphosis, reduced pupal weight and increased the mortality rate.

Insect bioassay with *M. nicotianae*

Although there were no apparent effects during the first seven to eight days when nymphs were grown up to adults, an apparent decline in survival was observed at three to six days after adult formation with aphids were fed on a transgenic line T2 expressing ASAII. In the controls this decline started later, namely between day 12 and 15 in the adult life. The other transgenic lines expressing ASAL, showed a survival pattern that was intermediate between transgenic line T2 and the control (T1). In all transgenic lines there was a significant effect ($p<0.05$) on the numbers of nymphs produced per adult per day, but this was most apparent with transgenic lines T2 and T4 expressing ASAII. Furthermore, different life table parameters such as intrinsic rate, total fecundity and doubling time were significantly affected ($p<0.05$) when the aphids were grown on transgenic plant material expressing ASAII and ASAL.

Insect bioassays with *A. pisum*

The snowdrop lectin (GNA) caused 95-100% aphid mortality at the highest concentration tested (1200 µg/ml). In controls, 85% of the nymphs survived. After curve fitting, an LC_{50} of 389 µg/ml (95% CL: 341-444) was calculated. In addition to mortality, GNA also inhibited nymphal growth.

Analysis of proteins from *S. littoralis* midgut interacting with GNA

Soluble as well as membrane proteins from *S. littoralis* midguts have been chromatographed on a column with immobilized GNA in order to purify putative receptors for the lectin. SDS-PAGE analysis of the proteins eluted from the GNA column revealed several GNA binding proteins. Identification and characterisation of some of these proteins is underway.

Conclusion

The present study demonstrates that ectopically expressed APA, ASAL and ASAII confer transgenic tobacco plants significant levels of protection against *S. littoralis* and *M. nicotianae*, respectively, which are considered two important pest insects since they cause severe yield losses in many economically important agricultural and horticultural crop plants. In addition, we elaborated an insect bioassay using an artificial diet which allows screening a pallet of different lectins for their insecticidal properties towards aphids. One of the main advantages of this bioassay is that only limited amounts of lectin are needed.

Assessment of ecotoxicity of a mixture of the herbicides imazapic and imazapyr over the earthworm *Amynthas gracilis* (Kinberg, 1867), Megascolecidae, in laboratory conditions

S M Alessandrini
Polytechnical University of Madrid, Spain
Email: seba_alessandrini@hotmail.com

R A Gimenez, A B Della Penna
Chair of Zoology, Faculty of Agronomy, Buenos Aire University. Av. San Martin 4453, CP 1417, Buenos Aires, Argentina

Introduction

The modern evaluations of the quality of soils include both analysis of physical-chemical and biological parameters in order to diagnose the soil health. The abundance and biomass of earthworms are an important tool to evaluate the impact of different farm systems and pollution of soils. It is necessary to develop standardised laboratory studies on side effects of pesticides on the function of the soil community. Buckerfield *et al.* (1997) and Jordan *et al.* (2004) used earthworms as indicators of sustainablility of agroecosystems. In this work the sublethal and lethal action of a commercial mixture of imidazolinones was evaluated over a specie of earthworm dominant in Bragado agroecosystems (Buenos Aires Province).

Materials and methods

The experiment was carried out with the IOBC guidelines for the Daniel funnel test (Bieri et al, 1989). The worms and soil of Ap horizon of a typic hapludol with 3.86% MOS were collected in a soybean field of Bragado. The worms were preconditioning before the trials. The used worms was 0.96 g/ind. (CV% 3.814) and with clitelum.

The treatments were:

1) Control;
2) mix of imidazolinone, commercial product OnDuty (BASF), imazapic 52.5% + imazapyr 17.5% WG, 114 g c.p./ha;
3) ten fold rate (1140 g c.p./ha).

The experimental design was DCA with five replications for treatment. The test conditions were 14°C +/-1.5°C, 80% H.R. and 12:12 h ligth/dark. Ten leaves of dried lucerne (*Medicago sativa* L.) were placed over the soils.

We recorded: withdrawn leaves (alimentary activity), live weight (11 earthworms with *clitelum* and empty gut content, were used for body weight determination of each treatment) and mortality in 15 days of exposition after the applications.

Results and discussion

The mean activity was 1.9, 1.87 and 1.89 leaves/day, the change of body weight (0.1, 0.09 and 0.001 g/ind.) were not different between the treatments, ANOVA and Tukey test ($p<0,05$) and any mortality was observed.

Table 1 Alimentary activity of earthworms

Treatment	Total withdrawn leaves (7 d)	Daily withdrawn leaves (leaves/d)	Total withdrawn leaves (14 d)	Daily withdrawn leaves (leaves/d)
Control	8	1.14	27	1.90
(D1=) 114 g/ha	9	1.29	26	1.87
(D2=) 1114 g/ha	8	1.17	26	1.89

Table 2 Body weight (g/ind.)at the end of the test (15d)

Replic.	Control	(D1=) 114 g/ha	(D2=) 1114 g/ha
1	0.783	1.442	1.000
2	1.659	0.832	0.823
3	0.745	0.928	0.839
4	1.062	1.025	1.023
5	1.452	1.044	0.655
6	0.783	1.442	1.000
7	1.659	0.832	0.823
8	0.745	0.928	0.695
9	0.672	1.054	1.023
10	1.452	1.044	0.655
11	0.672	1.025	0.695
Average	1.062	1.054	0.839
V.C. %	6.331	24.330	36.606

In conclution, the treatments with the used mix of imidazolinones was not toxic for the earthworm *Amyntas gracilis* in this experimental conditions.

Nevertheless, the high content of organic matter of the substrate utilized in this test, possible have been reduced the toxicity on *Amynthas gracilis* since Goss (1992) established that the organic matter is the characteristic of greater influence on the movement of the pesticides in the soil and the presence of organic layers produces extenuation of the flow of pesticides, protecting the terrestrial fauna of the effects of soil contaminants.

References

Bieri, M 1989. Guidelines for the Daniel funnel test- a laboratory test to measure side effects of pesticides on the earthworm *Lumbricus terrestris* L. In: *Guidelines for testing the effects of pesticides on beneficial organisms*. IOBC/WPRS Bulletin 1992:139-144. Ed. Hassan S A, Darmstadt, Germany.

Buckerfield J C; Lee K E, Davoren C W & Hannay J N 1997. Earthworms as indicators of sustainable production in dryland cropping in Southern Australia. *Soil Biology and Biochemistry*, **29**: 547-54.

Goss, D W 1992. Screening procedure for soils and pesticides for potential water quality impacts. *Weed Technology*, **6**:701-708.

Jordan D; Miles R J; Hubbard V C & Lorenz T 2004. Effect of management practices and cropping system on earthworm abundance and microbial activity in Sanborn Field: a 115- year-old agricultural field. *Pedobiologia* **48**: 99-110.

Potential reduction of growth regulator inputs by exploiting the leaf growth response to alkaline pH in oilseed rape (*Brassica napus* L.)

A K S Aronsson, P S Kettlewell, I G Grove, J P H Reade
Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, TF10 8NB, UK
Email: aaronsson@harper-adams.ac.uk

Introduction

Commercial oilseed crop canopies can often grow excessively large in the UK because early sowing and high seed rate are used as an insurance against poor establishment. As a result, the pre-flowering leaf canopy may exceed a green area index (GAI) of 3.5. A canopy above the GAI of 3.5 has been shown not to increase yield and also to reduce profitability due to the unnecessary inputs (Lunn *et al.*, 2001). As a consequence, fungicides exhibiting PGR effects to retard leaf area have become common practice in oilseed rape canopy management to increase yield. There are however increased concerns over the use of chemicals in agriculture. Therefore, more sustainable and cheaper alternatives are sought. Possibilities may lie in the leaf growth response to alkaline pH. Alkaline pH has been shown to reduce leaf elongation in barley fed with artificial xylem sap of different pH's (Bacon et al., 1998). In our study, two experiments using a leaf assay were carried out in a controlled environment with the aim of examining the effect of pH on oilseed rape leaf expansion.

