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Accepted Version

Antibiosis in wheat interacts with crowding stress to affect *Metopolophium dirhodum* development and susceptibility to malathion

Paul J. Clayson[1], J.P. Michaud[2] & Helmut F. van Emden[1]

[1]*School of Biological Sciences, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AJ, UK*; [2]*Department of Entomology, Kansas State University, 1232 240th Ave., Hays, KS, 67601, USA*

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Correspondence:

H.F.van Emden (corresponding author), School of Biological Sciences, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AJ, Email: h.f.vanemden@reading.ac.uk

Key words: aphids, body size, host plant resistance, toxicity, *Triticum aestivum*, winter wheat

Abstract

We used a laboratory study to compare the performance of rose-grain aphid, *Metopolophium dirhodum* Walker (Hemiptera: Aphididae), on the wheat cultivars 'Huntsman' (susceptible) and 'Rapier' (partially resistant) in both low density (uncrowded) and high density (crowded) colonies and examined the consequences for aphid susceptibility to malathion. Adult apterae that developed on Rapier wheat had their mean relative growth rate (MRGR) reduced by 6% and 9% under uncrowded and crowded conditions, respectively, whereas the crowding treatment reduced MRGR by 4%, but only in Rapier aphids. Rapier resistance also reduced adult dry weight by 13% and 14% under crowded and uncrowded conditions, respectively, whereas crowding reduced it by 34% and 35% in Rapier and Huntsman aphids, respectively. Development on Rapier substantially reduced the topical LC_{50} of malathion by 37.8% and 34.8% under crowded and uncrowded conditions, suggesting that plant antibiosis increased malathion susceptibility. By comparison, crowding only reduced the LC_{50} by 29.5 % and 26.0% on Huntsman and Rapier, The LC_{50} data showed that reductions on aphid body size on Rapier and through crowding did not fully explain the differences in LC_{50}. This was particularly the values for crowded aphids that were actually 80% higher than for uncrowded ones. This apparent tolerance of crowded aphids, however, may partly be due to loss of insecticide from small aphids at dosing. Evidence of synergy between plant resistance and insecticide susceptibility raises the possibility of using reduced concentrations of pesticides to control aphids on resistant crop cultivars, with diminished impacts on non-target and beneficial species important in IPM programmes.

Introduction

The rose-grain aphid, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae), is one of three aphid species that attack cereals in Western Europe (Carter et al., 1985) and cause considerable losses in the UK (Cannon, 1986). To mitigate the deleterious effects of cereal aphids, farmers often rely on IPM programmes that typically integrate traditional chemical controls with the use of resistant cultivars that impede aphid development and reproduction. Host plant resistance can be viewed as the anthropic exploitation of naturally-evolved plant defences against insects (van Emden, 1997). Resistant cultivars are those which express physical or chemical traits that are either deleterious to the biology of the pest (antibiosis), or deter the pest from attacking the plant (antixenosis) (Smith, 2005). Plant resistance is especially useful when crop value is relatively low, cosmetic damage unimportant and the pest's economic injury level relatively high (Painter, 1951), as is the case for cereal aphids on winter wheat.

Graminaceous crops exhibit a variety of resistance mechanisms that are specific to aphids. As they penetrate plant tissues, aphids secrete a continuous stylet sheath composed largely of lipoproteins and phospholipids that serves to protect the aphid's stylet from plant defensive responses while it feeds from the phloem (Dixon, 2000). Sheath formation is thought to be mediated by various enzymes in aphid saliva, notably polyphenol oxidase and peroxidases that interact with phenolic compounds mobilized by the plant (Urbanska et al., 1998). The primary chemical defences in wheat consist of phenolics and hydroxamic acids (Urbanska et al., 1998). The hydroxamic acids, in

particular the aglucone glucoside DIMBOA (2-O-B-D-glucopyranosyl-4-hydroxy-7-methoxy-1, 4-benzoxazin-3-one), are known to be important factors diminishing the susceptibility of young wheat plants to aphid feeding (Niemeyer et al., 1989). Fuentes-Contreras & Niemeyer (1998) showed that DIMBOA reduces the growth rate and body size of *Sitobion avenae* (F.) (Hemiptera: Aphididae), with concomitant detrimental effects on its parasitoid, *Aphidius rhopalosiphi* De Stefani-Perez (Hymenoptera: Braconidae). Consequently, this defensive compound also has the potential for negative effects on natural enemies that may diminish its net benefit to IPM programmes.

