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Tri-trophic effects of aphid-resistant wheat on a generalist predator, *Pardosa amentata*

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Abstract
This experiment was set up as a simulation of possible effects of genetically manipulated plants on polyphagous natural enemies. A partially aphid-resistant wheat cultivar (cv. Fold) was chosen as the "modified" model plant, the aphid *Sitobion avenae* (F.) as the model pest, and the wolf spider *Pardosa amentata* (Clerck, 1757) as the generalist natural enemy. *P. amentata* proved to be a suitable model generalist predator that showed sensitivity towards different types of prey. This sensitivity was detectable in mortality as well as growth rate. Aphids reared on the partially aphid-resistant wheat cv. Fold and the susceptible wheat cv. Terra were both poor quality food in terms of growth, but spiders showed higher mortality when were fed aphids reared on partially resistant wheat cultivar (55%) compared to aphids originating from sensitive wheat (25%). In terms of growth, there were no differences according to the origin of aphids, whether in pure or mixed feeding situation. The methods used in this experiment seemed suitable for testing the tri-trophic effects of GM-plants.

Key words: food quality, prey sensitivity, mortality, growth, natural enemy, environmental impact assessment

INTRODUCTION

Transgenic crops in 2001 covered a global area of 44.2 million hectares (James 2001). The main crop plans were soybean (58% of the total global GM-crop area), maize (23%), cotton (12%), and oilseed rape (7%), with the dominant traits of herbicide tolerance (74% of global area) and insect resistance (18%; James 2001). This rapid spread of transgenic crops has not been without controversy. In most countries, different regulations exist, but generally it has to be proven that outdoor planting has no "negative consequences". This general desire is imperfectly translated into specific tests required (National Research Council 2002). Genetically modified (GM) crops represent a technology that may offer significant benefits, ranging from more convenient and flexible crop management to higher productivity or profitability, and less pollution through decreased use of conventional pesticides (Carpenter & Gianessi 2001).

There are also several types of potentially negative side effects of transgenic crops. One of these is damage to beneficial ecological mechanisms (Lövei 2001), including the natural regulation of herbivorous insects that are potential pests of cultivated crops. GM crops can affect natural enemies by depleting their prey (by making the plant a less suitable host for the herbivores that are the predators' food) and thereby starving the predator. Indirectly, the GM-crops can influence the biochemical composition of the herbivore and thereby reduce their nutritional quality for the predator. Either way the transgenic plants may prevent the natural enemies from keeping potential arthropod pests below economically damaging density levels. Such potential effects need to be studied before a GM plant is approved for field planting, but there is no current consensus about the choice of model predators or methods to follow. The published experiments including natural enemies (for a review on Bt-crops, see Groot & Dicke 2002) all contain several experimental conditions (constant temperature, *ad libitum* feeding, single prey diet) that do rarely occur in nature and are thus unrealistic. Increasing the realism in these tests is an important condition to improve the reliability of pre-release testing of GM plants (Lövei 2001).

Common predators in European cereal fields include Carabidae, Staphylinidae, Linyphiidae, and Lycosidae (Crook & Sunderland 1984). Spiders are generalist predators and capable of eating different types of prey which also predestinates them for a beneficial role (Nyffeler & Benz 1988). In some crop fields spiders occur at high population densities (Kiss & Samu 2000) and manipulative experiments support the effectiveness of spiders as biological control agents (Riechert & Lockley 1984; Holland & Thomas 1997). Spiders are believed to be food limited (Riechert & Lockley 1984) but they also respond to the size (Riechert & Harp 1987), amino acid composition (Greenstone 1979) and quality of their prey (Toft & Wise 1999a,b; Mayntz & Toft 2001). It is therefore probable that spiders are sensitive to both direct and indirect changes generated by GM plants.

The aim of this experiment was to study effects of prey quality on a wolf spider (Lycosidae) kept under a limited feeding regime and fed on mixed prey, and to contribute to the development of GM plant " biosafety tests" using spiders as model natural enemies.

MATERIAL AND METHODS

Model organisms

The predator chosen for this experiment was the wolf spider *Pardosa amentata* (Clerck, 1757). This species occurs in a wide range of humid areas with a preference for grazed meadows. Wolf spiders are " sit-and-wait" predators. Flies (Diptera) make up around 70% of their prey in natural populations (Edgar 1970) but they will kill and consume a wider range of arthropods, including aphids. *P. amentata* is distributed throughout the Palearctic region (Alderweireldt & Maelfait 1988) and in Northern Europe it has an annual life cycle with reproduction from May to June (Vlijm et al. 1963).

Spiderlings used in the treatments were offspring of 10 female spiders collected with egg sacs in May 2000 in the fields around Flakkebjerg Research Centre, Slagelse, Denmark. After their first moult, the spiderlings were evenly distributed among the five treatments.

