

Manipulating biodiversity in arable farming for better pest suppression: which species and what scale?

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Field margins can attract and conserve predators and parasitoids, and may thus contribute to pest suppression. On 20 hectares of organic farming, a network of field margins has been laid down to investigate three main questions: 1. Does an increased biodiversity in a farming system contribute to the suppression of pest populations? 2. How far can field margins be apart? 3. What vegetation diversity is required in order to achieve pest populations suppression? Pitfall traps and yellow water pans in field margins and crops are used to assess antagonists' densities. Samples of key pests and damage assessments in different crops are related to the distance from the nearest margins. The first results out of these massive data sets are presented. In both wheat and potato, aphid incidence appeared unrelated to the distance from the nearest crop edge. However, aphid densities were much lower in the BIOdivers subsystem with field margins compared to the control system. A similar pattern was observed for carrot fly (*Psila rosae*; Diptera, Psilidae) damage in carrots. This reoccurring pattern suggests that in the field margin network of the BIOdivers system, the total assemblage of (generalists and specialists) predators and parasitoids exert a degree of pest suppression in several crops, over distances of at least 50 m.

Keywords: field margins, functional biodiversity, biological control, farming systems, Carabidae, spatial variation

During the last decades, biodiversity in agricultural landscapes in Western Europe has declined considerably. Road verges, watercourses and field margins have become the dominant refugia for biodiversity in agricultural landscapes. Consequently, the role of field margins in the conservation of plants, birds, mammals, butterflies and other groups has received a lot of attention (*e.g.* Boatman, 1994; Boatman *et al.*, 1999; Tamis *et al.*, 2001). Among many functions (Marshall & Moonen, 2002), field margins may play an important role in conserving pollinators, generalist predators and parasitoids, and may contribute to substantial degrees of natural control of agricultural pests in adjacent field crops (*e.g.* Thomas *et al.*, 1992; Meek *et al.*, 2002; Collins *et al.*, 2002).

In the open landscape of the Dutch Noordoostpolder, with very few natural landscape elements, we started a large-scale field experiment to investigate whether field margins can attract and conserve predators and parasitoids, and thus contribute to pest suppression. A network of permanent field margins sown with grass and perennials has been laid down on an organic farm to investigate three main questions: 1. Does an increased biodiversity in a farming system contribute to the suppression of pest populations? 2. What is the optimal or maximal distance between field margins? 3. What vegetation diversity is required in order to achieve pest populations suppression?

MATERIAL AND METHODS

At an experimental farm in Nagele, an organic farming system with a 6-year crop rotation of potato, summer wheat, iceberg lettuce, carrots, white cabbage and grass-clover is being studied. On a 10-hectare subsystem, hence called 'BIOdivers', a network of permanent field margins sown with different grass and wild flower mixtures has been laid down in spring 2001. Field margins were laid down in such a way that six fields were created (one for each crop) as follows (also see Fig. 1A): two large fields of 110 x 130 m, another two large fields which are subdivided each by field margins into four smaller plots of 50 x 60m, and the last two fields that are divided each by field margins into eight smaller plots of 36 x 50 m. Field margin width was varied in such a way that the surface ratio of plot versus surrounding margin remains constant. The East-West axis represents the network intensity gradient, and should clarify the maximum distance over which arthropod predators and parasitoids significantly reduce pest population densities. Field margins were sown and managed in such a way that a vegetation diversity gradient was created along the

North-South axis (Fig. 1A). This gradient is used to clarify how diverse field margins should be in order to host sufficient antagonists to reduce pest population densities. Theoretically, a biodiversity gradient towards the Northeast corner of the system will develop over the years, and insight will be gained in the optimal combination of margin heterogeneity and network intensity for pest suppression.

A second subsystem of 10 hectares (called 'BIOintensief') (Fig. 1B) has six large parcels (one for each crop) and has three narrow field margins along the top, middle and bottom of the subsystem, in East-West direction. This system serves as a reference ('control') system, in order to correct for background infestation levels of mobile (airborne) pest densities. Since both systems are not replicated, effects will be analysed with crops and years as variables.

Pitfall traps and yellow water pans, in use from mid-May until mid-October, were used to collect samples of the natural enemies in the systems. Pitfall sampling took place by sampling 80 locations distributed over both systems (see Fig. 3 for indication of locations) (three pitfalls per location, 5 m apart, combined into one sample). A total of 30 yellow water pans were distributed over both systems, but trapping results were very poor and will not be discussed in this paper. Pitfalls were filled with a 5% formaldehyde solution and emptied every 14 days. Catches were stored in 70% ethyl-alcohol at 5°C and were sorted and counted in the laboratory into functional groups (mostly at the order or family level).

Key pests, such as aphids (Homoptera, Aphididae), caterpillars (Lepidoptera, several families), root flies (Diptera, Psilidae), leaf beetles (Coleoptera, Chrysomelidae) and slugs (Mollusca, several families) were selected for each crop, and optimal sampling periods and methods were chosen for each pest. In 2002, pest densities (numbers), incidence (presence/absence per plant or shoot) or damage levels were assessed at 2, 15 and 50 m into the crop from the nearest field margin or crop edge.

