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Hydroxamic acids in wheat: antibiosis, antixenosis and effects upon aphid susceptibility to an insecticide

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Summary

A screen of 47 worldwide cultivars of Triticum (mainly T.aestivum) was conducted in which the concentration of the hydroxamic acid DIMBOA ranged between 1 and 8 mmol/kg fresh wt. When alatae of the aphid Sitobion avenae were released among replicated test seedlings, there were highly significant correlations between aphid antixenosis and DIMBOA levels in the seedlings.

The effects of DIMBOA levels in cultivars of wheat on the antibiosis and tolerance of the aphid Sitobion avenae to the insecticide, deltamethrin were investigated. Over 48 hours the mean relative growth rate was found to differ significantly between nymphs of S. avenae reared on wheat cultivars containing different levels of DIMBOA. Nymphs exposed to higher levels of DIMBOA suffered the greatest reduction in growth rate and showed a significantly greater susceptibility to deltamethrin, particularly at lower doses. The LD50, adjusted for weight, was reduced by 73% for nymphs reared on high DIMBOA seedlings. The value of these results in work leading to the production of aphid-resistant cultivars is discussed.

Introduction

Hydroxamic acids (Hx) are secondary plant chemicals present in Gramineae (Niemeyer, 1991; Copaja, Barría & Niemeyer, 1991a; Barría, Copaja & Niemeyer, 1991; Copaja, Niemeyer & Wratten, 1991b) showing significant deleterious effects on organisms such as fungi, bacteria and insects on cereals (Niemeyer,1988a; Xie, Arnason, Philogène & Lambert, 1990). Information from experiments in which aphids are reared on artificial diets containing DIMBOA, the main Hx in wheat (Niemeyer, Pesel, Franke & Francke, 1989; Niemeyer et al., 1988), and from electronic monitoring of aphid feeding (Argandoña, Corcuera, Niemeyer & Campbell, 1983; Givovich & Niemeyer, 1991), suggests that DIMBOA exerts both toxic and antifeedant effects, with complete inhibition of feeding by the aphid Schizaphis graminum (Rondani) at a concentration of 12mM DIMBOA (Argandoña et al., 1983).

The maximum recorded levels of Hx in cultivated wheat range-

from 1.4 to 10.9 mmol/kg fresh weight (Niemeyer, 1988b; Thackray et al, 1990; Copaja et al., 1991b). Higher levels are found in some wild Triticeae (Copaja et al., 1991a; Barría et al., 1991; Niemeyer et al., 1991), with wild Secale having a maximum concentration of nearly 40mmol/kg fresh wt (Barría, et al., 1991). There is therefore germplasm potentially availiable for breeding programmes aimed at producing cultivars with higher Hx levels.

The use of host-plant resistance to pests is of increasing importance as conventional chemical control has been shown to produce undesirable environmental side effects (Greig-Smith, Frampton, & Hardy, 1992); chemical control may also decline in efficiency through increased pesticide resistance displayed by some pests (Metcalf, 1983). New pesticides are becoming more expensive to develop with a less attractive monetary return for agrochemical research and development companies than some prospective host-plant resistant breeding programmes (Wratten, Martin & Rhind, 1990).

Previous research has shown the possibility of reducing pesticide inputs when these compounds are used in conjunction with host-plant resistance. The concentration of the insecticide parathion required to kill 50% of Myzus persicae (Sulz.) on a partially resistant chrysanthemum variety was less than half that needed on a susceptible variety (Selander, Makkula & Tiittanen, 1972). Tolerance of an organophosphate-resistant strain of the same aphid species to malathion was significantly decreased when aphids were reared on a moderately resistant Brussels sprout variety (Mohamad and van Emden, 1989). Raman (1977) compared the susceptibility to dimethoate of the leafhopper, Empoasa dolichi (Germar.) by spraying susceptible and resistant cowpea varieties. The same concentration causing 75% mortality on the susceptible variety gave 94% mortality on the resistant one. Similar increases in mortality were found with the cereal aphid, Metopolophium dirhodum (Walker) on resistant wheat cv. Emmer, compared with the susceptible cv. Maris Kinsman (Attah & Van Emden, 1992).