The grain aphid *Sitobion avenae* was used as prey. In temperate climates, *S. avenae* is an economically important pest on cereals (especially wheat) where they prefer to feed on the upper leaves and on the ears (Blackman & Eastop 2000). The pest status of *S. avenae* is in part a consequence of its ability to multiply rapidly on cereals during the summer (Acreman & Dixon 1989). Adult aphids and late instar nymphs were fed to predators.

Drosophila melanogaster was used as control prey as it gives the highest growth rate, the best survivorship and the lowest frequency of moulting problems for *P. amentata* (Mayntz & Toft 2001).

Rearing conditions

The aphids were kept on wheat plants at growth stages 19-22 (Zadoks et al. 1974), using susceptible (cv. Terra) and partially resistant (cv. Fold) cultivars grown in a temperature-regulated greenhouse. The wheat plants were kept at 23±1°C, 70% RH, 16h:8h L:D photoperiod during winter and natural photoperiod in the summer.

The fruit flies were reared on a mixture of basic Formula 4-24 Carolina medium (3.4 g, Carolina Biological Supply Company, USA) and crushed dog food (3.6 g, Pedigree Original, Masterfoods A/S, Helsinki) mixed with 15 ml distilled water; the flies were kept at 25±1°C, 16h:8h L:D photoperiod.

The spiders were kept in plastic tubes (diameter 20 mm, height 60 mm) with a base of plaster-of-Paris mixed with charcoal, at 23 ± 1°C, 65 ± 5% R.H., under 16h:8h L:D photoperiod. The charcoal base was wetted twice weekly to maintain sufficient humidity.

Experimental treatments

In order to make the experiment as realistic as possible the spiders in the five treatments were given a limited amount of food because spiders in the field usually are in a condition of underfeeding (Breymeyer & Jozwik 1975).

The feeding regimes were as follows:

- *Aphids only, susceptible wheat* (Treatment S): 3 *Sitobion avenae* (adult or late instar nymph, mean body mass = 0.331 mg, SD= 0.112 mg, N = 35) kept on aphid-susceptible wheat cv. Terra.
- *Aphids only, partially resistant wheat* (Treatment PR): 3 *Sitobion avenae* (adult or late instar larva, mean body mass = 0.294 mg, SD= 0.104 mg, N = 30) kept on partially aphid-resistant wheat cv. Fold.
- *Mixed, susceptible* (Treatment S-mix): 3 *Sitobion avenae* kept on susceptible wheat cv. Terra + 1 adult *Drosophila melanogaster* (mutant "vestigial winged" selected, because its impaired escape behaviour makes it an easy prey even for juvenile spiders. Mean body mass = 0.903 mg, SD= 0.200 mg, N= 30).
- *Mixed, partially resistant* (Treatment PR-mix): 3 *Sitobion avenae* kept on partially resistant wheat cv. Fold + 1 adult *Drosophila melanogaster.*
- *Control*: 1 adult *Drosophila melanogaster.*

Water was supplied twice a week and prey was given once a week. The experiment ran for 8 weeks after the first moult (= 9 weeks in total).

Measurements and evaluation

Growth rate and survivorship of the spiders were selected as surrogate fitness parameters. Spiders were weighed (sensitivity level of 0.0005 mg) weekly and mortality was checked twice weekly.

Analysis of survivorship data was performed using a modified log-rank test designed to compare mortality curves (Pyke & Thompson 1986). This test is a non-parametric test for comparing two or more samples with an option to compare " partial" mortality curves. In such cases the experiment is finished before all the experimental subjects die.

The growth curves were compared using pair-wise multiple comparison (GT2 test, Sokal & Rohlf 1998). Body mass data of the experimental treatment groups were analysed for week 0, week 3, week 5, and week 9 (end of the experiment). Due to the difference in food biomass received, we compared the treatments PR, S and C in one group, and treatments S-mix with PR-mix separately. For significance testing, we followed the advice of several statisticians who argue that for living systems, the significance level of $P<0.1$ should be considered acceptable (Sokal & Rohlf 1998).

RESULTS

Effects of prey quality on the survivorship of *P. amentata*

After 2 weeks all the spiders were alive but after 9 weeks (end of the experiment) the survivorship ranged from 45% (Treatment PR) to 93% (Treatment S-mix; Fig. 1). A test of all survivorship curves revealed a significant overall treatment effect (log-rank test, χ^2=9.49, *d.f.*=4, P=0.05).

There was little mortality during the first 4 weeks (Fig. 1). After this date, control and the mixed prey treatments continued to demonstrate little mortality. Treatment S displayed a gradual mortality, arriving to a 70% survival by week 9. The survivorship curve of spiders in treatment PR showed steeper decline that was nearly linear after week 4, and only 45%

of the animals remained alive by week 9 (Fig. 1).