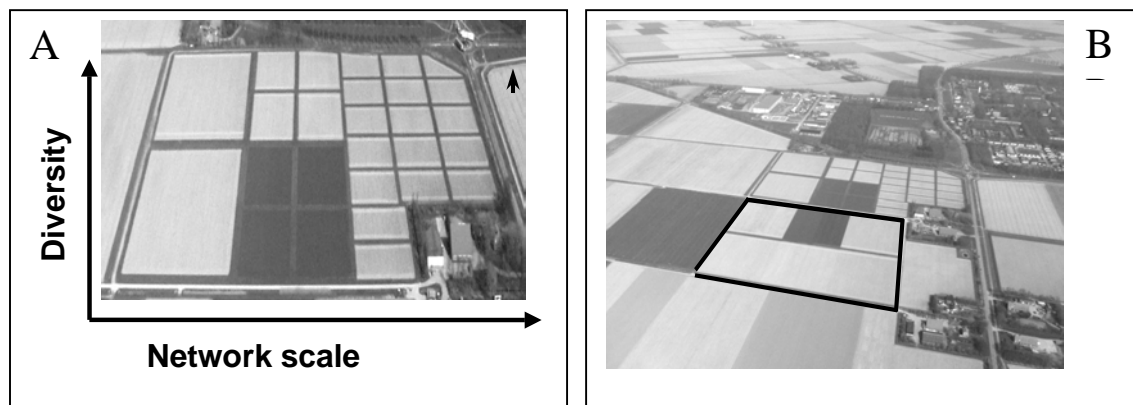


Figure 1. Aerial view of the experimental systems at Nagele. A. The BIOdivers subsystem with the network of field margins. B. The control system without a margins network (the black rectangle). Each subsystem is approx. 10 ha in surface; the top of the photographs is facing North.

RESULTS

To this date (December 2002), about 40% of the pitfall samples of the 2001 season have been sorted and counted (62800 arthropods sorted). Spiders (Araneae; predominantly dwarf spiders, Erigonidae) were the dominant group with 30400 specimens (48% of total catch). Carabid beetles (Coleoptera, Carabidae) were presented by 15900 individuals (25%) and rove beetles (Coleoptera, Staphylinidae) constituted 7900 (13%) of the catches. The pitfall samples of the 2002 season will be sorted and counted in January 2003, and could therefore not be included in this paper. Data of the pitfall traps presented here fall into two groups. The first group presents results from three sampling locations (in a white cabbage plot, in a field margin between the white cabbage field and

the carrot field, and in a grass-clover field) in the BIODivers subsystem, that have been sorted for the whole 2001 sampling season (time series from mid-May until mid-October 2001). The second group represents two sampling periods (week 20-22, *i.e.* second half of May, and week 30-32, *i.e.* late July and early August 2001) in which all traps in the system were sorted (spatial overviews).

To illustrate the first findings in this experiment, we present the trapping results for carabid beetles (Fig. 2A) and spiders (Fig. 2B) as a time series throughout the 2001 sampling season (May-October) from three locations in the BIODivers subsystem. Carabid beetles (Fig. 2A) seemed to prefer the open plots of white cabbage above the densely grown grass-clover plots and grassy field margins. In contrast, spiders (Fig. 2B) were more abundant in the field margins and grass-clover plot than in white cabbage field.

An example of the spatial variation in the systems is given in Fig. 3, for carabid beetles caught in August 2001. Highest carabid densities were found in the plot of white cabbage of the BIODivers system (Fig. 3A, lower left corner).

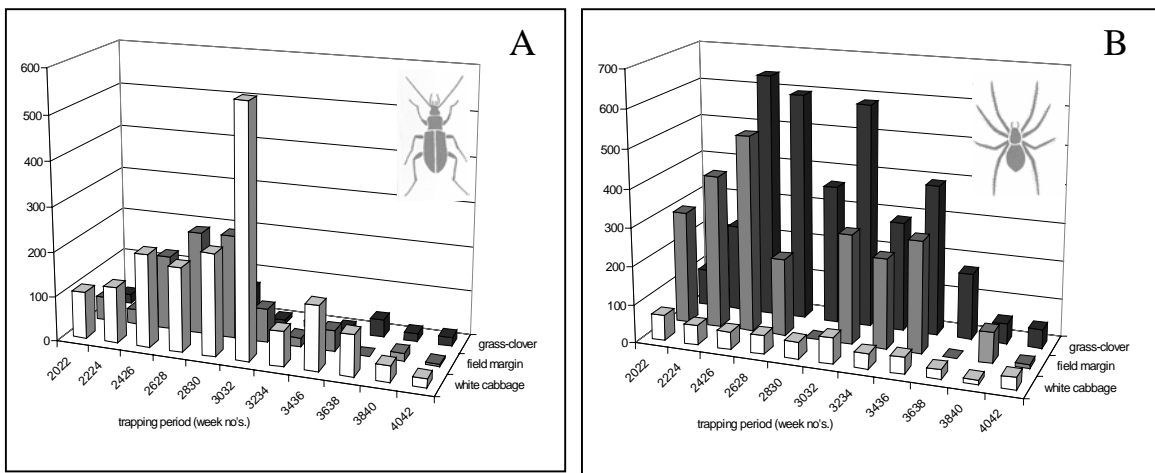


Figure 2. Results from 3 pitfall traps in the BIODivers subsystem (+ margins) during the 2001 trapping season (11 biweekly samples from week 20, early May, until week 43, end October). A: Carabid beetles. B: Spiders.

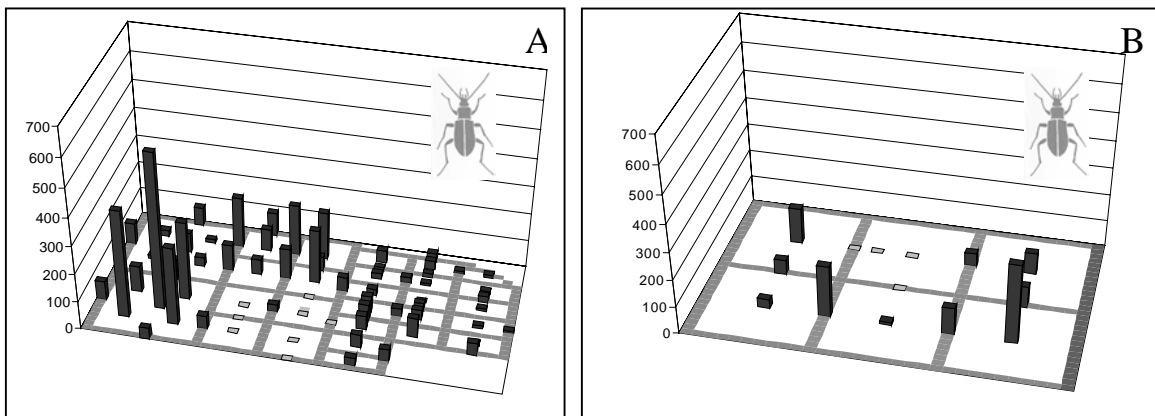


Figure 3. The numbers of carabid beetles caught in each pitfall trap in week no. 30-32, August 2001. A: in the BIODivers subsystem (+ margins) and B: in the BIOintensief subsystem (no margins).