Givovich et al. (1992) found that the glucoside DIMBOA was excreted in the honeydew of *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae), indicating that the chemical does occur in phloem elements. At low concentrations DIMBOA was passively ingested, but at high concentrations, aphid feeding was deterred, yielding a biphasic pattern of honeydew production when plotted against hydroxamic acid concentrations. Thus, this chemical expressed antixenotic and antibiotic activity, first deterring aphid stylet penetration, and then diminishing feeding rate. Furthermore, Nicol et al. (1992) showed that the settling rates of alate *S. avenae* in a field trial of 47 *Triticum* cultivars were inversely correlated with the DIMBOA concentrations expressed in the cultivar, suggesting the antixenotic effect could reduce rates of aphid colonization.

Copaja et al. (2006) used electro-penetration graph technology to highlight the antifeedant effect of DIMBOA by manipulating the chemistry of barley leaves. Jiao et al. (2005) increased concentrations of DIMBOA in wheat by spraying plants with jasmonic acid; such plants diminished the performance of *S. avenae* directly, and also defended themselves indirectly by emitting volatiles attractive to natural enemies. Gianoli et al.

(1996) conducted a field trial with 26 wheat cultivars and concluded that high DIMBOA accumulation was not costly in terms of yield. Silva et al. (2006) arrived at similar conclusions studying changes in DIMBOA concentrations in 15 wheat cultivars in response to drought stress and aphid infestation.

Overall aphid performance can be inferred from changes in fresh weight over time, i.e., growth rate, and Leather & Dixon (1984) showed that the intrinsic rate of increase (r_m) is closely correlated with mean relative growth rate (MRGR). When aphids are reared under sub-optimal conditions, body weight and overall size tend to decrease (Dixon, 2000). Since the size of aphids at maturity is strongly affected by colony density (Dewar, 1976), crowding can be used as a means of stressing aphids so as to compare their performance under optimal versus suboptimal conditions. The fresh weight of aphids can also be reduced by plant crowding (Dewar, 1976) allowing one to control for age-weight interactions that might affect toxicological susceptibility. For example, a small adult aphid might be more resistant to a toxin than a large adult aphid due to differences in overall mixed function oxidase enzyme titre (Leszczynski et al., 1993). The use of crowded/uncrowded cultures is also a means of detecting potential interactions between host plant resistance and other aphid stress factors. For example, Nicol et al. (1993a, 1993b) found that the LD_{50} of deltamethrin for *S. avenae* nymphs was reduced by 73% when these were raised on seedlings with high DIMBOA content. These nymphs also showed the greatest reduction in growth rate, this translated to a reduction of 91 % in the LC_{50}. In a study by Loayza-Muro et al. (2000), *S. avenae* showed overall greater induction of cytochrome P-450 and NADPH-cytochrome C reductase when reared on wheat cultivars with high concentrations of hydroxamic acids.

Selander et al. (1972) were the first to show, using parathion and aphid-resistant chrysanthemum cultivars, the effectiveness against the aphids of a reduced dose on such cultivars. Since then, it has become well-established that host plant resistance to insects synergises positively with plant resistance, and van Emden (1999) was able to list 12 examples of this phenomenon; three of these examples concerned aphids.

The objective of this study was to test whether wheat plant resistance traits interact with the aphid stress factor crowding to reduce the LC_{50} and LD_{50} following acute pesticide exposure. Aphids were reared in cages on potted plants in a greenhouse at two densities on two wheat cultivars that differed in susceptibility to *M. dirhodum*. We measured the fresh weight, dry weight, hind tibia length and MRGR of aphid nymphs and adults from each culture. These cultures were then tested for susceptibility to various concentrations of an organophosphorus insecticide, malathion, applied topically with a microapplicator.