Comparing the experimental treatments S and PR with the control treatment revealed significantly lower survivorship for spiders given aphids reared on sensitive (log-rank test, Z=1.63, *d.f.* = 59, P=0.0516) or partially resistant wheat (log-rank test, Z=3.51, *d.f.* = 60, P=0.0002). There was no difference in survivorship between the control treatment and the spiders given mixed diets. Neither S-mix treatment and the PR-mix treatment nor treatment S and treatment PR differed from each other.

Effects of prey on the growth of immature *P. amentata*

The mean body mass of the spiders after the first week was almost the same in all treatments (Fig. 2, no statistically significant differences, see Table 1). The mean starting mass values of experimental groups ranged from 0.99 mg (SD = 0.18 mg, N=30) to 1.03 mg (SD = 0.23 mg, N=31). Differences appeared during the experiment, and after 8 weeks, the fresh body mass values ranged from 1.27 mg (SD = 0.28 mg, N=14) to 4.74 mg (SD = 0.69 mg, N=26).

In the treatments S and PR there was only a small increase in the mean body mass from 0.99 mg in week 1 to 1.31 mg (SD = 0.18 mg, N=21) (S) and 1.27 mg (SD = 0.25 mg, N=14) (PR) in week 8 (Fig. 2). Treatment S-mix showed the greatest increase in body mass, from 1.03 mg (SD = 0.23 mg, N=31) in week 1 to 4.74 mg (SD = 0.69 mg, N=28) by week 8. The growth curve in treatment PR-mix was

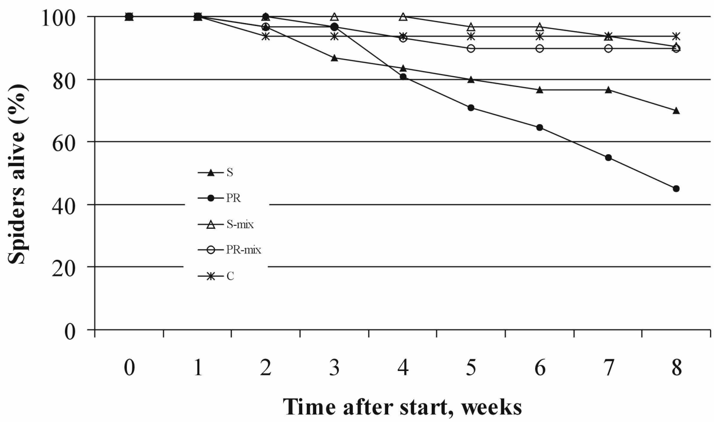

Fig. 1. Survival of *P. amentata* in five treatments with different diets: S (aphids reared on susceptible wheat, cv. Terra), PR (aphids reared on partially resistant wheat, cv. Fold), S-mix (aphids reared on susceptible wheat + fruit flies), PR-mix (aphids reared on partially resistant wheat + fruit flies), C (control, fruit flies).

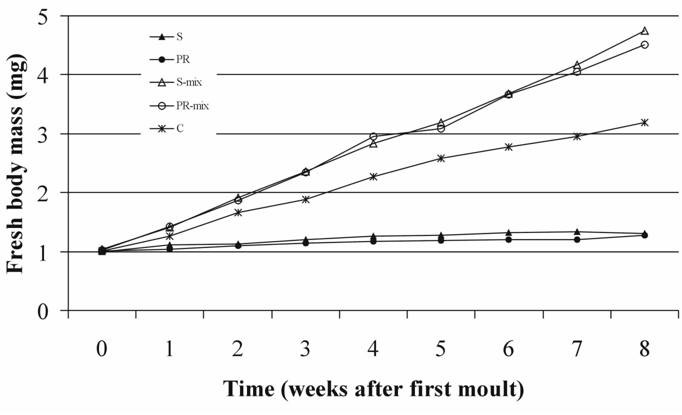

Fig. 2. The growth of immature *P. amentata* on five different diets: S (aphids reared on susceptible wheat, cv. Terra), PR (aphids reared on partially resistant wheat, cv. Fold), S-mix (aphids reared on susceptible wheat + fruit flies), PR-mix (aphids reared on partially resistant wheat + fruit flies), C (control, fruit flies). Data points are means of the spiders in each treatment.