Materials and methods

Spring oilseed rape cv. Mozart was sown in a controlled environment cabinet (SANYO, Japan) under a 16 h photoperiod with a PAR of ~200 µmol m-2 s-1. The temperature was set at 18/12°C and 16/10°C day/night for Exp. 1 and Exp. 2 respectively. Five week old plants with the third leaf still expanding were cut under distilled water before being placed in 50 ml sarstedt tubes with artificial xylem sap containing 1.0 mM KH2PO4, 1.0 mM K2HPO4, 1.0 mM CaCl2, 0.1 mM MgSO4, 3.0 mM KNO3, 0.1 mM MnSO4 (Wilkinson & Davies, 1997). Eight treatments ranged from pH 4-11 with 15 replicates for each treatment. The pH was adjusted with 1 M HCl or KOH and the tubes sealed with parafilm to prevent evaporation. The controls consisted of shoots with their roots intact placed in sarsted tubes with vermiculite. The controls were watered with Hoagland nutrient solution at the start and at the second day of the experimental period. All the tubes were moved back to the growth cabinet in a randomized block design. The area of the third leaf was measured over four days with a LiCor portable leaf area meter (LiCor Biosciences, Nebraska, USA). The pH of the artificial xylem sap was measured for each treatment at the end of the treatment period.

Results

The pH of the artificial xylem sap kept fairly stable over the treatment period. The leaf expansion rate was less in Exp. 2 compared to Exp. 1 potentially because of the lower temperature in Exp. 2. ANOVA analysis with Genstat 9th edn. (Lawes Agricultural Trust) showed in Exp. 1 that plants fed with alkaline artificial xylem sap had a significant reduction in leaf expansion over four days compared to the Control plants ($p = 0.023$), with the pH 8 treatment showing a 80% reduction in leaf area expansion. In Exp. 2, pH 8 treated plants showed a significant reduction in leaf area expansion (60%) compared to Control plants ($p = 0.034$, Table 1).

Table 1. The effect of pH on leaf area increase (cm^2) over four days for oilseed rape cv. Mozart under two temperature regimes (Exp. 1, 18/12°C day/night. Exp. 2, 16/10°C day/night)

Treatment	Mean	
	Exp. 1 (n=15)	Exp. 2 (n=15)
Control	3.05	1.13
pH 4	2.27	0.57
pH 5	1.87	0.82
pH 6	1.57	0.99
pH 7	1.36	0.75
pH 8	0.48	0.44
pH 9	1.27	0.49
pH 10	1.34	0.87
pH 11	0.98	0.72
Exp. 1, p 0.023, df 86, SE 0.28, CV 77%.		
Exp. 2, p 0.034, df 88, SE 0.15, CV 82%		

Discussion

The results show that a slightly alkaline pH of artificial xylem sap can decrease leaf area in oilseed rape. A similar effect of alkaline pH has been found in leaf elongation of barley (Bacon *et al.*, 1998). In this basic experiment it is difficult to determine the underlying mechanisms behind the response to alkaline pH. Two mechanisms have however been suggested to be responsible for the reduction in leaf area by high pH. Leaf cell expansion can be mediated by low pH, also known as the acid growth theory (Cleland, 1991). The high pH fed to the leaves may slow down this process, retarding leaf area expansion. The second suggested mechanism involves the growth regulating hormone ABA. The leaf apoplast under normal conditions is slightly acidic and ABA is undissociated and taken up by mesophyll cells. The alkaline pH dissociates the ABA and instead of being taken up by mesophyll cells it reaches guard cells and induces stomatal closure which results in reduced leaf expansion (Wilkinson & Davies, 1997; Wilkinson, 1999). The experiments showed a high variability (Table 1) and the cause must be investigated. Further experiments will explore which of the mechanisms may be responsible for the reduction in leaf area from alkaline pH. Research will also examine if sprays of alkaline pH can be used as a growth retardant.

References

Bacon, M A, Wilkinson, S, Davies, W J, 1998. pH-regulated leaf cell expansion in droughted plants is abscisic acid dependent. *Plant Physiology*, **118**, 1507-1515

Cleland, R E (1991) Auxin-induced growth of Avena coleoptiles involves two mechanisms with different pH optima. *Plant Physiology*, **99,** 1556-1561

Lunn, G D, Spink, J H, Stokes, D T, Wade, A, Clare, R W, Scott, R K, 2001. Canopy management in winter oilseed rape. *HGCA Project Report No. OS49*

Wilkinson, S, Davies, W J, 1997. Xylem sap pH increase: A drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. *Plant Physiology*, **113**, 559-573

Wilkinson, S, 1999. pH as a stress signal. *Plant Growth Regulation*, **29**, 87-99

Plant growth promoting rhizobacteria (PGPR) applied to biological control and to improve sugar beet, pumpkin and tomato crops production

G S Pérez-Minayo, J I Reguera-Useros, D J López-Robles
Microbiology Department, Faculty of Sciences, University of Burgos, Plaza de Misael Bañuelos s/n, E-09001, Burgos, Spain
Email: gsacristan@ubu.es

Objectives and introduction

Rhizobacteria are bacteria from the rhizosphere of plants that exhibit active root colonization in the presence of the native microorganisms. Rhizobacteria that exert beneficial effects on plant development are referred to as plant growth promoting rhizobacteria (PGPR), because their application is often associated with increased rates of plant growth (Kloepper *et al.*, 1980).

To consider a rhizobacteria as a PGPR is a complex issue since the number of bacteria present in the rhizosphere of plants is very high. To avoid this controversy, four defining characteristics for this group of bacteria have been established (Jiménez Delgadillo *et al.*, 2001): i) they do not require internal penetration of the radicular tissue to exert their activity, as occurs in the case of mycorrhizal fungi; ii) they have high levels in the rhizosphere after inoculation in plants, since if their presence diminishes rapidly they are poor competitors against native microbial soil populations; iii) they are capable to colonize the roots of plants so as to be able to influence plant growth positively, and iv) they are not harmful to humans or to the environment.

The modes of action of PGPR are very different and in some cases, more than one mode will overlap. Some researchers categorize PGPR into bioprotectors, biofertilizers and biostimulators. Generally, the different modes of action are based on the suppression of plant pathogens (bioprotectors), stimulation of the acquisition of nutrients (biofertilizers) and the production of plant phytohormones (biostimulators).

The application of PGPR to agricultural systems is very widespread and they have been used in numerous plant crops. In our work, *Pseudomonas fluorescens* has been evaluated as PGPR against damping off produced by *Rhizoctonia solani* in sugar beet crop. The most common symptom of *R. solani* disease is reported as damping off. Damping off is characterized by non-germination of severely infected seeds whereas infected seedlings may be killed both before and after emergence. Also we inoculated, *in vitro* and in field, a culture of *P. fluorescens* in sugar beet (*Beta vulgaris*), pumpkin (*Cucurbita maxima*) and tomato (*Lycopersicon esculentum*).

Material and methods

Seeds of sugar beet crop were inoculated for 5 h with a culture of *Pseudomonas fluorescens* PfO-1 ($6x10^9$ CFU/ml) and they were sown in a naturally infected with *Rhizoctonia solani* soil after, to study the effect of *P. fluorescens* on *R. solani* (Ps+R assay). *P. fluorescens* was isolated from an agricultural soil in Pampliega, Burgos (Spain), and was selected because of its *in vitro* inhibition effect against *R. solani* AG-4 in PDA medium. The effects occurred in Ps+R assay was observed daily and seedlings emergence was determined over 36 d. Also other tray was used as control where the sugar beet seeds were sown in a non infected with *R. solani* soil, named T (-) assay.

Other seeds of sugar beet, pumpkin and tomato crops were inoculated (Ps assays) with a culture of *P. fluorescens* PfO-1 ($3x10^8$ CFU/ml) and they were sown after as in a climatic chamber (*in vitro* assays) as in agricultural plots (field assays). After 30, 26 and 87 d for sugar beet, pumpkin and tomato crops, respectively it was noted the changes occurred in Ps assays carried out *in vitro*. In field assays the results were noted at the end of productive cycle. In both assays, Ps and T, we recorded the soil pH, total, aerial and root biomass and length, and microbial counts. Also, in field Ps assays of sugar beet crop we recorded the sucrose concentration at the end of the productive cycle.

Also, others assays were carried out with the seeds inoculated only with BHI broth (T assays).

Results and discussion

Damping-off due to *R. solani* infection of these pre-emerged seedlings was reduced by *P. fluorescens* inoculation. The mean value of final sugar beet seedling emergences was 25 (50%) and 35 (70%) plants for Ps+R and T (-) assays, respectively. Damping off due to *R. solani* infection of these emerged seedlings was observed in seven and three plants for R+Ps and T (-) assays, respectively.