Materials and Methods

Wheat cultivars

We selected two commercial wheat cultivars (cv) for our experiments based on their differential susceptibility to aphids and ease of obtaining seeds. These cvs were: 'Maris Huntsman', a susceptible variety (Attah, 1984) and 'Rapier' which has demonstrated partial resistance to cereal aphids, both in the laboratory (Attah 1984; Ul-Haq & van Emden, 2002) and the field (Lowe, 1982). All plants used in experiments and for rearing aphids were grown in plastic pots (9 cm diameter) filled with potting mix (Levington®

John Innes No. 2, The Scotts Miracle-Gro Company, Surrey, UK) in a greenhouse under a photoperiod of L14:D10 that was maintained by supplementing natural sunlight with high intensity mercury vapor lamps during winter months. Shading was employed during summer months to prevent excessive heating. Daily minimum and maximum temperatures ranged from 16 – 21.5 ºC and 23 – 36.5 ºC, respectively.

For experiments, seeds of both cultivars were sown in pots in the greenhouse, 30 pots per week. Seven seeds were sown per pot and then thinned to four plants after three weeks. To prevent infection by powdery mildew (*Erysiphe graminis* L.) (Ascomycotina: Erysiphales), the fungicide 'Impact' (Flutriafol®, PP-450, Syngenta, Surrey, UK) was applied as a soil drench (0.03 g a.i. per pot) when plants were seven days old. This fungicide has been shown to have minimal effects on aphids and associated parasitoids (Jansen, 1999). Four pots of each variety were placed in separate labelled cages. The position of pots in each cage was altered by randomization once per week.

Aphids

A stock culture of *M. dirhodum* was established and maintained on potted Huntsman wheat plants from material collected from the main laboratory stock culture of aphids (also maintained on Huntsman) originally obtained from Rothamsted Research, Harpenden, Hertfordshire, UK. In preparation for culture rotation, four week old wheat plants were thinned to four plants per pot and placed in a cage constructed of wood and clear Perspex™ (45 cm x 40 cm x 45 cm). The sides were covered with muslin fabric and the front of each cage was fitted with a muslin sleeve, which was tied off when not in use. Once a week, the stock culture was restarted by removing a few leaves from a

heavily infested plant and placing them on caged clean plants. The stock culture was held in the greenhouse under the conditions described above except during periods of summer heat when cages were moved into an insectary. The insectary was illuminated with 'daylight' fluorescent light tubes in a L14:D10 identical to the conditions for the plant rearing above.

For experiments, two different aphid densities, each comprising four cages of each cultivar, were established by introducing different numbers of *M. dirhodum*: low density (ca. 100 per pot), and high density (> 500 per pot). All plants were watered daily and changed weekly. Aphid cultures were introduced into clean cages and allowed to reproduce for a period of four weeks before approximately 125 apterous adult aphids were carefully removed from each cultivar/treatment combination and placed into clip cages, five per cage, on clean plants of the corresponding cultivar.

The clip cages used throughout these experiments were of the design of Adams & van Emden (1972). Two clear, Perspex rings (20 mm diam x 10 mm high) were attached to opposing prongs of a hair-curl clip. The upper rim of each ring was covered with a fine nylon mesh (to prevent aphid escape) and the lower rim was lined with polystyrene foam (to prevent leaf damage). Aphids were confined on the abaxial leaf surface with the leaf sandwiched between the two foam-lined rims. So the plant would not bear the weight of the clip cage, the latter was affixed to a small stake pushed into the soil.

Adult clip cages contained five adults per cage and were used to generate up to five nymphs per day of known provenance. These cages were opened daily and all first instar nymphs removed with a fine paintbrush. Live nymphs were then placed in separate clip cages (five per cage) on separate clean plants of the appropriate cultivar. All clip

cages were changed daily throughout the course of aphid development to prevent any build up of honeydew and exuviae and then remounted on previously unused areas of leaves to prevent local deterioration of plant quality. Used clip cages were soaked in domestic detergent, rinsed in distilled water and thoroughly dried and examined before re-use. Aphids were moved to and from clip cages with a moist paintbrush.