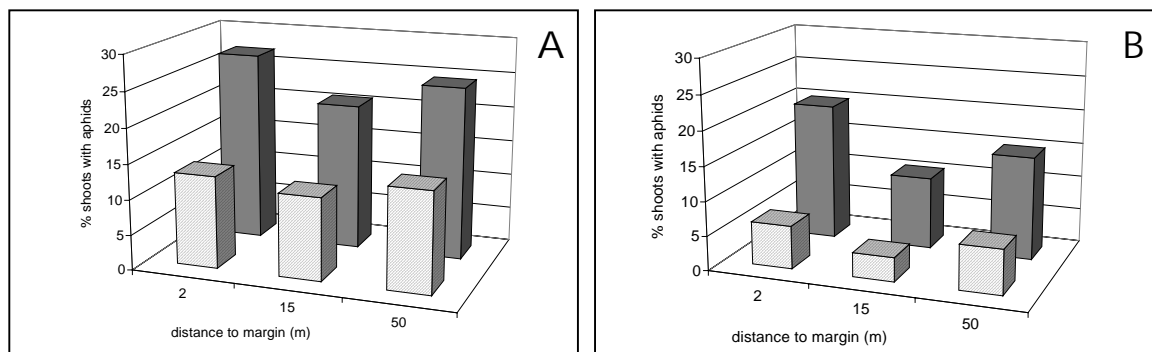


Figure 4. Percentages of shoots infested by aphids at different locations within plots in relation to the distance to the nearest crop edge. Hatched columns: in the BIOdivers subsystem (+ margins); filled grey columns: in the BIOintensief subsystem (no margins). A: in summer wheat, June 2002 and B: in potatoes, July 2002.

Sampling of key pests revealed lower levels of leaf-feeding beetle (*Lema* sp.; Coleoptera, Chrysomelidae) damage along the margins of summer wheat, compared to the centre of large plots (data not shown). In contrast, in white cabbage and Brussels sprouts, the density of Diamond-back moth (*Plutella xylostella*; Lepidoptera, Plutellidae) appeared highest along field edges (data not shown).

In summer wheat, aphid incidence in June 2002 appeared unrelated to the distance to the nearest crop edge. However, aphid densities were much lower in the BIOdivers subsystem than in the BIOintensief system (Fig. 4A). A similar pattern was also observed for (other) aphids in potato in July (Fig. 4B) and for carrot fly (*Psila rosae*; Diptera, Psilidae) damage in carrots in October 2002 (data not shown).

DISCUSSION

The data presented here provide only the first glimpses of what is going on in this complex system. More time and effort, more seasons and further processing and analyses of data are required to answer our research questions. The 2001 season yielded approx. 150,000 arthropods in the pitfall traps alone, about 50% of them belonging to the spiders, 25% to the carabid beetles and 13% to the rove beetles. So far, only 40% of the 2001 samples have been sorted. Numbers (as an indication of activity) of these generalist predators vary widely in time and space, representing seasonal phenology but also distinct habitat preferences (Figs. 2A, 2B and 3A). Habitat preference may be influenced by vegetation structure, microclimate, prey abundance, agricultural activities, and other factors. The pitfall samples of the 2002 season will be sorted and counted in January 2003 and will improve our insight in the spatial and temporal dynamics of the functional groups in our experimental system.

In 2002 key pest populations were sampled for the first time in every crop. For only a few pests, density gradients in relation to the field margins were observed. Damage of leaf-feeding beetles (*Lema* sp.) was lower along the margins of summer wheat, compared to the centre of large plots (data not shown). This suggests pest suppression by predators (with a limited mobility) from the field margins. In contrast, in white cabbage and Brussels sprouts, the density of Diamond back moth (*P. xylostella*) appeared highest along field edges (results not shown).

An interesting pattern of aphid densities (for the total species complex) was found in summer wheat and potato. In both crops, aphid incidence appeared unrelated to the distance from the nearest crop edge. However, aphid densities were much lower in the BIOdivers subsystem compared to the BIOintensief system (Fig. 4A and B). A similar pattern was observed for carrot fly (*P. rosae*) damage in carrots. This reoccurring pattern suggests that in the field margin network of the BIOdivers system, the total assemblage of (generalists and specialists) predators and parasitoids exert a degree of pest suppression in several crops, over distances of at least 50 m.

Since most key pests were sampled only once or twice in the 2002 season and data of the antagonists monitoring are not yet available, causal relationships and underlying mechanisms cannot be analysed at present. In the 2003 season, pest sampling will be focussed on two or three key pests and crops only, and carried out with a (bi)weekly frequency, in order to get a better insight in population dynamics and rates of predation and parasitism. Possibly, exclusion experiments with barriers and cages to exclude antagonists at different distances from field margins, can be carried out to analyse the effects of pest suppression and the contributions of antagonists from the field margins. Marking and release experiments could demonstrate the dispersal distances of predators, and whether field margins act as sources or sinks for antagonists in crops. The BIODivers field margin system thus offers great opportunities to study different hypotheses with regard to functional biodiversity in agricultural systems. We hope to set up co-operation with other research groups in order to tackle several of these interesting questions.

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Insect diversity on yellow Asteraceae in road verges in the Netherlands

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Yellow Asteraceae, which are very common in Dutch road verges, are known to attract many insect species. Insect visitors on three most common yellow Asteraceae flowers (*Hieracium laevigatum*, *Hypochaeris radicata* and *Leontodon autumnalis*) were studied. Over 80% of the insect species (mostly syrphids, and also some oligolectic bee species) visited at least one of those three plant species. Insect diversity (Shannon index) appeared to be positively related to flower diversity. In a flower constancy experiment *Eristalis tenax* discriminated flowers of two of the three plant species, presumably because those flowers differ in diameter size or UV-reflection.