Biomass and aerial part length values from *in vitro* Ps assays were increased in sugar beet (34.61%, 8.13%), pumpkin (22.26%, 13.03%) and tomato (16.25%, 29.57%) with regard to T assays, respectively. Sucrose concentration mean value was increased (21.95% Brix) for the sugar beet plants (Ps assays) in field, with regard to T assays results (20.45% Brix).

The development and growth of seedlings were notably increased in the Ps+R assays. Others authors have checked the synergic effect by the combination of two PGPR, *Bacillus subtilis* RB14-C and *Burkholderia cepacia* BY, to control *R. solani* damping off (Szczech & Shoda, 2004). *P. fluorescens* applied to control damping off caused by *Rhizoctonia solani* is viable.

The stimulation of plant growth due to PGPR colonization has been studied in several works, and in our case it is reasonable to consider *P. fluorescens* as a PGPR in these crops.

References

Jiménez Delgadillo R, Virgen Calleros G, Tabares Franco S, Olalde Portugal V. (2001). Bacterias promotoras del crecimiento de plantas: agro-biotecnología. *Avance y Perspectiva* **20**, 395-400.

Kloepper J W, Leong J, Teintze M, Schroth M N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**, 885-886.

Szczech M, Shoda M. (2004). Biocontrol of Rhizoctonia damping-off of tomato by *Bacillus subtilis* combined with *Burkholderia cepacia*. *Journal of Phytopathology* **152**, 549-556.

Genetic structure of the Swedish population of *Phaeosphaeria nodorum*

E Blixt, Å Olson, N Högberg, A Djurle, J Yuen
Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, PO Box 7026, SE-750 07 Uppsala, Sweden
Email: eva.blixt@mykopat.slu.se

Introduction

The loculoascomycete *Phaeosphaeria nodorum* along with its anamorph *Stagonospora nodorum* causes glume blotch on wheat. There are three main ways to start an epidemic and researchers have discussed the most important source of primary inoculum for a long time. First through clonal reproduction where conidia are dispersed by splashing water droplets and infect during humid conditions. Second, seeds may serve as an inoculum source if the spike is infected and transfer the disease to the next growing season. The third source of primary inoculum is wind borne ascospores that are produced when the two mating types of *P. nodorum* meet. Ascospores may therefore blow in from fields nearby and initiate epidemics.

The aim of the study was to investigate the genetic structure of the population of *P. nodorum*, and the possibility of sexual reproduction in the pathogen, in order to better understand the epidemiology of glume blotch in Sweden.

Material and methods

Hierarchical samplings of diseased leaves were made at 50 sampling sites in five unsprayed fields in three regions of Sweden during 2003-2005. One isolate was taken from each sampling site but multiple isolations were made at some sites in four of fields.

The two mating types (MAT1-1 and MAT1-2) were identified using PCR-primers for the two loci. The genetic structure was analysed by simple sequence repeats (SSR) markers that were used to identify the alleles in five loci. Statistical calculations made were χ^2-test, genotypic diversity, gene diversity, Index of association (I_A, the measurement of linked alleles) and the *M*-value for detection of demographic bottlenecks, calculated as the mean ratio of the number of alleles to the range in allele size.

Results

The field collections resulted in 227 isolates from 177 sampling sites. In addition, eight ascospore isolates were caught with the spore trap at one of the sampled fields during the samplings in 2005. The mating type distribution within all fields, including the ascospores, was not significantly different from a 1:1 ratio when using clone corrected data. Both mating types were present in 16 out of 50 sampling sites where more than one isolate was collected.

One minisatellite and four microsatellite markers, of which one was monomorphic, as well as the mating type marker were used to determine the genotype of the isolates. The number of alleles at each polymorphic locus varied at both field level and at locus level. Three of the loci had a large majority of one allele and that resulted in a low genetic diversity.

In total 93 genotypes were found in the five populations of which 69 occurred only once in the total population. In each population there were between 23 and 38 genotypes found of

which 9-20 were unique isolates. The index of association (I_A), was not significantly different from zero in any of the five fields *i.e.* no difference was found in the variance of pair wise distances between individuals. The *M* value of the five populations were about 0.5, significantly denoting that the populations have experienced genetic bottlenecks compared to the expectations under a mixed mutation model.

Conclusion

Three strong indications of sexual recombination were found:

i) the two mating types were found equally distributed within fields;

ii) the genetic structure is diverse with many unique genotypes;

iii) the lack of correlation between alleles (measured with I_A) indicates that genetic recombination (a result of sexual reproduction) is frequent.

However, the effect of asexual reproduction and infected seeds cannot be neglected since identical genotypes were found within the field and that fungicide treatment of seeds is not sufficient. The fungal population has experienced a demographic bottleneck some time in the past validated by low gene diversity and an *M*-value of 0.5. At three of the SSR loci one allele was in abundance and at one of the SSR locus no polymorphism were found at all hence the low gene diversity.

Quantitative resistance of tomatoes (*Lycopersicon* ssp.) against *Phytophthora infestans*

A F Butz, M R Finckh
Faculty of Organic Agricultural Sciences, Department of Ecological Plant Protection, University Kassel, Nordbahnhofstr. 1, D-37213 Witzenhausen, Germany
Email: abutz@uni-kassel.de

Introduction

Late blight, caused by *Phytophthora infestans* (Mont) de Bary is one of the most important diseases in outdoor tomato (*Lycopersicon ssp.*) production worldwide sometimes causing total crop failure. Qualitative resistances (based on hypersensitive reactions) to late blight are easily broken by pathogen adaptation. Therefore, the focus in tomato breeding for resistance to late blight should be on quantitative resistance which are not based on hypersensitive reactions.

Methods

A total of 125 tomato accessions obtained from various international and local gene banks were screened with detached leaf tests, for their reactions against a set of 12 *P. infestans* isolates representing a wide spectrum of virulence and aggressiveness to potato and tomato. For each tomato variety, four separate leaflets were spot-inoculated with 20µl of sporangial solution with $5*10^4$ sporangia ml^{-1}. The percent diseased leaf area and the type of lesions were recorded six days and 12 days after inoculation (DAI). The area of the leaflets was recorded with image recognition, allowing for the calculation of absolute lesion size independent of leaf size.

The maximum number of varieties that could be tested together was 12. Therefore, one variety was used as a standard in each set and all data were standardised against this variety.

Results and discussion

Of the original 12 isolates used, two isolates were unreliable in their sporulation and therefore had to be omitted from the overall analysis.

The majority of the apparently resistant isolate by variety interactions, based on the assessment 6 DAI sporulated 12 DAI while a few interactions remained completely resistant, however often showing necroses indicating the existence of qualitative resistance probably based on hypersensitivty.

Besides the qualitative resistances there were cases with reduced infection efficiency and cases with slowed down disease development once infection took place, indicating various types of quantitative or partial resistance.

An analysis of quantitative resistance based on disease development was done omitting qualitative resistance and the cases with no infection where there were not four replications with successful infection. Omitting two more isolates with obviously important avirulences, a total of 39 varieties could be analyzed for differences in quantitative resistance based on disease development. In order to keep the information on the inherent variation in the reactions observed, a cluster analysis (Ward) was done based on the

Sanghvi-T2-Distance (Shangvi, 1953). This analysis accounts for the variance of the isolate by variety interaction based on the mean overlap of the Student's T-distributions.

The cluster analysis split the 39 varieties into 6 groups with one highly susceptible group containing seven varieties, three moderately resistant groups with two to 14 members, and one group of five varieties with a high mean resistance. The variety Philovita fell into a separate group and was highly resistant.

The reason for the different groupings with the same overall resistance level is to be found in race-specific interactions in partial resistance. Thus, while all isolates sporulated on all varieties, the aggressiveness on the different varieties differed sometimes considerably. For example, three isolates were highly aggressive to the group six members but the same three isolates did not differ from the other five when tested against the group two members. This indicates that different resistance genes and/or mechanisms are acting in the different varietal groups. It remains to be studied if the reactions within the groups towards the isolates are homogeneous based on aggressiveness and histological reactions. If this were the case, then the combination of parents from different groups should result in a broader resistance base than the combination of parents from within a group.

It should be possible to include varieties with qualitative resistances to certain isolates in the analysis by omitting these isolates and thus gaining information on differences in quantitative resistance backgrounds.

Conclusions

Possible variation in underlying resistance mechanisms may be the reason for isolate specific differences. These mechanisms need to be studied histologically, biochemically, and genetically. If true genetic differences can be identified, then combining parents from different groups with different race-specific interactions for breeding may be an interesting option.