Determination of fresh weight and MRGR

Synchronous aphid cohorts were obtained by caging 75 apterous adults on each of 4 plants of each respective variety /density for a period of 24 h, removing them, and leaving all deposited nymphs caged on that plant. The leaf positions of cages and nymphs were changed daily in all treatments. Fifteen aphids were then removed from each plant on day 3 and collected in clean, labeled 5 cm glass test tubes. These were plugged with cotton wool and the aphids anaesthetised with CO_2 to facilitate handling. The fresh weight of each aphid was then recorded using a microbalance. Two days later another batch of 15 aphids from each plant were weighed, and MRGR was calculated according to van Emden (1969):

MRGR ($\mu g^{-1} \mu g^{-1} day^{-1}$) = ($\log_e$ final weight (μg) – $\log_e$ initial weight (μg)) / number of days between the two measurements.

Determination of dry weight

Fifteen adults of the same cohort were randomly selected from each of four plants of each culture type, placed in a clean, labelled glass Petri dish and put in a 150 ºC oven for ca.

12 h (overnight). Preliminary observations revealed this to be sufficient time to ensure complete desiccation. After drying, the dish was carefully removed and placed in a desiccator to cool, after which time each aphid was removed from the dish and weighed individually.

Determination of hind tibia length

Hind tibia length has been shown to be a reliable indicator of body mass in aphids that is insensitive to variation in water content (Nicol & Mackauer, 1999). The hind tibia length was measured for 100 individual adults from each of three replications for each cultivar/ treatment combination. A single drop of glycerol was placed in the centre of a clean, labeled microscope slide, whereupon an individual adult aphid was placed in the glycerol and the hind leg separated with forceps. A cover slip was slowly lowered onto the drop and then the hind tibia was measured under a binocular dissecting microscope in graticule units. At the end of the experiment, graticule units were converted to µm following calibration with a stage micrometer.

Insecticide bioassay

A stock solution of one percent a.i. malathion (Fyfanon®, technical grade, 96 – 97 % Cheminova Agro, Lemvig, Denmark) was made using butanone (methyl-ethyl-ketone) as a solvent. Serial dilutions were prepared from this stock solution at the beginning of each experiment. Adult aphids for testing were removed from clip cages with a moist paint brush and placed in a clean glass Petri dish painted with black enamel paint and coated

with Fluon™ around the rim. An Arnold microapplicator (Arnold, 1965), consisting of a Hamilton™ RN syringe coupled to a precision syringe driver, was calibrated to deliver precisely droplets of 0.75 µl to the dorsal surface of each insect (Needham & Devonshire, 1973). This volume remained constant throughout the experiment; dosage was varied by altering the amount of a.i. in the solution. On the basis of preliminary experiments and the results of Attah (1984) and Smith (1990), six doses were tested ranging in concentration from 38.6 to 724.0 p.p.m. a.i: 0.02, 0.04, 0.048, 0.06, 0.07, and 0.08 mg l^{-1}; butanone was used as a control. Approximately 100 individuals were tested at each dose with three replications.

Droplet application resulted in a rapid darkening of the aphid's coloration until complete evaporation of the droplet had occurred. Aphids that did not exhibit this color change were excluded from the experiment. Treated aphids were transferred to a clip cage, five per cage, and held on a leaf on a fresh plant as above; mortality was assessed after 24 h.

Results

The MRGR of *M. dirhodum* (Figure 1) was 7.44 % lower on the partially resistant Rapier than on Huntsman ($F_{1,56}$ = 23.15, P < 0.001) but the 3.00% reduction caused by crowding was only marginally significant ($F_{1,56}$ = 3.58, P = 0.064). The 'cultivar x crowding' interaction was not significant ($F_{1,56}$ = 0.66, P = 0.421).

There was a significant effect of both cultivar ($F_{1,6}$ = 689.11, P < 0.001) and crowding ($F_{1,6}$ = 5 903.42, P < 0.001) on the dry weight of *M. dirhodum* adults. The

aphids on Rapier weighed 13.40% less than Huntsman aphids and crowding greatly reduced adult dry weight on both cultivars (Figure 2). The weight reduction was 34.26% on Rapier and 35.20% on Huntsman. These reductions led to a significant 'cultivar*crowding' interaction ($F_{1,6}$ = 45.85, $P < 0.001$) due to the very small residual variation (giving a SE. for even the smallest mean of less than 1%). Therefore, this interaction was not considered to be biologically significant.