Keywords: insect diversity, plant diversity, yellow Asteraceae, flower constancy

In agricultural landscapes many flowering plants can only survive in linear (habitat) fragments, like road verges and ditch banks. Besides their function as a refuge for plant species, road verges may also play an important role as corridor between nature areas, for both plants and animals. The management of those sites, which is often done by local farmers, can affect insect diversity, both directly and indirectly via plant diversity. Plant species flowering at the same time and place may interact for pollinator visits in a variety of ways: plants can have a facilitative function by attracting more pollinators (Rathcke, 1983). On the other hand, competition among flowers for pollinators is possible (Thomson, 1978; Kwak *et al.* 1998; Chittka & Schürkens, 2001).

The family of the Asteraceae is one of the largest families of flowering plants, with 25,000 species over the world (Proctor *et al.*, 1996). Asteraceae grow mostly in sunny (micro-)habitats. A relatively large number of Asteraceae can be found in grasslands and road verges, especially on dry to moderately wet habitats (Weeda *et al.*, 1988). The attractiveness of flowers for insects depends on floral traits, such as volume and sugar composition of nectar, and corolla tube length of flowers (Torres & Galetto, 2002). Most entomophilous plants are visited by a large variety of insects (Proctor *et al.*, 1996). Yellow Asteraceae have flowers in which nectar and pollen are easily accessible, so many insect species can visit these plant species. Insect visitors can be divided into two groups: the generalists (polylectic species) and the specialists (mono- and oligolectic species). Specialist insects can be found among the bees: almost one-third (76 out of 224) of bee species in the Netherlands are oligolectic (Peeters *et al.* 1999). Thirteen of the oligolectic bee species are specialists on Asteraceae.

In this paper we present data on the insect diversity on three yellow Asteraceae common in Dutch road verges: *Hieracium laevigatum*, *Hypochaeris radicata* and *Leontodon autumnalis*. The relation between insect and flower diversity was investigated. In addition we examined if insects can discriminate between flowers of different yellow Asteraceae species.

MATERIALS AND METHODS

Study area

The study was performed in the North of The Netherlands, near Assen. In the Drentsche Aa area five sites (road verges and ditch banks), near Bovensmilde four sites and near Ekehaar one site were selected.

Flower and insect diversity: transect observations

Flowering plants and insects were counted in transects of 100 by 1-1.5 meters at each site. Flowers of yellow Asteraceae appeared to close during the day, depending on site, date and species between 13.00 and 15.00 h. Therefore transect observations were carried out between 9.00 to 13.00 h.

Numbers of flowers of all flowering plant species interesting for insects were recorded one to three times at all sites. Depending on the plant species, single flowers or flowering units were counted (*e.g.* in Asteraceae the number of heads). Flower units are units that a medium-sized bee has to fly, rather than walk, between (following Dicks *et al.*, 2002). All flower-visiting insects in combination with the plant species were recorded one to five times at all sites, walking at a slow speed.

Plant species were identified using Van der Meijden (1995), if there was uncertainty about a species. Insects were recorded at species level as far as possible. When uncertainty about a certain species existed, individual insects were caught. Hoverflies were identified in the field, using Reemer (2000); bees (Apidae) were labelled for later identification. All insects longer than 3 mm were included. Smaller insects are easy to miss, they can be extremely difficult to identify while walking a transect, but first of all they cannot be comprehensively collected without disturbing insect-flower interactions (Dicks *et al.*, 2002). Insects were grouped in orders: Syrphidae, Diptera rest, Apidae and Lepidoptera.

Transect observation data of flowers and insects were used to calculate diversity, with the Shannon index of diversity (Magurran, 1991). This formula incorporates number of species as well as a measure of abundance. The relation between the diversity of insects and plants was investigated.

Flower constancy experiment

Heads of two of the three target species were experimentally arranged in a patch. All (three) combinations of two target species were examined. Test-tubes filled with water attached to wooden sticks were placed in the ground, in a way that the distances between each flower is equal, *i.e.* 10 cm. Therefore the plot had a total dimension of 40 by 35 cm. Visitation of individual insects was recorded and note was taken on changes between flowers. Observations of individual insects were summed for analysis, with a minimum of 15 transitions per insect species.

We used the following measure for flower constancy: the number of intraspecific transitions per plant species divided by the total number of transitions. This constancy has a range from 0 to 1. Insects can only show flower constancy if they can distinguish flowers of different species in a certain way. Therefore flower diameter size was measured of thirty flowering heads of each target species.

Statistics

The relation between insect diversity and flower diversity was analysed using linear regression. Possible differences between numbers of transitions in the flower constancy experiment were analysed with a χ^2 goodness of fit test.

RESULTS

Flower and insect diversity: transect observations

In total, 55 flowering species were recorded, of which 6 species of yellow Asteraceae. The number of flowering plant species varied from 4 to 20 species per transect, depending on site and date. The number of flowering units varied from 144 to 3187 per site. Depending on month and site, yellow Asteraceae flowers can make up to even 96% of all the flowers. The plant diversity, calculated with the Shannon index (H), varied from 0.48 to 1.96.

In total, 48 insect species were recorded. The number of insects species varied from 2 to 22 species per transect; the total number of insects varied from 3 to 79 individuals per transect. The insect diversity varied from 0.64 to 2.54. The number of insects, as well as the diversity declined in the season ($R^2=0.16$, $p=0.01$ and $R^2=0.39$, $p<0.001$, respectively). The insect diversity was positively correlated with the flower diversity ($R^2=0.24$; $p=0.02$) (Fig. 1).

The three target plant species were visited by many different insect species (Fig. 2), but mostly by Syrphidae species. We observed at least three oligolectic bee species specialised on yellow Asteraceae. Over 80% of all insects were observed to visit at least one of the three target plant species.