Reference

Sanghvi, L D 1953. Comparison of Genetical and Morphological Methods for Study of Biological Differences. American *Journal of Physical Anthropology* **11**:385-404.

Effects of wetness and temperature on maturation of *Leptosphaeria maculans* and *L. biglobosa* ascospores in pseudothecia on oilseed rape debris

Z Liu, B D L Fitt, A O Latunde-Dada
Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK
Email: arthur.liu@bbsrc.ac.uk

A M Hall
University of Hertfordshire, Hatfield, Herts. AL10 9AB, UK

Introduction

Phoma stem canker, caused by *Leptosphaeria maculans/L. biglobosa*, is a world-wide oilseed rape disease causing considerable yield losses (Fitt *et al.*, 2006). It is initiated by ascospores formed in pseudothecia after fungal sexual reproduction. Further understanding of factors affecting ascospore release will improve strategies for forecasting the severity of the disease. This paper describes work to study the effects of wetness and temperature on maturation of ascospores of *Leptosphaeria* species in pseudothecia.

Materials and methods

Winter oilseed rape (cv. 'Apex' in 2003; cv. 'Courage' in 2004) stem debris with phoma lesions was collected from Rothamsted after harvest. Pseudothecial development was monitored by weekly microscopic examination (50 fruiting bodies were assessed each time) according to the criteria described by Toscano-Underwood *et al.* (2003): at stage A pseudothecia are differentiated but ascospores are undifferentiated, at stage B pseudothecia are further developed, at stage C ascospores start to differentiate with < 8 spores/ascus and < 4 cells/spore, and at stage D pseudothecia are mature and have ascospores with eight spores/ascus and ≥ 4 cells/spore. To study the effects of wetness on maturation, one batch of debris was artificially sprayed with rain-water twice a day on days without rain. To study the effects of temperature on maturation, three groups of debris (30-40 stems each) were prepared. One was exposed to natural conditions on 12 August 2004 (T_1), while the others were stored dry at 4 °C and exposed at monthly intervals (T_2 on 23 September; T_3 on 4 November 2004). Data were analysed using GenStat software.

Results

Pseudothecial maturation differed between 2003 and 2004. No pseudothecia were observed in stem base lesions in late September 2003, but in 2004 a few pseudothecia were mature at that time. In mid-December 2004, over half of the pseudothecia were mature and 26% had already released their ascospores, but in 2003 only 46% of the pseudothecia were mature at that time.

On the debris with extra watering, pseudothecia at stem bases reached stage A 36 days after exposure while those on unwatered stem bases started to develop after 44 days. The time to pseudothecial maturation (stage D) was 111 days for the pseudothecia on unwatered debris and was only 75 days for the watered debris (Table 1).

Pseudothecia matured differently when debris was exposed to natural conditions on different days (Table 2). After seven weeks of exposure, $> 50\%$ of the pseudothecia assessed from T_2 stem bases were at stages C and D, while $< 30\%$ of T_3 pseudothecia and only 14% of T_1 pseudothecia were at the same stages. Meanwhile, 32% of the pseudothecia

from upper stems of T_2 group were at stages C and D but none from T_1 and T_3 had reached those stages.

Table 1. Effects of watering on maturation of *Leptosphaeria* pseudothecia on winter oilseed rape (cv. 'Courage'), showing the difference in time (in days) from debris exposure to 50% of the pseudothecia reaching stage D

Treatment		LD_{50}	s.e.
Debris without extra watering	Stem base	110.9	5.94
	Upper stem	109.0	2.83
Debris with extra watering	Stem base	75.4	1.88
	Upper stem	77.8	1.91

Table 2. Pseudothecial maturation on winter oilseed rape (cv. 'Courage') after debris was exposed to natural conditions for seven weeks (no. of pseudothecia in each stage)

	Stem base				Upper stem			
	A	B	C	D	A	B	C	D
T_1	24	17	7	0	29	1	0	0
T_2	0	24	23	3	14	20	14	2
T_3	14	22	9	5	48	2	0	0

Discussion

The pseudothecial maturation process was affected by wetness and temperature. In 2004, the process was faster than in 2003 when the summer was hotter and drier (met data, not shown). The influence of wetness was reiterated by extra watered debris showing about one month earlier than un-watered one. Differences in pseudothecial maturation between debris exposed outdoors on different times highlighted the influence of both wetness and temperature on the maturation process. Pseudothecia matured faster on debris exposed in September (T_2) when there was more rainfall before/after the exposure (met data, not shown). Pseudothecia on debris initially exposed in November (T_3) matured more slowly, possibly due to the lower temperature. Though pseudothecia on debris exposed in August (T_1) had the highest temperature during the first seven weeks, their maturation process was halted most likely by the less rainfall at that time.

Acknowledgements

The authors thank the China Scholarship Council, Perry Foundation, Henry Lester Trust, and Great Britain-China Education Trust for funding. Rothamsted Research is sponsored by the Biotechnology and Biological Sciences Research Council.

References

Fitt B D L, Brun H, Barbetti M J, Rimmer S R. 2006. World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). *European Journal of Plant Pathology* **114,** 3-15.

Toscano-Underwood C, Huang Y J, Fitt B D L, Hall A M. 2003. Effects of temperature on maturation of pseudothecia of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape stem debris. *Plant Pathology* **52,** 726-736.

Nonlinear regression analysis to determine infection models of *Colletotrichum acutatum* causing anthracnose of red pepper using logistic equation

W S Kang, E W Park
Department of Agricultural Biotechnology, Seoul National University, Seoul, 151-921, Korea
Email: ewpark@snu.ac.kr

S C Yun
Department of Applied Biological Sciences, Sun Moon University, Asan, 330-744, Korea

Introduction

Anthracnose is one of the most damaging diseases of red pepper in Korea. A forecasting model would be useful to control the disease with minimum sprays. We developed a model for estimating possible infections by the anthracnose pathogen, *Colletotrichum acutatum*, on red pepper fruits.

Methods and results

Pepper fruits were harvested from pepper plants (cultivar: 'Dabotop') grown in green house. Conidial suspension of the pathogen was prepared from the fungal cultures on potato-dextrose agar media. After spray inoculation of the suspension on the surface of pepper fruits, the fruits were incubated under humid conditions at 20, 23, 25, 28, 30, and 33C. Appressorium formation rate on the surface of the fruits was measured for each temperature under light microscope at 0, 6, 12, 15, 18, 21, 24, 30, and 36 hours after inoculation. The logistic model ($P = A/(1+e^{B-kW})$), where P is appressorium formation (%), and W is wetness period (hr), was fitted to the appressorium data for each temperature using NLIN procedure of SAS software. The asymptote value (A) for each temperature treatment was fixed to the maximum percent of appressorium formation at each temperature (Table 1). The estimates of position and rate parameters (B and k, respectively) of logistic models for six temperatures were obtained from the nonlinear regression analyses (Table 1). Responses of A, B, and k to temperature were determined using nonlinear regression analyses (Table 2). The asymptote A was fitted to a model for enzyme activity with temperature. The other parameters, B and k, were fitted to reciprocal functions of temperature.

Table 1. Parameter estimates of the logistic model ($P = A/(1+e^{B-kW})$) for percent appressorium formation of *Colletotrichum acutatum* during wetness periods at different temperatures

Parameter	Temperature (C)					
	20	23	25	28	30	33
Asymptote (A %)	23.33	32.67	29.00	33.67	38.00	18.33
Position (B)	59.04	2.48	11.54	9.52	1.87	1.54
Rate (k)	3.78	0.25	0.87	0.66	0.23	0.17
R^2	0.92	0.99	0.99	0.99	0.99	0.95

Table 2. Responses to temperature of asymptote (A), position (B), and rate (k) parameters of the logistic model ($P = A/(1+e^{B-kW})$) for appressorium formation of *Colletotrichum acutatum* on the surface of pepper fruits under different regimes of temperature and wetness duration

Model	F	R^2
$A = \dfrac{52746.88 \cdot T \cdot e^{-\frac{5276.4}{T}}}{1+e^{64216.7(\frac{1}{305.15}-\frac{1}{T})}}$	118.72**	0.99
$B = \dfrac{30.47}{T-19.48}$	64.82**	0.97
$k = \dfrac{2.59}{T-19.31}$	59.10**	0.97

T : temperature; W : wetness duration

**: highly significant (P<0.01)

By combining the temperature models for A, B, and k, into the logistic model, The following equation was obtained determining percent appressorium formation (P) depending on wetness duration (W) and hourly temperature (T).

$$W = \left\{ \frac{30.47}{T-19.48} - \ln\left(\frac{52746.88 \cdot T \cdot e^{-5276.4/T} / (1+e^{64216.7(1/305.15-1/T)})}{P} - 1 \right) \right\} \cdot \frac{T-19.31}{2.59} \qquad \text{(Eq. 1)}$$

Conclusion

If a certain percentage of appressorium formation is used as a critical point for anthracnose development, infection risk levels can be determined every hour by calculating W using hourly temperature and accumulating reciprocals of W every hour. A similar infection model as Eq. 1 can be obtained by applying multiple linear regression on appressorium formation data against temperature and wetness duration. However, the infection model resulted from linear regression analysis overestimated infection risks consistently when compared with Eq. 1. Further studies are needed to determine an infection risk level that can be used as the threshold for suggesting farmers to spray fungicides to control the disease.