Due to differences between some wild Gramineae and modern wheats, in agronomical traits such as grain quality, yield, growth habit and adaptation to different environments, , it may be advantageous to demonstrate usefully high levels of Hx in modern hexaploid wheat cvs. To investigate this potential, a screen of 47 worldwide cultivars of Triticum (mainly T.aestivum) was conducted. Ten of these cvs were selected and assessed for their suitability for aphids via antibiosis and antixenosis bioassays using the pest aphid species Sitobion avenae (Nicol, Copaja, Wratten & Niemeyer, 1992), . The effects of DIMBOA levels on the tolerance of S. avenae to the insecticide, deltamethrin were then investigated using two cvs of high and low DIMBOA content, respectively. The value of these results in work leading to the production of aphid-resistant cultivars is discussed in relation to enhanced natural predation levels arising from reduced pesticide input.

Materials and Methods

Quantification of DIMBOA content in wheat seedlings

The method of extraction and quantification closely followed that of Copaja et al., (1991b). The shoot of a seedling, cut at its junction with the seed, was macerated progressively with three batches of 0.33ml distilled water using a pestle and mortar. After 15 minutes, 1-2 drops of 0.1N H_3PO_4 were added to the extract to bring it to pH 3. The sample was then centrifuged at 13,000 rpm for 15 minutes and the supernatant filtered (0.45μm). Aliquots of 50 or 100μl were then injected into a Gilson 712 HPLC using a Lichrospher 100RP-18 (5μm) column (125 x 4mm). The gradient profile of solvent A (MeOH) and solvent B (0.5ml H_3PO_4 in 1l H_2O) was 0 - 9 min: 30% A to 50% A; 9 - 9.5 min: 50% A to 30% A; 9.5 - 10 min: constant at 30% A. Flow rate was 1.5 ml/min and the detection was carried out at 263nm. The retention time of DIMBOA at 263nm was 3.5 ± 0.2 mins. The reference compound DIMBOA was obtained from ethereal extracts of Zea mays (L.), as described (Queirolo, Andreo, Corcuera & Niemeyer, 1983).

Screening of cvs for DIMBOA content

A screen of 47 worldwide cultivars of Triticum (mainly T.aestivum) was conducted. For each cv. approximately 15 seeds were planted in each of ten 6-cm-diameter plastic pots containing vermiculite and allowed to germinate in a plant growth room. The temperature was 20°C with a range of 2°C; relative humidity ranged from 45 - 65 %; photoperiod was 12h and light intensity was 440 $\mu E\ m^{-2}s^{-1}$. Four, five, six and seven days after planting, one healthy seedling of representative size from each pot was cut at the junction with the seed and its length was measured, The sample was weighed and frozen at -20°C ready for subsequent Hx analysis.

Aphid antixenosis experiments

Ten wheat cvs previously analysed for DIMBOA (Nicol et al., 1992) were selected to include DIMBOA concentrations representing the full range found during screening. Within this range, cvs were selected which were at the same height at four days; also as wide a range of countries of origin as possible was included.

For each selected cv. four seeds were planted in each of 27 plastic spittle pots of 4 cm diameter and 4 cm depth containing vermiculite. The pots were placed in 27 randomised blocks, each block containing a full replicate of the ten cvs, in the growth room described above. On the morning of the fourth day after planting seedlings were thinned to one seedling per pot, providing plants of similar size in each of ten pots per cv. These pots were re-arranged in a randomised block design of ten distinct blocks, each block containing a full replicate of the ten cvs, within a metal arena measuring 0.5 x 0.5 x 0.15m. The arena was then filled to just above pot level with vermiculite;

PTFE suspension had previously been painted on the inside edge of the arena to prevent the escape of crawling aphids. Four hundred alate S.avenae collected from a non-clonal culture 24 hours previously and stored without food in polyestyrene specimen tubes were then dispersed from the tubes evenly over 25 equally spaced sites in areas between the plants. Three counts were made at two, four and six hours after infestation, respectively, of the number of alatae settled on each seedling. Immediately after the last count, aphids were removed gently with a fine brush and the seedlings cut, measured, weighed and frozen ready for Hx analysis, as described above.