Flower constancy of insects: an experiment

The flowering heads in the experimental arrangement were visited by a range of different insects. However, only 3 out of 19 visiting insect species made more than 15 transitions. *Eristalis tenax* showed a significant flower constancy for *H. laevigatum*, when it had a choice between *H. laevigatum* and *L. autumnalis* ($\chi^2=5.18$; $df=1$; $p<0.05$). When *E. tenax* could choose between *L. autumnalis* and *H. radicata* it preferred the first one ($\chi^2=20.38$; $df=1$; $p<0.05$) (Table 1). Other insect tested showed no significant discrimination behaviour.

Table 1. Number of visited flowers per offered combination and flower constancy for three insect species.

Insect species	flower combination	# visited flowers	sign.	flower constancy		sign.
<i>Eristalis tenax</i>	HL-HR	12-19	ns	0.04	0.23	ns
<i>E. tenax</i>	HR-LA	1-15	<0.05	0	0.85	<0.05
<i>E. tenax</i>	HL-LA	55-36	<0.05	0.33	0.14	<0.05
<i>Sphaerophoria</i> sp.	HL-HR	13-11	ns	0.25	0.13	ns
<i>Panurgus banksianus</i>	HR-LA	10-13	ns	0.05	0.14	ns

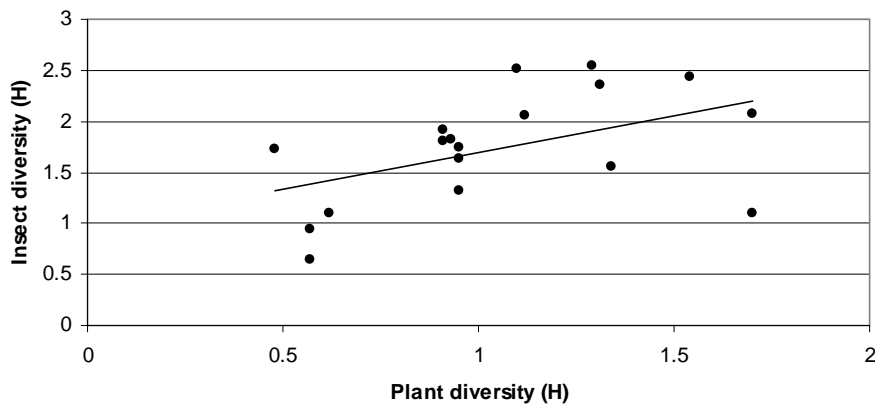


Figure 1. Insect diversity (Shannon index) as a function of flower diversity and regression line ($y = 0.73x + 0.97$; $R^2 = 0.24$; $N = 18$; $p = 0.02$)

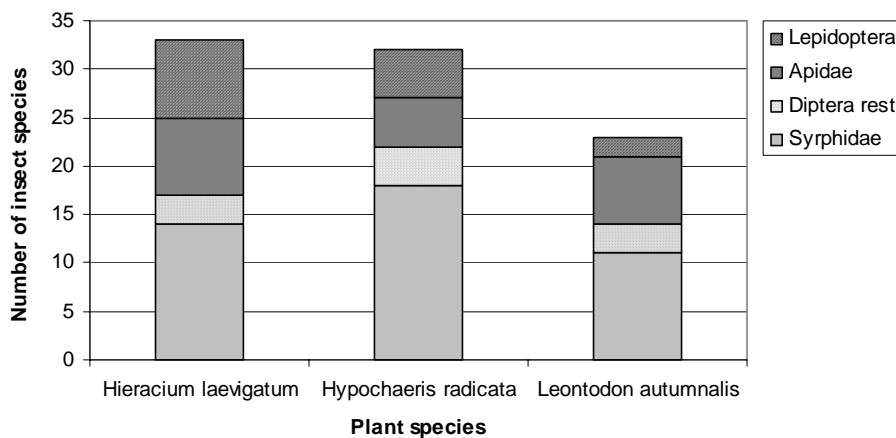


Figure 2. Number of insects species on three common yellow Asteraceae species.

DISCUSSION

Plant diversity differed between sites. Overall, insect and plant diversity were positively related. Also insect diversity differed largely between sites and decreased during the season. Part of the variation can be explained by the mowing regime. After mowing it took some time before plants flowered again.

Yellow Asteraceae are dominant flowering species in road verges. A high percentage (over 80%) of the insect species observed during transect observations were recorded at least on one of the three common yellow Asteraceae species in road verges. The three investigated plant species did not differ in insect species composition. Syrphidae were the largest visitor group, however, they were also frequently seen on other plant species like *Valeriana officinalis*. Specialist bees have no alternative but to forage on yellow Asteraceae.

Eristalis tenax appeared to be the only insect species to show a significant flower constancy. Flower constancy of syrphids has rarely been shown (Goulson & Wright, 1997). It can be said that *E. tenax* is able to discriminate between the three target plant species, although not when the combination *H. laevigatum*-*H. radicata* was offered. Except for the diameter of the flowers, which was smaller for *L. autumnalis* than for the other two plant species, insects can also discriminate between flowers due to their difference in UV-reflection. The yellow heads of *H. laevigatum* do reflect UV radiation, whereas the heads of *L. autumnalis* do not (L. Chittka, pers. comm.). No data on the UV-reflection of the third species, *H. laevigatum* were found, but since *E. tenax* did not discriminate between flowers of this species and flowers of *H. radicata* we presume that *H. laevigatum* does also not reflect UV radiation.

Other insect species did not choose one of the two target plant species over the other. However, this can also mean that those plant species had the same reward at that moment, which means that there was no reason to discriminate between the flowers.

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Malathion Resistance in *Drosophila melanogaster* (Diptera: Drosophilidae)

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Malathion resistance developed in a *Drosophila melanogaster* population that has been exposed to malathion for almost 20 years. Genetic analyses showed that malathion resistance in this population is under control of several genes that are located on the first, second and third chromosome. The second chromosome contributes the most to resistance; the factor(s) involved may be (partly) dominant or additive and additional recessive genes may also be present on this chromosome. Although to a lesser extent, the third chromosome also contributes to resistance, the factors being mostly recessive or only expressed in interaction with the second chromosome. On the X-chromosome, a factor with a very small effect on resistance was observed.