Genetic variation in inducibility of resistance in tomatoes against *Phytophthora infestans*

K Sharma, M R Finckh
Faculty of Organic Agricultural Sciences, Department of Ecological Plant Protection, University of Kassel, Nordbahnhofstr. 1a, D-37213, Witzenhausen, Germany
Email: kalpana@mail.wiz.uni-kassel.de

Introduction

Considerable knowledge has accumulated in recent years on the potential use of resistance induction in plant protection. Especially the mechanisms of induction and potential inducers are being focused on in research. Despite the numerous instances in which induced responses have been demonstrated, they have not found their way into practical plant production. One reason might be that inducibility for resistance is not a trait that breeders currently select for. This is also due to the fact that there is almost no knowledge on genetic variation within species for inducibility of resistance, a prerequisite for breeding for this trait.

Tomatoes have served as a successful model system for induction of resistance to many pathogens including *Phytophthora infestans*, the causal agent of late blight. Different researchers have used different cultivars of tomatoes to test induced resistance against late blight achieving different protection levels but only in a few studies more than one variety was used. It is unclear if different protection levels reported were only due to differences in the inducers and experimental conditions or to the genetic background of the tomato cultivars themselves.

The main purpose of this research is to determine if there is genetic variation among tomato cultivars in their ability to be induced for resistance against *P. infestans*.

Methods

A total of 32 tomato cultivars were grown in standard soil containing no basic nutrients in the glasshouse. Plants were watered daily and fertilised once a week with mineral fertilizer (8:8:6 NPK). Four week old plants were sprayed near run off with BABA (DL-3-amino butyric acid) at 1g l^{-1} of demineralised water seven days before inoculation while control plants were sprayed with demineralised water only (four replicate plants per treatment). Leaves directly treated with BABA (old) and newly grown leaves (young) were included in the test.

Two *P. infestans* isolates, collected from tomatoes in 2004, viz 101.04 and 108.04 were used. The two first lateral leaflets of the leaves were detached and placed lower side up in plastic trays lined with wet fleece and filter paper and covered with plexi glass. Each leaflet was inoculated with a 20µl drop of a solution of 5×10^{4}sporangia ml^{-1}. Trays were kept in the dark for 24 h at 17 °C. After 24 h, 16-h light/ 8-h dark cycle was maintained and leaves were sprayed with sterile demineralised water every two days. Percent diseased leaf area was assessed daily from day five to seven.

As only six to eight cultivars could be handled at a time five separate inoculations were carried out. The cultivar Supermarmande was used as an internal control and included in each inoculation.

Area under the disease progress curve (AUDC) was calculated from the raw data and standardized against Supermarmande within each set. AUDC for Supermarmande was very consistent among inoculations varying from 6.20 to 17.09 cm^2 for isolate 101 and 7.99 to 26.27 cm^2 for isolate 108. Data were log-transformed and then analyzed together using SAS PROC mixed.

Results

There was significant variation in the degree of susceptibility of the cultivars to the two isolates ranging from 0 to 3.25 times the AUDPC of Supermarmande.

Resistance induction on the young leaves was in most but not in all cases higher than on the old leaves that had been directly treated by BABA.

The degree of induction varied depending on the isolate and cultivar used (significant cultivar x treatment interactions). At all resistance levels there were cases in which no disease reduction was achieved through the use of BABA and others with significant disease reduction. Some cultivars were equally susceptible to both isolates and disease reductions due to BABA were similar. However, there were also cases in which resistance induction only worked with one of the two isolates or the degree of induction was isolate dependent.

Discussion and conclusions

The results presented in this paper confirm that there is variation among tomato cultivars in their ability to be induced for resistance against *P. infestans*; and both leaf age and isolate used for challenge inoculation interact with inducibility. The level of induction that was achieved seems to be dependent on tomato cultivars and not related to the resistance level. While reactions of Supermarmande were consistent across inoculations, additional repeats should be done with other cultivars to confirm these results. Also, there is a need to determine if the plants will react in the same way to other resistance inducers.

The higher degree of induction on young leaves might be because of a combined effect of local and systemic induction of resistance since the young leaves were not fully developed while spraying BABA. However, in some cultivars BABA was found to give better induction on old leaves suggesting that local induction levels may be more important in these cultivars.

While these results suggest that the degree of inducibility may be variable, genotype by inducer by pathogen isolate and environment interactions need to be understood before efficient selection for this trait will be possible.

Phytotoxic effect of *Artemisia aucheri* on germination and growth of *Amarantus retroflexus*

H S Zadeh
Laboratory of Tropical and Subtropical and Ethnobotany,Coupure Links 653,B-9000 Gent, Belgium and Faculty of Natural Resources & Desert Studies,Yazu University, Yazd, Iran
Email: hsodaee@yahoo.com

K Steppe
Department of Applied Plant Ecology, Faculty of Bioscience Engineering, Coupure Links 653, B-9000 Gent, Belgium

P Van Damme
Laboratory of Tropical and Subtropical and Ethnobotany, Coupure Links 653, B-9000 Gent, Belgium

Introduction

Weeds drastically reduce crop yields. Traditional methods for controlling weeds are time-consuming and labour intensive. On the other hand, imprudent use of chemical herbicides in agriculture causes environmental problems. In sustainable agriculture, we need new strategies to improve weed and pathogen management. Allelopathy as a rather new method of weed control could lead to reduced labor costs and increased efficiency, while taking into account a number of environmental conservations. Several *Artemisia* species have allelopathy potential and contain chemicals compounds that can inhibit germination and growth of other plants (Escudero *et al.* 2000, Modallal & Al-Charchafchi, 2006). One species of this genus, *Artemisia aucheri*, is widely distributed in the desert area of Iran. Earlier field observations revealed that communities dominated by *A. aucheri* have reduced density of other associated herbaceous species. Besides competing with other plants for nutrients, *A. aucheri* might be allelopathic and presence of this plant may negatively influence growth of certain crops or weeds. Therefore, the present study was conducted to evaluate allelopathic effects of *A. aucheri* by determining the phytotoxic effects of aqueous extracts and soil incorporated with *A. aucheri* above-ground on growth of one weed species (*Amarantus retroflexus*) under laboratory and greenhouse conditions.

Materials and methods

16 g fresh above-ground material of *A. aucheri* was cut into 1 to 2-cm parts. Plant material was soaked in a flask containing 100 ml distilled water for 24h on an orbital shaker (Edmand Bahler, VKS-75). The extract was filtered through two layers of cheese cloth and centrifuged at 5000 rpm for 20 min. The supernatant was filtered again through two layers of Whatman No.1 filter paper. The extract was diluted with sterile distilled water to give the final concentrations of 4, 8, 12 and 16% (w/v). Seeds of *A. retroflexus* were surface-sterilized with 5% aqueous solution of sodium hypochlorite for two minutes, rinsed five times with distilled water and dried between two papers towels. To determine the effect of the *A. aucheri* extract effect on seed germination, 25 seeds of *A. retroflexus* were placed in Petri dishes with 9-cm of Whatman no.1 filter paper, containing 5 ml of each *A. aucheri* aqueous extract (or distilled water for control). The Petri dishes were placed in a Shellab 1535 incubator at 25 ± 1°C. The number of germinated seeds was recorded daily for eight days. After this final germination percentage and mean period of final germination (MPFG) were calculated. The above mentioned procedures were also followed for 25 germinated

seeds to evaluate the effect of the aqueous extract of *A. aucheri* on seedling growth of *A. retroflexus*. Therefore, the seedlings were placed in a climatised room at 25 ± 1°C with a 16/8 h light–dark photoperiod. After eight days root and shoot (coleoptile) length and seedling dry weight were measured.