Cultivar and crowding had very similar effects on both fresh and dry weight. Aphids on Rapier weighed 23.11% less than those on Huntsman ($F_{1,6}$ = 1 339.98, $P < 0.001$), but crowding caused a much larger fresh weight reduction of 53.24% ($F_{1,6}$ = 14 978.34, $P < 0.001$, Figure 3). A significant interaction between the two factors ($F_{1,6}$ = 112.36, $P < 0.001$) again related to a very small difference between the weight reduction with crowding on Rapier (62.91%) and that on Huntsman (59.14%). As for dry weight, the interaction only attained statistical significance because of extremely low residual variation.

For hind tibia length (HTL), the cultivar x crowding interaction was significant ($F_{1,6}$ = 499.85, $P < 0.001$) (Figure 4). Crowding significantly reduced the HTL of aphids on both Rapier and Huntsman (by 20 and 28% respectively). Whereas HTL did not differ between cultivars under crowded conditions, when not crowded, aphids on Huntsman had an HTL 15% longer than on Rapier.

Both cultivar and crowding had an effect on the LC_{50} of malathion ($F_{1,6}$ = 429.66, $P < 0.001$ and $F_{1,6}$ = 231.06, $P < 0.001$, respectively, Figure 5). For the main effects, the antibiosis expressed in Rapier wheat reduced the LC_{50} value by 36.7% and crowding reduced the LC_{50} of aphids by 28.19%. The interaction, although statistically significant

($F_{1,6}$ = 19.16, P < 0.01) was biologically unimportant in that the main effects would suggest that the LC_{50} of 68.67 μg ml $^{-1}$ of uncrowded aphids on Huntsman should reduce to 30.64 for crowded aphids on Rapier, and the actual LC_{50} was hardly different at 31.56.

Since insecticide toxicity is a function of body weight, it is important to recalculate bioassay results as LD_{50}s, the toxicity as the dose of a.i. per unit body fresh weight (Mohamed & van Emden, 1989). If the smaller size of crowded aphids and those reared on Rapier was wholly responsible for the reduced LC_{50} values, LD_{50}s would not be affected. Yet there were significant differences, and in an unexpected direction (Figure 6). The increase in sensitivity to malathion of aphids on Rapier compared with those on Huntsman (the LD_{50} was reduced by 19.27%; $F_{1,6}$ = 45.90, P < 0.001) revealed that the lower weight of aphids on that cultivar only partly accounted for the increased sensitivity. However, in contrast with the LC_{50} data, LD_{50}s were actually over 80% higher for crowded aphids ($F_{1,6}$ = 758.23, P < 0.001), in spite of their smaller size. This overall effect of crowding arose from a near doubling in LD_{50} on Rapier combined with a somewhat smaller (72.56%) increase on Huntsman, but this interaction was not statistically significant ($F_{1,6\ d.f.}$ = 0.10, P > 0.05).

Discussion

Aphids reared on the susceptible cultivar Huntsman had a lower mean relative growth rate (MRGR) than aphids reared on the partially resistant cultivar Rapier, regardless of whether they were crowded or not (Figure 1), indicating that the antibiosis present in the latter variety delayed aphid development. However, the crowding treatment did not

affect the growth rate of aphids on Huntsman. The crowding treatment had a bigger effect than cultivar in reducing adult size, whether measured as dry weight (Figure 2) or fresh (Figure 3) or as hind tibia length (Figure 4), but Rapier aphids were still smaller than Huntsman aphids in both treatments.