Calculation of mean relative growth rate (MRGR) of aphids

To establish that the cvs chosen because of their different Hx concentrations, did differ in their levels of resistance under the conditions of the experiment, seedlings of the Triticum cvs Altar and Dollarbird were planted in 6cm-diameter plastic pots containing vermiculite, in separate culture boxes under the same conditions as the aphid culture. Fifty nymphs of S. avenae were individually weighed using a torsion balance with sensitivity of ± 0.005mg, and placed singly in separate clip cages. Twenty-five cages were subsequently clipped onto 4-day-old seedlings of each of the two cvs, respectively. After 48 h the nymphs that had settled were reweighed individually and their MRGR calculated using the following formula (van Emden, 1969):

$$\frac{\log_e \text{final weight (mg)} - \log_e \text{initial weight (mg)}}{}$$

Effect of pesticide on aphids reared on different cultivars

Approximately 25 seeds of Triticum aestivum (L.) cv. Dollarbird and Triticum durum (L.) cv. Altar were planted into each of three pots, respectively. The pots were placed in separate boxes under the same conditions as those for aphid culturing. After four days 150 adult apterous S. avenae from the stock culture were transferred to the test seedlings in each of the two respective culture boxes. Three days later, 120 first instar nymphs were removed from each culture with a fine paintbrush and weighed in batches of five individuals. Ten of these aphids were then transferred back into each of 12 small polyestyrene tubes per cv. ready for subsequent pesticide testing on the same day. Twenty four hours prior to this latter analysis, 1ml of pesticide of known concentration had been pipetted onto filter paper in each of two Petri dishes per dose for each cv. The concentrations used were 0.1, 0.03125, 0.0156, 0.01 and 0.0067 field concentration. For the analysis, ten nymphs cultured on either of the two cvs were placed into each of the Petri dishes and the number of aphids alive, moribund or dead were recorded at the same time intervals as in the initial dose range test. The aphids were subsequently transferred to clean Petri dishes containing only barley leaves and the number that were live, moribund or dead were recorded after a further 24 h.

Correcting LC50 values

The equation below was used to correct the LC50 values for the difference in the mean weight of the aphids reared on the two different cvs, respectively; the units of LC50wt are mg/g aphid fresh wt.

$$LC50wt = \frac{LC50\ (mg/l)}{\text{fresh wt of 10 aphids (mg)}}$$

Results

The DIMBOA concentrations of the cvs studied by Nicol et al., 1992 ranged from 0.99 to 8.07 mmol/kg fr. wt. The majority of seedlings had maximal levels of DIMBOA at four days after planting. In every cv. the amount of DIMBOA decreased rapidly two days after the maximal level and it was assumed that whole plant concentrations would not rise to this level again (Argandoña, Niemeyer & Corcuera, 1981).

The relationship between the mean number of alate aphids per cv. and mean DIMBOA concentration per cv.is shown in Fig. 1. There was a significant negative relationship between the mean number of alate S.avenae present on each cv. after each of the assessment periods 2, 4 and 6 h and the mean DIMBOA level per cv. However, the relationship did not differ significantly (intercept or slope) so the data for the three periods was pooled. The relationship for the pooled data was: ($\log y = 0.84 - 0.22x$, $r = -0.81$, $P < 0.001$). There was no significant relationship between the mean number of alate S. avenae present on each cv. and the mean seedling height per cv.(pooled assessment periods: $\log y = -0.46 + 0.22x$, $r = 0.31$, $P > 0.05$). The ranking of the DIMBOA content of the ten cvs selected for the bioassay, analysed under non - bioassay and bioassay conditions was strongl positively correlated (Spearman's rank correlation; $r_s = 0.77$, $P < 0.02$), (Nicol et al., 1992).