In another set of experiments, three concentration of shade-dried powder of above-ground *A. aucheri* material (3, 6 or 9 g dry weight per 1000 g soil) were mixed with sandy loam soil in plastic pots, which were placed in the greenhouse. Control pots were filled with soil without residue. 50 ml tap water was added to each pot and after one day, 15 *A. retroflexus* seeds were sown in each pot. Plants were harvested 30 days after planting and leaf area, root length, plant height and dry weight of *A.retroflexus* plants were measured. Treatments were arranged in a randomized complete block design with three replicates. Data were subjected to an analysis of variance and the means were compared using least significant difference (LSD) at the 5% level.

Results and discussion

Aqueous extract of *A. aucheri* fresh biomass showed strong inhibitory effect on seed germination of *A. retroflexus* and significantly increased MPFG. At the highest concentration of the extract, 98% reduction in germination was observed when compared to the control. Mean period of germination increased progressively with increase in extract concentration. In concentration of 4,8,12 and 16%, MPFG increased 24.5, 87,109 and 182% respectively, with respect to the control. Both root and shoot lengths were severely affected and the effect was higher on root length than on shoot length. When a concentration of 16% was used, root and shoot length of seedlings decreased 73 and 43%, respectively, compared to the control. The dry weight of seedlings was significantly reduced at all the aqueous extract concentration, except for 4%. This reduction was approximately 51% for the highest extract concentration. Incorporation of above-ground powder of *A. aucheri* affected the growth of *A .retroflexus* at all concentrations. Leaf area, root length, plant height and biomass of test plants were lower in the soils amended with residues when compared with a no-residue control. The inhibitory effect was a function of the amount of residues added to the soil and consequently greatest inhibition was observed in soils with the highest amount of above-ground amendment. Leaf area of *A. retroflexus* declined progressively with increased concentration. A 73% reduction in leaf area with respect to the control was observed at 9 g debris per 1000 g soil. Decrease in root and shoot length was concentration-dependent. 9 g per 1000 g residue inhibited root length at 47% of control values. Dry material from *A. aucheri* plants at 3, 6 and 9 g kg^{-1} reduced dry weight of *A. retroflexus* by 58.4,73 and 81%, respectively, when compared to the control. In conclusion, a bioassay on allelopathic effects of extracts or residues demonstrated that (1) *A. aucheri* plant has allelopathic potential and decreased germination and growth of *A. retroflexus*, and (2) the activity differed depending on concentration. This potential may be a valuable means of biological weed control based on natural plant compounds.

References

Escudero, A, Albert, M, Pita, J, Garcia, F, (2000). Inhibitory effects of *Artemisia herba-alba* on the germination of the gypsophyte *Helianthemum aquamatum.Plant Ecology*.**148**:71-80

Modalla, N B, Al-Charchafchi, F M R, (2006).Allelopathic effect of *Artemisia herba-alba* on germination and seedling growth of *Anabasis setifera*. *Pakistan Journal of Biological Science*.**9**:1795-1798

Evaluating the role of cytochrome P450s in pyrethroid resistance of the diamondback moth, *Plutella xylostella* (L.)

M A M Bautista, K Miura, T Miyata, T Tanaka
Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa-ku, Furo-cho 464-8601, Japan
Email: maanita_bautista@yahoo.com

Introduction

Resistance of the diamondback moth (DBM), *Plutella xylostella* (L.), to many insecticides, is a worldwide problem and a continuing threat to crucifer production. One mechanism involved in DBM resistance to pyrethroids is increased detoxification by cytochrome P450 monooxygenases (P450s) via elevated expression of P450s (Feyereisen, 1999), hence frequently found over-expressed in insecticide-resistant insects, including some agricultural pests of economic importance. P450-mediated insecticide resistance has been comprehensively explored in other insects, but mediation in DBM resistance, particularly to pyrethroids, remains elusive. Identifying P450 (s) involved using overexpression and inducibility as criteria, and demonstrating functional role(s) in resistance will provide valuable information in understanding resistance mechanisms.

This study, therefore, aims to understand the mechanism of permethrin, a synthetic pyrethroid, resistance in DBM, initially, by evaluating the role of cytochrome P450 isolated from a permethrin-resistant DBM strain. Knowledge on the role of P450 offers support in development of tools essential for monitoring, possibly predicting, preventing, and reversing the development of resistance to permethrin and possibly other pyrethroids.

Experimental procedures

Isolation of P450s

A polymerase chain reaction (PCR)-based technology was used to isolate P450s from permethrin-resistant DBM strain.

P450 expression and induction

Transcript expression levels were determined by quantitative real-time PCR (QRT-PCR). Induction experiments were done by exposing third instars to different concentrations of permethrin at different times. Transcript and resistance levels were analyzed after each treatment by QRT-PCR and bioassays, respectively.

Permethrin toxicity and P450 activity assays

Standard procedure for LC_{50} determination was used to investigate the effect of pre-exposing larvae to permethrin. On the other hand, a discriminating dose (LC_{10} equivalent) was used to determine effects of P450 RNA interference i resistance to permethrin. Total P450 activity was determined by a slightly modified procedure for p-nitroanisole *O*-demethylation assay (PNOD).

RNA interference

Fourth instars were either droplet-fed buffer (0.5 µl) or buffer containing 250 ng of CYP6BG1 dsRNA produced by *in vitro* transcription of a 345 bp PCR product. QRT-PCR analysis was done to determine knockdown of CYP6BG1, and target- specificity of dsRNA used. Total P450 activity assays and toxicity tests were also done to determine effects of CYP6BG1 silencing to enzyme activity and resistance of the strain used, respectively.

Results and discussion

Six predominantly occurring P450s in DBM fourth instars were amplified by PCR (Bautista, 2007). These P450s were named and classified under CYP4 and CYP6 P450 families, which are often associated with insecticide resistance. QRT-PCR results indicated that two P450s, CYP6BG1 and CYP6BG2, were over-expressed (4.9- and 3.8-fold, respectively) in fourth instars of the resistant compared to the susceptible strain, which suggests a potential major involvement in permethrin resistance. However, based on a more recent study, overexpression does not necessarily confer resistance (Kuruganti *et al.*, 2007). Because this may complicate identification of P450s with role in resistance, inducibility by permethrin was used as another criterion. Induction is said to have evolved as an adaptive response and is often linked with increased tolerance to insecticides (Brattsten & Wilkinson, 1973). Its control primarily occurs at the transcription level.

Induction experiments indicated that pre-exposure to permethrin at 230 and 350 ppm for 6h induced (8.4- and 3.6-fold, respectively) CYP4M11, which is an isoform that is not over-expressed in resistant strain. On the other hand, pre-exposure to 350 ppm for 16 h induced CYP6BG1, which is an over-expressed P450 in resistant strain, transcript level to 2.2-fold. Other P450s were either at their basal levels or were down-regulated. Surprisingly, CYP6BG2, which is an over-expressed P450 in the resistant strain, was down-regulated by certain doses of permethrin. Induction also resulted to increased resistance to permethrin (up to 6.3 -fold).

Functional analysis, by RNAi, for the over-expressed and inducible CYP6BG1 was subsequently performed to investigate whether it mediates permethrin resistance and to determine the extent of mediation. Results demonstrate that oral delivery of dsRNA to fourth instars reduced transcript level of CYP6BG1 up to 250-fold at 24h post feeding. At the observed reduced level, overall CYP450 activity and resistance had a 20 and 36% reduction, respectively, which serve to indicate partial involvement of CYP6BG1 in resistance to permethrin of *P. xylostella.* Moreover, possibilities that other P450s are involved cannot be ruled out.

References

Bautista M A M; Tanaka T; Miyata T (2007). Identification of permethrin-inducible cytochrome P450 in the diamondback moth, *Plutella xylostella* and the possibility of involvement in permethrin resistance. *Pesticide Biochemistry and Physiology* **87**, 85-93.

Brattsten L B; Wilkinson C F (1973). Induction of microsomal enzymes in the southern Armyworm (*Prodenia eridania*). Pesticide Biochemistry and Physiology 3, 393-407.