Keywords: *Drosophila melanogaster*, insecticide resistance, malathion, localisation

During the second half of the last century, synthetic insecticides were intensively used to control pest insects (Casida & Quistad, 1998). However, in many cases the treated pest insects rapidly developed resistance to the applied insecticides. Consequently, development of resistance has become a significant problem for pest management in agriculture. Understanding the evolution and genetic basis of resistance, therefore, is of utmost importance in this applied field.

The general aim of our project is to determine the genes involved in insecticide resistance in *Drosophila melanogaster* populations that for a long period (nearly 20 years) have been treated with insecticides and particularly to study the possible evolution of modifier genes that decrease the cost of resistance. Before we can study the evolution of modifiers, we first have to locate the primary genes responsible for resistance. This is the aim of this present study, where we focus especially on malathion resistance. The organophosphate malathion was applied in 1952 for the first time (Casida & Quistad, 1998). With other organophosphates, such as trichlorfon and dimethoate, malathion belongs to the aliphatic derivatives, which are compounds with straight carbon chains (Pedigo, 1989). In *D. melanogaster*, previous studies have indicated that malathion resistance is under polygenic control (e.g. Halpern & Morton, 1987; Houpt *et al.*, 1988). In these studies, genetic factors affecting resistance were observed on both the second and third chromosome of *Drosophila*.

Here, we report the chromosomal localisation of genetic factors involved in malathion resistance by means of visible genetic markers and balancer chromosomes (King & Sømme, 1958; Hartl & Clark, 1997).

MATERIAL AND METHODS

The *Drosophila melanogaster* populations

From the base population '50 x 50' (see Bijlsma & van Delden, 1977, for description) two replicated cage populations were started in 1983. One of the offshoots (designated by **R** throughout the paper) was initially reared on food supplemented with 0.15 ppm malathion. This concentration was gradually increased until 1987, when a final concentration of 0.9 ppm was reached. From then on, the **R** population has been continuously reared on 0.9 ppm malathion. In 1987, the **R** population had already become highly resistant to malathion (R. Bijlsma & A.C. Boerema, unpublished). The other offshoot was maintained on normal medium and therefore likely to remain susceptible (designated by **S**) to malathion. As such, the latter population served as control.

The *Drosophila* populations (population size about 2000-5000 flies) have been reared in population cages (for description and construction of the cages see Beardmore *et al.* (1963)) at 25°C and 50-70% RH. In a rotating scheme, every week 5 of the 15 food cups in the cage were

replaced by fresh food cups containing 40 ml of standard medium consisting of 26 g dead yeast, 54 g sucrose, 17 g agar and 13 ml Nipagin solution (10 g Nipagin in 100 ml 96% ethanol) per 1000 ml water. For the population treated with malathion, malathion was added to the standard medium to a final concentration of 0.9 ppm. In all experiments, flies were cultured in an incubator room at 25°C and 50-70% RH.

Resistance measure

Malathion resistance was measured by determining egg-to-adult survival (viability) on normal (control) and malathion-supplemented medium. For each malathion concentration, 10 vials (10 cm high, Ø 1.5 cm, containing 8 ml medium) were established with 100 eggs each. All emerging flies from these vials were collected and the number of adults was determined.

RESULTS AND CONCLUSIONS

Sex-linkage and dominance of the factor(s)

Sex-linkage was investigated by assessing differences in viability between the sexes among the offspring from the crosses of **R** females with **S** males, cross **R** x **S**, and the reciprocal cross **S** females with **R** males, **S** x **R** (Fig. 1). The mean observed sex ratios were tested for deviation from the expected sex-ratio of 1 with a t-test. Table 1 shows no significant deviation from sex ratio = 1 for the **S** x **R** cross on all concentrations ($P > 0.05$). However, for the **R** x **S** cross, on the two highest malathion concentrations tested, significantly more males survived (0.9 ppm: $P < 0.02$; 1.5 ppm: $P < 0.001$). The males from the **R** x **S** cross received their X-chromosome from their resistant mother, whereas the **S** x **R** males received their X-chromosome from their susceptible mother. No differences in chromosomal composition of the females were present. Since only the number of males in the F_1 of the **R** x **S** cross is different on higher malathion concentrations, a minor genetic factor affecting malathion resistance is located on the X-chromosome.

Comparing the viability of the above crosses to that of the parental susceptible (**S** x **S**) and resistant (**R** x **R**) stock, enables us to get some information of the degree of dominance of the resistance factors involved. Figure 2 shows that on 1.5 ppm malathion the resistant population still survives nearly as well as on control medium, whereas the susceptible population already suffers greatly from a low concentration of 0.5 ppm malathion. This clearly shows that the long-term exposure to malathion leads readily to resistance for this insecticide. Although on control medium the resistant population shows a significantly lower viability ($P < 0.001$) than the susceptible population, it survives much better on increasing concentrations of malathion than the heterozygous offspring of the crosses **S** x **R** and **R** x **S**. On 0.9 and 1.5 ppm, the survival of the reciprocal crosses **S** x **R** and **R** x **S** was similar, but it was highly significantly lower than that of the resistant (**R** x **R**) parental stock. The fact that the resistance of reciprocal crosses is somewhere in between that of the parental stocks indicates that the factors involved in malathion resistance are not completely dominant nor completely recessive. This may mean that either most factors involved act in an additive fashion or that part of the genes involved are (partly) dominant while others are (partly) recessive.

Table 1. The mean (\pm s.e.) sex-ratios ($\text{♀} : \text{♂}$) of the crosses **S** x **R** and **R** x **S**. * = Significant deviation from sex-ratio = 1, tested with a t-test ($P < 0.05$).