There was a significant difference ($t = 3.279$; $P < 0.005$, d.f. = 40) between the mean values of MRGR over 48 h for nymphs of S. avenae cultured on two different Triticum cvs. The mean MRGR of nymphs cultured on cv. Altar was 0.150 mg/mg/day, 46.4% lower than that for cv. Dollarbird, which was 0.280 mg/mg/day. The initial weight of nymphs did not differ significantly between the two cvs ($t = 1.943$; $P > 0.05$, d.f. = 40).

The regression lines in Fig. 2 represent probit mortality after 45 mins at different doses of deltamethrin for nymphs reared on cv. Altar ($\chi^2 = 5.689$; $P > 0.05$, d.f. = 8)and cv. Dollarbird ($\chi^2 = 9.099$; $P > 0.05$, d.f. = 6), respectively. The χ^2 values indicate that the relationships did not differ significantly from the (pre-transformation) sigmoid dose-response model. The LC50 of nymphs cultured on cv. Altar was 0.119 mg a.i./l, significantly lower than that of nymphs from the cv. containing lower DIMBOA levels, which was 0.528 mg a.i./l ($t = 8.954$; $P < 0.001$, d.f. = 16). The aphids cultured on cv. Altar were slightly (2%), but significantly lighter ($t = 3.304$; $P < 0.005$, d.f. = 46) than those on cv. Dollarbird. When the values were adjusted for weight the LC50wt (0.074 mg a.i./g aphid fresh

wt) for nymphs on Altar was 73% lower than the LC50wt (0.324 mg a.i./g aphid fresh wt) for nymphs on cv. Dollarbird (t = 8.874; P < 0.001, d.f. = 16).

Discussion

Nicol et al., 1992, demonstrated a wide range of DIMBOA concentrations in the 47 cvs screened. The range is very similar to that demonstrated by Copaja et al. (1991b) for a screen of cvs from within one country (Chile), implying that the DIMBOA levels in currently grown wheat seedlings may not extend far beyond those in the earlier Chilean screen. However, the levels of DIMBOA displayed confirm the potential of Hx as possible aphid resistance factors in modern cvs.

The fact that the range of DIMBOA concentration of the ten selected cvs was strongly positively correlated between non-bioassay and bioassay conditions, suggests that despite some individual variation, the relative difference in DIMBOA content between the cvs remained similar under both sets of conditions. Any individual variation recorded may be due to the effects of induction from aphid feeding or the different conditions experienced by the wheat seedling, such as the lower density of planting under bioassay conditions. Most published work on Hx levels and aphid resistance has concerned antibiosis, in which aphids have usually been confined to a plant of a particular cultivar (Argandoña, Luza, Niemeyer & Corcuera, 1980; Bohidar, Wratten & Niemeyer, 1986; Thackray et al., 1990), or dual choice tests between two cvs. This work is the most complete antixenosis bioassay to date concerning this aphid/biochemical interaction.

Aphids reared on the Triticum cv. Altar, containing a higher level of DIMBOA than cv. Dollarbird, had a significantly lower mean relative growth rate over 48 h, demonstrating an effect upon aphid antibiosis.

Analysis of probit mortality showed that the effect of the pesticide was significantly different for nymphs reared on the two cvs. The nymphs that experienced higher levels of DIMBOA were less tolerant to the insecticide. Some effect may be expected due to their slightly smaller size, but when LD50 values were adjusted for weight the effect was still significant.

This interaction between host-plant resistance and pesticide effects, if shown in the field, could allow reduction of pesticide dosage. In addition, the lower rate of pesticide application may subsequently have less detremental effect upon beneficial insects (van Emden, 1987; van Emden & Wratten, 1990).