Feyereisen R (1999). Insect P450 enzymes. *Annual Review of Entomology* 44, 507-533.

Kuruganti S; Lam V; Zhou X; Bennett G; Pittendrigh B; Ganguly R (2007). High expression of *Cyp6g1*, a cytochrome P450 gene, does not necessarily confer DDT resistance in *Drosophila melanogaster*. *Gene* 388, 43-53.

Residual fate and metabolism of oxadiargyl in paddy

N Sanyal
Pesticide Residue Laboratory, Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741 252, Nadia, West Bengal, India
Email: sanyalnilanjan@rediffmail.com.

A Chowdhury
Department of Agricultural Chemistry and Soil Science, Institute of Agricultural Science, University of Calcutta, Kolkata 700 019, India

Introduction

Oxadiargyl (5-tert-butyl-3-[2, 4-dichloro-5-(prop-2-ynyloxy) phenyl]-1, 3, 4-oxadiazol-2(3H) -one) is a novel protoporphyrinogen IX oxidase herbicide, active pre-emergence on both annual monocotyledons and dicotyledons. The product acts at germination, as the new shoots come in contact with treated soil particles. Oxadiargyl is active via contact activity, with very limited translocation following shoot uptake.

Materials and methods

A three season field trial with oxadiargyl following application at recommended (90 g a.i. ha^{-1}) and double the recommended (180 g a.i. ha^{-1}) doses was conducted in paddy under tropical monsoon climatic conditions. Oxadiargyl was applied three days after transplanting and samples were collected at 1, 7, 15, 30, 45 and 60 days after application for residue analysis. Soil sample of the last day (60 d) was subjected to Q-tof micromass spectroscopy for structure elucidation of the metabolites formed. All soil samples were subjected to microbiological and biochemical analyses i.e. microbial biomass carbon (MBC), basal soil respiration (BSR) and fluorescein diacetate hydrolyzing activity (FDHA).

Samples (crop, cropped soil and flood water) were extracted with acetone: water (8:2, v/v) and partitioned into dichloromethane. However the flood water samples were directed subjected to partitioning after filtration. The organic fractions was evaporated to dryness, the concentrated mass was transferred, for adsorption chromatography, over silica packed SPE cartridge. The SPE cartridge was washed with 10 mL hexane and the analytes were eluted with 10 mL of acetone: hexane (80:20, v/v). The eluate was evaporated to dryness by rotary vacuum evaporator at 40^0C and subsequently the residues were rinsed with HPLC grade acetonitrile and filtered (0.2 µm) for HPLC analysis.

For analysis of harvest residue in grain and straw, the samples were extracted with soxhlet apparatus using acetone: hexane (7:3, v/v). It was then filtered and concentrated to dryness. The solid mass was then taken in water and partitioned into dichloromethane (100 + 50 + 50 mL). Then it was carried out same way as adopted for soil/plant samples.

The residual oxadiargyl was separated on an Intersil 150 x 4.6 mm ODS 2, 5 µ (RP C_{18}) using a mobile phase of acetonitrile and water (80:20, v/v) at a flow rate of 1 mL min^{-1} and column temperature at 40 ^{0}C. Quantification was performed against oxadiargyl standard at a wave length of 210 nm. Under this condition the retention time of the compound was 4.4 min whereas the limit of detection and the limit of quantification of the method were 0.001 mg and 0.005 mg kg^{-1} respectively. The average recovery was found to be 85-97% and thus adopted for the experiment.

The MBC was determined by fumigation extraction method (Joergensen, 1995) followed by determination of K_2SO_4 extractable C (Vance *et al.*, 1987). Biomass C was estimated as: Biomass C = 2.64 Ec, where Ec is the difference between K_2SO_4 extractable C from the fumigated and unfumigated soils. BSR and FDHA content of the soil samples were determined by the methods described by Alef (1995a) and Alef (1995b) respectively.

Results and discussion

Interestingly it was observed that in paddy no residue of oxadiargyl was found in any sample which is found to be well in agreement with the basic mechanism of action of the compound (Dickmann *et al.*, 1997). The result showed that the initial deposit of this herbicide in soil varied in the range of 0.0362 - 0.0772 mg kg^{-1} and 0.0166 - 0.0308 mg L^{-1} in soil and water respectively irrespective of treatment level and seasons. In surface water the half-life was ranged between 2.959 - 3.129 d and 3.596 - 3.864 d for single and double dose of applications respectively.

However, in cropped soil the dissipation of this compound was very rapid initially up to a range of 44.684 - 53.369% within first seven days of application and then disappeared steadily and the half-lives were ranged between 18.133 - 18.813 and 19.803 - 20.338 d for single and double rates of application. In all cases the degradation of the herbicide occurred following the first order kinetics. From the experiment it was revealed that the compound had no adverse effect on the soil microbial parameters as at both the application rates the soil microbial biomass carbon (MBC), basal soil respiration (BSR) and fluorescein diacetate hydrolyzing activity (FDHA) was increased significantly over control. So it was understood that at both application levels oxadiargyl was utilized as the 'C' source by the soil microbial community. The Q-tof micromass spectra of soil samples (60 d) revealed the presence of at least three metabolites. From the MSMS data the structures were elucidated as N-(2,4-Dichloro-5-prop-2-ynyloxy-phenyl)-N-(-1-hydroxy-2,2-dimethyl-propylidene)-hydrazine, 2,4-Dichloro -1-prop-2-ynyloxy-benzene and N-(3-Chloro-5-prop-2-ynyloxy-phenyl)-N-(2,2-dimethyl-propionyl)-hydrazine carboxylic acid methyl ester. The metabolite identified indicates that the probable transformation of oxadiargyl in field occurs via break down of active oxadiazoline moiety and thus less toxic products are expected.

References

Alef K (1995a). Estimation of soils respiration. In: *Methods in Applied Soil Microbiology and Biochemistry.* Alef K and Nannipieri P (eds.) Academic Press. London. pp. 215-216.

Alef K (1995b). Estimation of the hydrolysis of fluorescein diacetate. In: *Methods in Applied Soil Microbiology and Biochemistry.* Alef K and Nannipieri P (eds.) Academic Press. London. pp. 232-233.

Dickmann R; Melgarejo J; Loubiere P; Montagnon M (1997). Oxadiargyl: a novel herbicide for rice and sugarcane. *Brighton Crop Protection Conference: Weeds.* BCPC, UK. pp. **1**: 51-57.

Joergensen R G (1995). Microbiol biomass. In: *Methods in Applied Microbiology and Biochemistry.* Alef K; Nannipieri P (eds.). Academic Press. London. pp. 382-386.

Vance E D; Brookes P C; Jenkinson D S (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry.* **19**: 703-707.

Sexual fertility and vegetative compatibility status of Fusarium verticillioides from maize in Iran

A M Gohari, M Javan-Nikkhah, G A Hedjaroude,
Plant Protection Department, Faculty of Agriculture, University of Tehran , Karaj, Iran
Email: amirzady@yahoo.com

M Abbasi
Botany Department, Plant Pests and Diseases Research Institute, Tehran, Iran

V Rahjoo
Department of Seed and Plant Improvement Institute, Karaj, Iran

Abstract
Forty-four isolates of *Fusarium verticilliodes* from stalks and seeds in the maize producing areas in Iran, during 2004-2005 growing season were recovered. Sexual crosses with standard tester strains identified 44 isolates of *F. verticillioides*, all of which were *MAT-1*.

We identified 25 vegetative compatibility groups among 44 isolates of *F. verticilliodes* by pairing *nit* mutants. It is concluded that genetic diversity within *F. verticillioides* population in Iran is a result of sexual reproduction rather than of asexual recombination.

Introduction

Fusarium verticillioides is a member of the *Gibberella fujikuroi* species complex, which contains at least nine mating populations or biological species, denoted by the letters A-I. Sexual fertility is an important parameter when biological species concepts are being applied, as these species concepts usually require some evidence of sexual cross-fertility before two strains are assigned to a common species (Leslie & Summerell, 2006). To date, most population genetics studies of *F. verticillioides* have been conducted using the vegetative compatibility group as a marker for genotyping fungal isolates.

Our specific objectives were:

to determine mating type frequencies to calculate the effective population number and sexual fertility for *G. moniliformis;*

to assess the vegetative compatibility among *F. verticillioides* isolates originating from maize in Iran.