Malathion (ppm)	S x R	R x S
0	0.86 \pm 0.063	1.02 \pm 0.100
0.5	1.09 \pm 0.053	1.13 \pm 0.175
0.9	1.58 \pm 0.454	0.63 \pm 0.085*
1.5	0.68 \pm 0.143	0.43 \pm 0.095*

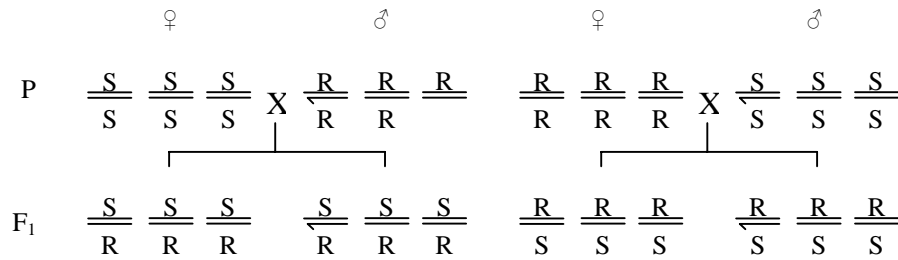


Figure 1. Crossing schemes to determine sex-linkage and dominance of the resistance factor(s). R designates chromosomes of the resistant strain, S those of the susceptible strain.

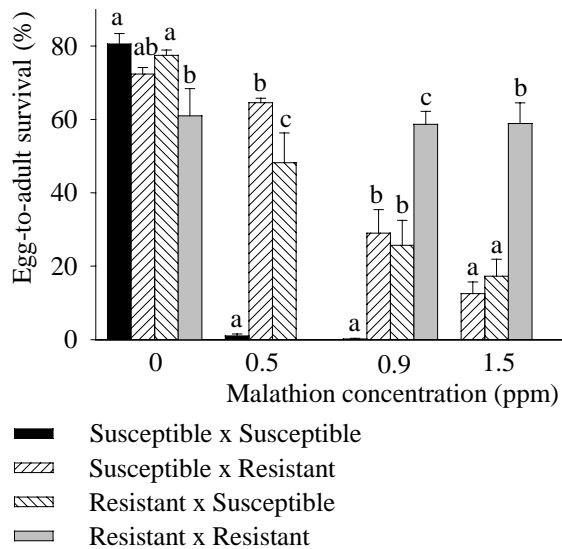


Figure 2. Mean egg-to-adult survival (+ s.e.) of the two reciprocal crosses and the two parental strains on different malathion concentrations to determine the degree of dominance of the resistance factor(s). The survival of Resistant strain (Resistant x Resistant) was not tested on 0.5 ppm. The survival of Susceptible strain (Susceptible x Susceptible) was not tested on 1.5 ppm. For each concentration different letters denote significant differences in mean survival between the different crosses. The same letter indicates no significant difference in survival on that concentration.

Dominant autosomal factor(s)

To determine the presence of dominant factors (collectively denoted by 'R' in Fig. 3) involved in malathion resistance on chromosome 2 and 3 (the small fourth chromosome of *D. melanogaster* was ignored), we crossed the **R** with the recessive *bw//bw;st//st* eye-colour marker strain (*bw*, brown eyes, and *st*, scarlet eyes, are located on chromosome 2 and 3, respectively). As depicted in Fig. 3, females of the (susceptible) marker strain were crossed with malathion resistant males. Next, F₁ males were backcrossed with *bw//bw;st//st* females. Egg-to-adult survival of the F₂ was determined for 3 malathion concentrations.

The survival of the four possible genotypes, that in the absence of malathion are expected in the ratio 1:1:1:1, is shown in Fig. 4. In our experimental design this means expected numbers of 25 for each genotype. Even though on control medium already some significant differences in survival are observed (probably due to the presence of the eye colour chromosomes), the most important finding is that the genotypes *bw//bw;st//st* and *bw//bw;st//R* do not survive on malathion

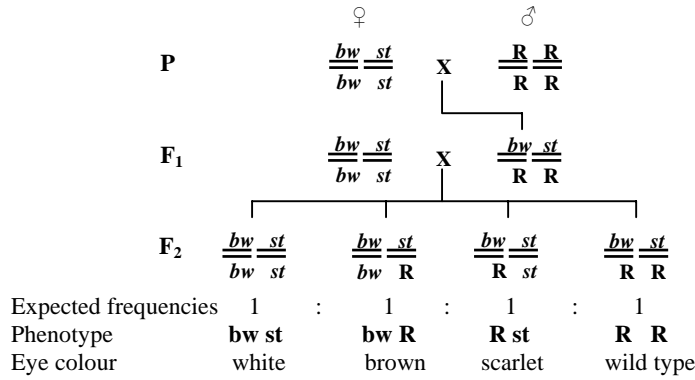


Figure 3. Crossing scheme to investigate the presence of dominant factor(s) on the autosomes by means of recessive markers. *bw*=brown eyes (chromosome 2); *st*=scarlet eyes (chromosome 3); the combination of both markers in homozygous condition produces white eyes.

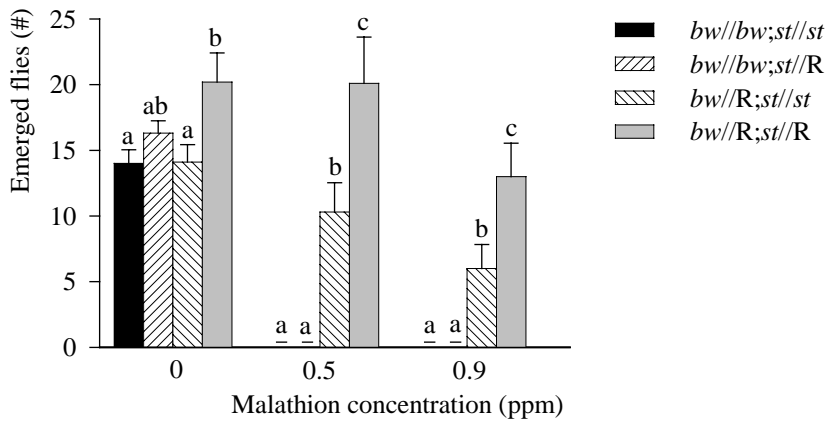


Figure 4. Mean egg-to-adult survival (+ s.e.) for different malathion concentrations. For each concentration, bars marked by the same letter indicate no significant difference in survival between genotypes for that concentration.

at all, whereas the genotypes *bw//R;st//R* and *bw//R;st//st* show high to moderate survival. This indicates that one or more (partly) dominant factors are located on the second chromosome. Moreover, survival of *bw//R;st//R* genotype was significantly higher than of *bw//R;st//st* genotype on both malathion concentrations (tested with an One-Way ANOVA after angular transformation). Thus, the third chromosome seems to harbour factor(s) that increase(s) resistance to malathion only when at least one second chromosome of the resistant strain is also present, indicating a significant genetic interaction between factors on the second and third chromosome.