Table 1. Pairwise multiple comparisons of growth curves for *P. amentata* using the GT2-test. Body mass data of the five treatments with different diets: S (aphids reared on susceptible wheat, cv. Terra), PR (aphids reared on partially resistant wheat, cv. Fold), S-mix (aphids reared on susceptible wheat + fruit flies), PR-mix (aphids reared on partially resistant wheat + fruit flies), and control (fruit flies) were analysed for week 0, week 3, week 5, and week 8 (end of the experiment). Sample sizes for the test were: Week 0: S (N=30), R (N=31), S-mix (N=30), PR-mix (N=29), C (N=31). Week 3: S (N=26), R (N=30), S-mix (N=30), PR-mix (N=28), C (N=29). Week 5: S (N=24), PR (N=22), S-mix (N=30), PR-mix (N=27), C (N=29). Week 8: S (N=23), PR (N=17), S-mix (N=29), PR-mix (N=27), C (N=29). ** P<0.05

	GT2-values for the weeks analysed			
Comparison	Week 0	Week 3	Week 5	Week 8
Control *vs*. S	0.259	6.675**	12.725**	13.813**
Control *vs*. PR	0.353	7.555**	13.274**	12.844**
PR *vs*. S	0.353	0.615	0.818	0.209
S-mix *vs*. PR-mix	0.054	0.137	1.461	1.793

similar to treatment S-mix and the mean body mass was 4.51 mg (SD = 0.60 mg, N=26) after 8 weeks (Fig.2). The body mass of spiderlings in the control gradually increased from 1.00 mg (SD = 0.18 mg, N=31) in week 1 to 3.19 mg (SD = 0.35 mg, N=29) in week 8.

In the beginning of the experiment (week 0) there was no significant difference among any of the treatments (Table 1). By week 3, week 5, and week 8 there was still no significant difference between treatment S-mix and treatment PR-mix and between treatment S and treatment PR (Table 1). All other comparisons showed significant differences (Table 1).

DISCUSSION

Single diet effects

This study showed that spiders fed single diets of aphids have a greater mortality and a lower growth than the spiders fed mixed diets. This confirms the results of Toft & Wise (1999a) who obtained similar results on another wolf spider, *Schizocosa* sp.

Survivorship, growth and development of the spiders fed single-diets showed that aphids alone, irrespective of their host plant, were insufficient nutrition for *P. amentata*. Spiders fed aphids from the partially resistant wheat cultivar showed a higher mortality in the second half of the experiment than spiders fed aphids kept on sensitive plants. Aphids from partially resistant plants could be lower quality prey for *P. amentata* than aphids from sensitive plants. As the treatments did not include a starved control, we could not test whether the aphids were toxic prey for the spiderlings.

Apart from mortality, there were other signs showing that single-diets were worse than mixed-diets. None of the spiders fed only aphids developed further than the second nymphal stage while spiders fed mixed-diets reached the fourth nymphal stage (Pedersen & Lövei, unpublished results). The confounding effect here is the difference in prey biomass received by the mixed vs. single diets, so the effect of prey quantity and prey quality could not be separated.

In summary, aphids were low-quality prey for the spiders. Tri-trophic effects were also observed. The survivorship of spiders fed PR-aphids decreased more steeply towards the end of the experiment than that for spiders fed S-aphids. The two types of aphids are likely to have different biochemical compositions and this seemed to influence spider performance. The limited growth of the spiders fed fruit flies might be a result of underfeeding. However, qualitative effects cannot be excluded as Riechert & Harp (1987) reported that spiders were not able to complete their development to reproductive maturity on a diet of only *D. melanogaster*.

Mixed diet effects

The spiders in treatment PR-mix and treatment S-mix showed equal mortality and growth. This indicates that the quality of the prey had less influence when prey was mixed. This confirms the results of Toft (1995) who found that the cereal aphid *Rhopalosiphum padi* was low quality prey when offered as single-diet but had synergistic effects when was part of a mixed-diet with fruit flies. Toft (1995) applied *ad libitum* feeding. Our results indicated that the effect may exist even under limited food conditions.

Suitability of *P. amentata* and *S. avenae* as model organisms for environmental impact assessment of GM-plants

Although there were no large differences between most spider parameters measured with respect to the origin of aphids, spiders demonstrated sensitivity towards prey quality. Aphids are also a sub-optimal prey, but spiders will accept them and aversion to sub-optimal prey can be of short duration (Toft & Wise 1999b). The spiders were sensitive to the quality of prey and this was expressed in both mortality and growth rate. This speaks in favour of the use of *P. amentata* or, in a different region, an appropriate relative in future environmental impact assessment of GM-plants before field release. The results of this experiment also showed the importance of including limited and mixed diet in the environmental impact assessment experiments. The limited feeding and mixed diets are more realistic conditions when dealing with generalist predators. The " ecological realism" of experiments performed in laboratory and glasshouse is always a crucial consideration when trying to "scale up" the results obtained at smaller scales. Setting up experiments with a higher level of such realism gives us a better chance to forecast what the possible effects of GM-plants will be in the field.

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