Materials and methods

Sexual crosses and diagnosis of mating type

Crosses to confirm mating population and to identify mating type were made in duplicate on carrot agar according to the techniques of Klittich and Leslie (1988) with known standard tester strains 7600 (*MAT-1*), 7603(*MAT-2*).

Vegetative compatibility grouping

Nitrate-nonutilizing (*nit*) mutants were recovered on minimal medium amended with 1.5% potassium chlorate (KClO3) and were assigned to one of three phenotypic classes (*nit1*, *nit3*, or Nit M).

Vegetative compatibility grouping

Nitrate-nonutilizing (*nit*) mutants were recovered on minimal medium amended with 1.5% potassium chlorate (KClO3) and were assigned to one of three phenotypic classes (*nit1*, *nit3*, or Nit M) based on their growth on basal medium containing different nitrogen sources. Complementation tests between *nit* mutants were performed on minimal medium in 24-well hybridoma plates as previously described by Klittich and Leslie (1988).

Results

Mating type and sexual cross-fertility

Forty-four isolates examined were *MAT-1*. All of them were fertile as males with one of the two standard tester strains for these species.

Assignment of *F. verticillioides* isolates to VCGs

Based on positive complementation reactions, we identified 25 VCGs among 44 isolates, of which 21 had only one member, and the remaining 23 belonged to four multimember VCGs.

Discussion

The mating type ratio (44:00, *MAT-1-MAT-2*) was the inverse of that reported by Leslie & Klein (1996) for some populations.

Some explanations for this discrepancy include: mating type (*MAT-2*) has been eliminated or maybe the frequency of *MAT-2* is so little or even only *MAT-1* has been imported to Iran.

The high degree of VCG polymorphism in this study supports the assumption that the genotypic diversity of *F. verticillioides* is primarily caused by recombination during the sexual reproduction.

In conclusion, four multimember VCGs observed within the species *F. verticillioides* indicate population differentiation and genetic diversity within subpopulations, suggesting that sexual reproduction occurs frequently in the analyzed population.

Acknowledgements

We thank John F Leslie, Kansas State University, for critically reviewing the manuscript.

References

Klittich, C J R and Leslie, J F (1988). Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics* **118**: 417-423.

Leslie, J F, & Summerell, B A (2006). *The Fusarium labratory manual*. Iowa State University Press.

Leslie, J F and Klein, K K (1996). Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **44**: 557-567.

Growth, yield and yield components of maize (*Zea mays* L.) as affected by density and the time of redroot pigweed (*Amaranthus retroflexus* L.) emergence

M AghaAlikhani
Department of Agronomy, Faculty of Agriculture, Islamic Azad University - Varamin Branch, Varamin, Iran
Email: maghaalikhani@modares.ac.ir

S A M Modarres Sanavy, S Soufizadeh, F Etemadi
Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, PO Box 14115- 336, Tehran, Iran

Introduction

Maize damage by weeds is estimated to be 10 to 100% if they left uncontrolled. However, interference of weeds against crop varies based on weed density, competitive ability and crop growth stage. In Iran, weed control in this crop has been relied almost exclusively on herbicide applications. However, it is strongly recommended today to use an integrated approach to control weeds in order to reduce negative effects of herbicides on ecosystem and environment. Following this line of thought, a thorough understanding about quantitative effects of competition intensity and its effect on crop growth and yield is needed.

Materials and methods

A field experiment was conducted at the research field of Seed and Plant Improvement Institute, Karaj (35° 59′N, 50° 75′E; 1313m above sea level) in 2005. The soil texture was clay loam. The experiment was conducted as a randomized complete block design with factorial arrangement of treatments and three replications. Treatments were three times of redroot pigweed emergence and four densities of redroot pigweed. The three times of redroot pigweed emergence was defined according to crop growth stages; i.e. emerging simultaneously with the crop, emerging at two- to three-leaf stage and emerging at four- to five-leaf stage of maize. Redroot pigweed densities were equivalent to 3, 5, 8, and 10 plants m^{-1} of the crop row. In addition to these treatments, the full-season hand weeded and full-season weed infested plots were also included in the experiment. Each plot consisted of four 8-m rows, spaced 0.75m apart. The distance between the crops within rows was 0.20m. The seedbed was prepared using a moldboard plow followed by disking, and smoothing with land leveler. Maize (*Z. mays* cv. Mohaghegh; SC 647) was planted on 25 May 2003 using a four-row pneumatic corn planter. Redroot pigweed seed was gathered in the previous year from different fields located near the study area. These seeds were kept at 4°C and dry condition to inhibit the loss in seed longevity. Redroot pigweed seeds were planted 15cm from crop in both sides of the crop row at high density. The weed planting was done immediately after maize planting, and one-week and two-weeks after maize emergence so that weed competition started at the determined times of crop growth stages. By emergence of redroot pigweed, the weed population was thinned to the determined densities. All weed species except for redroot pigweed were removed by hand during entire growing season. Maize was harvested one week after physiological maturity from 3m of the center two rows of each plot after excluding 1-m portion of both the front and rear of each plot to minimize neighborhood effects. The crops were oven-dried at 75°C for 72h and weighed to determine biological yield. Each crop was then divided into stem, leaf and ear, and dry weight of each component was determined. Leaves were separated from the stem by cutting the lamina at the ligule. Number of grain rows/ear, number of grains/ear row and ear length were measured. Grains were shelled by hand to determine grain yield and 1000- grain weight.

Harvest index was calculated as the proportion of grain yield to biological yield multiplying by 100. Percent maize grain yield and biological yield reductions were calculated by dividing grain yield and biological yield in the weed infested plots by grain yield and biological yield in the full-season weed free plot and multiplying by 100. Data were analyzed statistically by PROC GLM procedure in SAS statistical software. Assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. The Duncan multiple range test (DMRT) set at 0.05 was used to determine the significance of the difference between treatment means.

Results and discussion

Analysis of variance showed no significant interaction between weed density and its time of emergence in case of all traits, so the results were presented based on the main effects of experimental factors. Biological yield, stem and leaf weight significantly differed among different times of weed emergence so that the highest amounts of these traits were obtained when redroot pigweed emerged at four- to five-leaf stage of maize. The lowest biological yield, stem and leaf weight were achieved in plots in which redroot pigweed emerged simultaneously with maize. These results showed that maize is especially susceptible to early weed competition. This could mainly be attributed to competition for light as the primary cause of crop yield loss in many crop-weed associations. Interestingly, different densities of redroot pigweed could not significantly affect the above traits. However and in contrast to biological yield and leaf weight, stem weight increased by increase in weed density. This indicated that a change in the pattern of dry matter partitioning could be occurred by weed density enhancement. Significant differences existed among different densities and times of redroot pigweed emergence for percent biological yield reduction. At all densities of redroot pigweed, the lowest percent biological yield loss belonged to the latest time of weed emergence, although the reduction increased by increase in weed density (except for five redroot pigweed m^{-1} of crop row). The difference in percent biological yield reduction between the first and second times of redroot pigweed emergence was highest in plots where three redroot pigweed m^{-1} of crop row emerged. But the difference reduced in higher weed densities. This might be attributed to intraspecific competition between redroot pigweed at higher densities. Similar result was obtained in case of grain yield; i.e. grain yield was significantly higher at the latest time of redroot pigweed emergence than the other two emergence times. Percent grain yield reduction also agreed with this result so that the reduction was highest when redroot pigweed was emerged simultaneously with maize and at two- to three-leaf stage of the crop, respectively.

As observed, in the first and last times of weed emergence the highest yield loss was occurred where 10 redroot pigweed m^{-1} of crop row existed (58.1 and 19.2%, respectively). Generally, maize grain yield was reduced between 18.5 and 58% at the weed densities between three and 10 plants m^{-1} of crop row. It was also observed that percent grain yield loss occurred at simultaneous emergence of maize and redroot pigweed at three weeds m^{-1} of crop row was more than that of 10 weeds m^{-1} of crop row which emerged at two- to three-leaf and four- to five leaf stages of maize. This also highlighted the importance of the time of weed emergence compared with weed density. Number of grain rows/ear, number of grains/ear row and ear length were significantly affected by the time of weed emergence but not by weed density. 1000- grain weight and harvest index, however, were not significantly influenced by both experimental factors. This indicated that weed competition has mainly influenced pre-anthesis growth of maize crop and post-anthesis growth was affected to a little extent. Overall, it could be concluded that maize is very susceptible to early growth competition. In addition, the time of weed emergence plays more important role than weed density in crop-weed interaction.