Recessive autosomal factor(s)

The presence of recessive resistance factors (collectively denoted by ‘r’ in Figs. 5 and 6) was investigated using the dominant marker strain *Cy//Pm;Ser//Sb*. *Curly* and *Plum* are located on chromosome 2, whereas *Serrate* and *Stubble* are located on chromosome 3. Resistant females were crossed to males from the marker strain. F₁ males with the genotype *Pm//+;+//Sb* were backcrossed to resistant females, as shown in Fig. 5. Egg-to-adult survival of the F₂ flies from this cross was determined for different malathion concentrations and, after angular transformation, tested for significant differences with an One-Way ANOVA. In the absence of malathion, the four different genotypes from this cross are expected to be present in a 1:1:1:1 ratio.

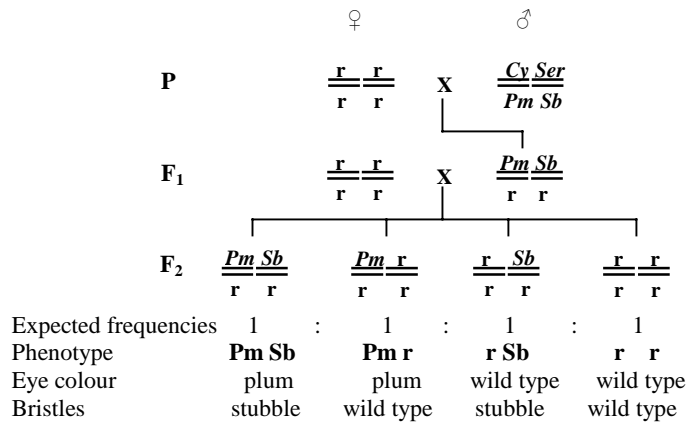


Figure 5. Crossing scheme to investigate the presence of recessive factor(s) on the autosomes by means of dominant markers. *Pm*=plum eyes (chromosome 2); *Sb*=stubbled bristles (chromosome 3). *Cy*=Curly wings (chromosome 2); *Ser*=Serrated wings (chromosome 3)

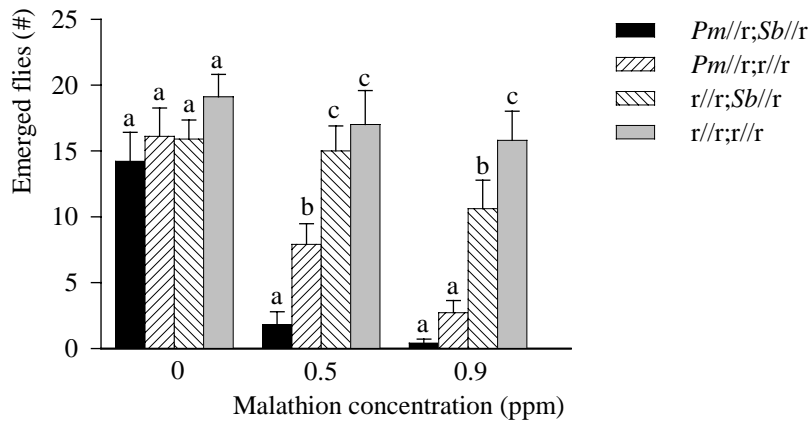


Figure 6. Mean egg-to-adult survival (+ s.e.) for different malathion concentrations. For each concentration, bars marked by the same letter indicate no significant difference in survival between genotypes for that concentration.

Figure 6 shows that on control medium the four genotypes are found in the expected ratio, as ANOVA revealed no significant differences ($P=0.344$). On malathion, the survival of the four genotypes was found to be significantly different ($P<0.001$) with *r//Pm;r//Sb* showing the lowest survival. Substituting the non-resistant marker chromosomes for both the second and third chromosome by resistant chromosomes increase survival, indicating that factors on both these chromosomes contribute to malathion resistance. The effect of the second chromosome seems to be stronger than that of the third chromosome, corroborating the observation that the second chromosome carries a factor improving resistance in heterozygous condition, as shown in the previous section.

General Conclusions

Several genetic factors contribute to malathion resistance in this *D. melanogaster* population, confirming earlier findings that malathion resistance is polygenically determined (Halpern & Morton, 1987; Houpt *et al.*, 1988). The X-chromosome, the second as well as the third chromosome contribute to malathion resistance, but clearly the second chromosome contributes

the most. This is in agreement with the localisation of several genes that have been reported previously to be involved in malathion resistance. Hout *et al.* (1988) suggested that two cytochrome P450 genes (one on the second and one on the third chromosome) are involved in resistance. Also other studies suggest involvement of cytochrome P450's in malathion resistance (references in Wilson, 2001). Other genes associated with malathion resistance are glutathione-S-transferases, mapped both on the second and third chromosome (Morton, 1993), acetylcholinesterase, located on the third chromosome (Fournier *et al.*, 1989) and glucose-6-phosphate dehydrogenase on the X-chromosome (Morton & Holwerda, 1985). Further research mapping the factors involved in malathion resistance in this population in more detail, within the first, second and third chromosome, will clarify whether these 'candidate' genes are also responsible for the resistance observed in the present study.

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