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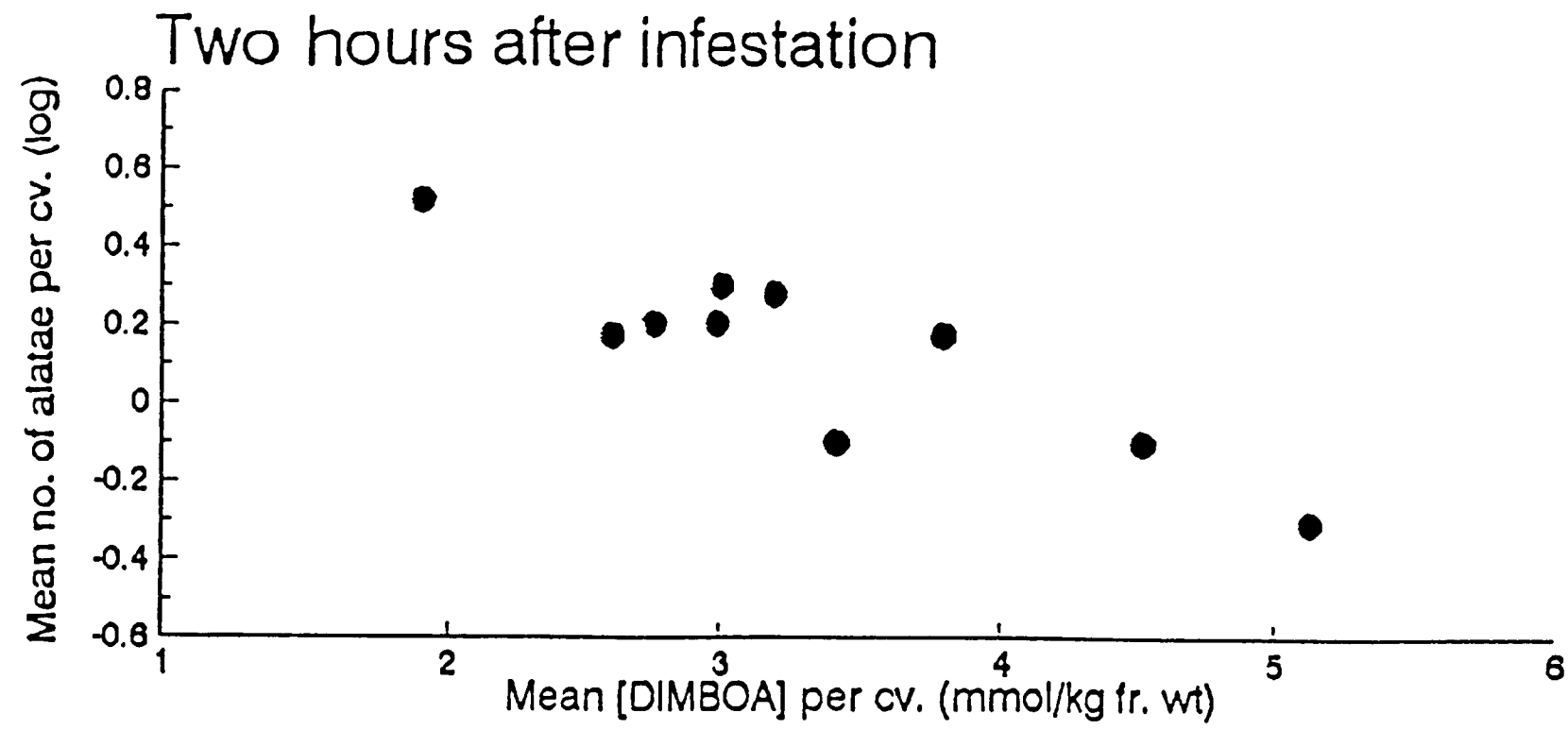
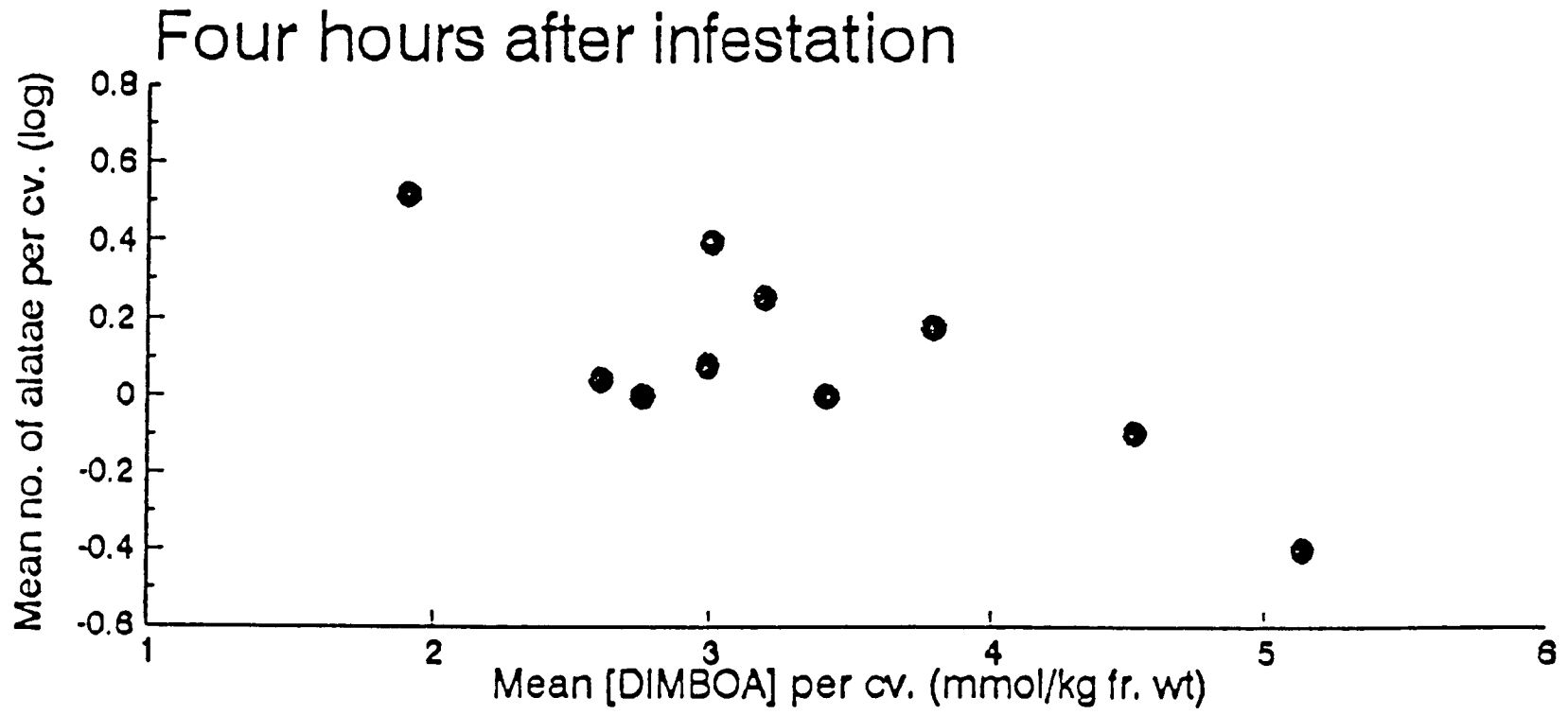
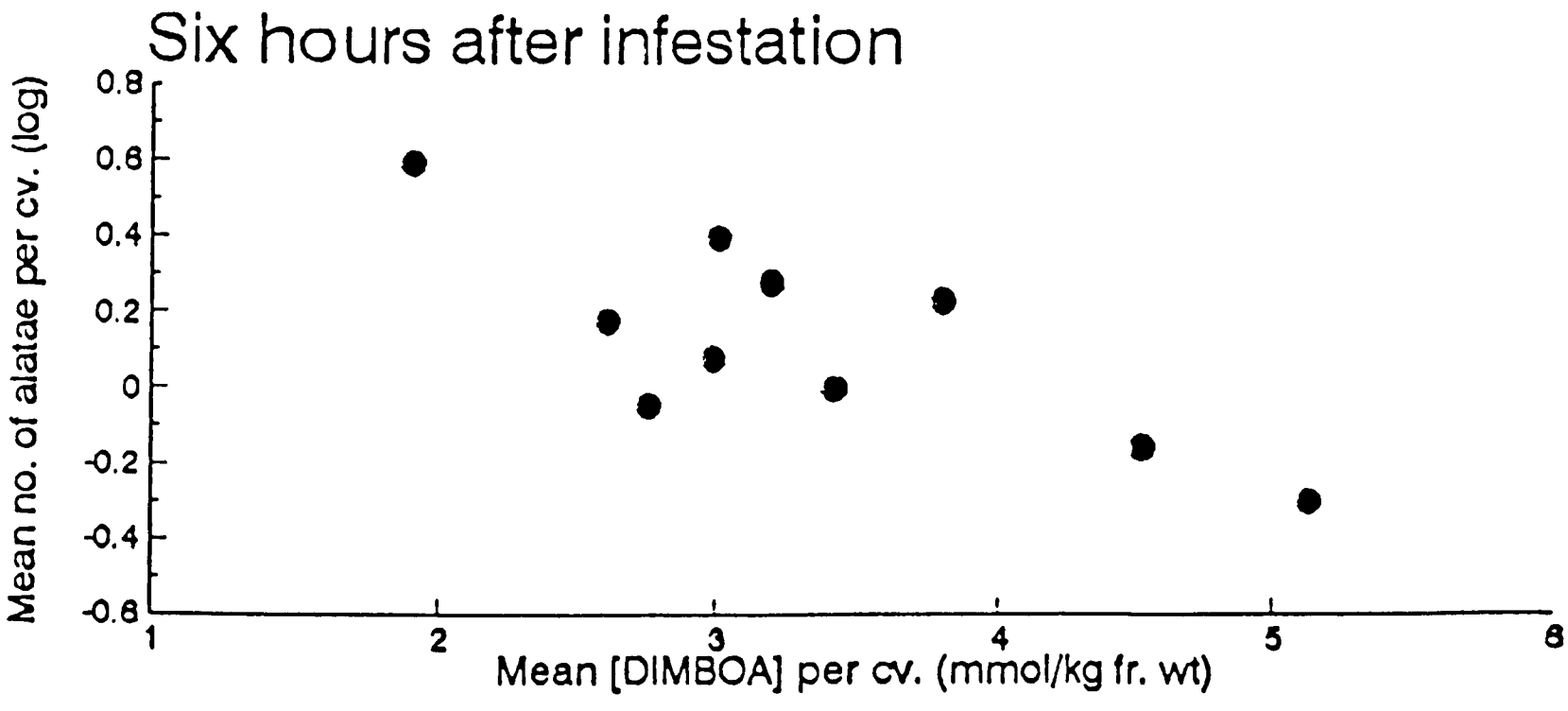
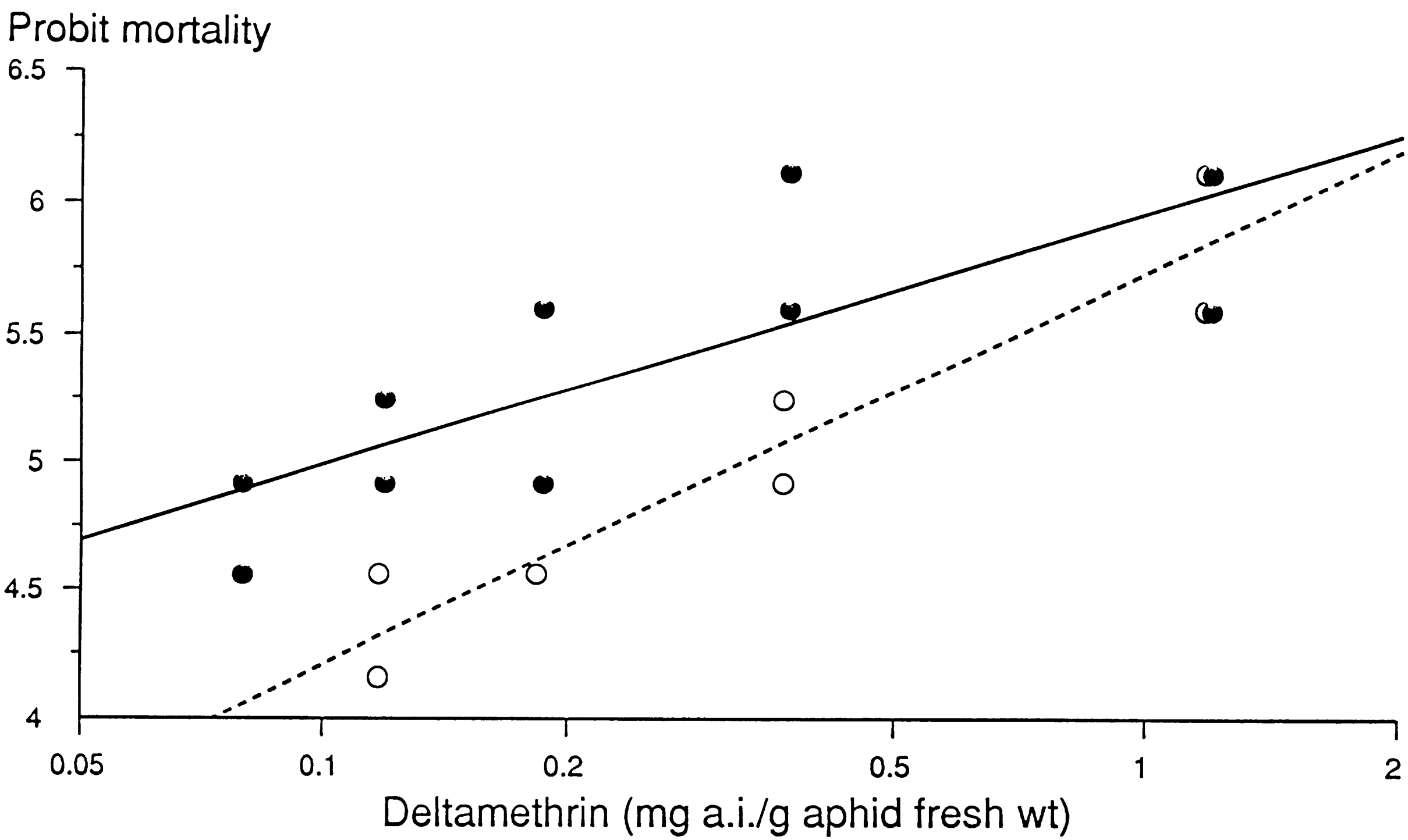

Two hours after infestation
Mean no. of alatae per cv. (log)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
1
2
3
4
5
6
Mean [DIMBOA] per cv. (mmol/kg fr. wt)


Four hours after infestation
Mean no. of alatae per cv. (log)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
1
2
3
4
5
6
Mean [DIMBOA] per cv. (mmol/kg fr. wt)


Six hours after infestation
Mean no. of alatae per cv. (log)
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
1
2
3
4
5
6
Mean [DIMBOA] per cv. (mmol/kg fr. wt)


Probit mortality
6.5
6
5.5
5
4.5
4
0.05
0.1
0.2
0.5
1
2
Deltamethrin (mg a.i./g aphid fresh wt)