The crowding treatment reduced aphid body dry weight by more than one third on both cultivars (Figure 2), and fresh weight to an even greater extent (Figure 3). The proportional reduction in dry weight was reasonably similar, if slightly greater than, the reduction in LC_{50} as a consequence of crowding. Thus, a parsimonious explanation might attribute much of the effect of crowding on increased susceptibility to malathion concentrations to the effect of this treatment on body size. Crowding in an aphid colony results in competition for food and is thus a potential source of stress, eventually triggering many physiological changes such as the development of alatae in many species (Dixon 2000). It is therefore conceivable that crowding stress could have adversely affected the titres of enzymes involved in detoxification processes, in addition to stunting aphid growth. In the case of the cultivar effect, the LC_{50} of malathion for aphids grown on the resistant Rapier was about one third lower than that of aphids grown on Huntsman (Figure 4), even though the former were only 13-14% smaller. Thus, the smaller size of Rapier aphids relative to Hunstman aphids cannot fully account for their greater malathion sensitivity. Similarly, Mohamed & van Emden (1989) found that rearing *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) on a resistant cultivar of Brussels sprouts reduced the LC_{50} of malathion by more than 50%, when the plants were between 13 and 16 weeks old, and also found that size reduction only accounted for part of this reduction. Detoxification of glucosinolates and isothiocyanates present in the

Brassicaceae has been shown to occur in *M. persicae* via the induction of glutathione S-transferases (GST) (Francis et al., 2005), and these may also be involved in detoxification of malathion. The efficacy of such enzyme systems may be compromised when they are forced to detoxify plant-based and synthetic toxins simultaneously. For example, Luchao et al. (2007) found that *Aphis citricola* van der Goot (= *Aphis spiraecola* (Patch)) (Hemiptera: Aphididae) resistance to malathion and other insecticides correlated with levels of GST and other detoxifying enzymes in the aphids that, in turn, varied as a function of their host plant.

Hydroxamic acids such as DIMBOA have often been implicated as compounds that confer insect resistance in seedling cereals, and this chemical group may be involved in the antibiosis expressed by Rapier to *M. dirhodum*. These compounds are normally correlated with reduced activities of key detoxification enzymes in feeding aphids (Loayza-Muro et al., 2000) and may be partly responsible for the lower LC_{50} observed for Rapier aphids. For example, Mukanganyana et al. (2003) fed a hydroxamic acid (DIMBOA) to *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) in artificial diet and found that it significantly inhibited the activities of key esterases, and to a lesser extent glutathione *S*-transferases, effects that should reduce insecticide tolerance. Thackray et al. (1990) observed that resistance to *S. avenae* and *R. padi* were both correlated with increased levels of hydroxamic acids in six *Triticum* cultivars. However, Castañeda et al. (2009) found that hydroxamic acids in wheat did not induce increased levels of key detoxification enzymes in *S. avenae*. These inconsistencies may reflect the fact that DIMBOA is metabolised by different enzymes from those used to detoxify insecticides (Figueroa et al., 1999). Interactions between plant resistance traits and the insecticide

susceptibility of spider mites have been shown (Gould et al., 1982) but the possibilities of similar interactions in aphids have received little research attention to date.

Crowding, like the partial plant resistance of Rapier, reduced aphid size, and also lowered the LC_{50} on both cultivars (Figure 4). However, when toxicity of malathion to the aphids was expressed as dose per unit fresh weight (LD_{50}), plant resistance and crowding showed effects in opposite directions. As for LC_{50}, the LD_{50} was lower for aphids on Rapier than on Huntsman, confirming that the reduction in LC_{50} could not be fully explained by the smaller size of the aphids. In contrast, crowding raised the LD_{50} of the smaller aphids resulting from it. However, this may not represent a reduction in 'physiological' sensitivity, since it may have resulted from run-off onto the substrate of some of the insecticide applied to the smaller aphids; had 20% of the malathion run off crowded aphids, the LD_{50} would not have differed from that for uncrowded ones.

A likely body length for *M. dirhodum* on susceptible wheat (i.e., Huntsman) is about 2.5 mm (Blackman & Eastop, 2000). Simplifying the aphid body shape to a sphere gives a volume of 8.2 µl, which is more than 10 times that of the 0.75µl drop of malathion applied. However, the reduction in length of the aphid's HTL on Huntsman when crowded would suggest a body length of only 1.79 mm. This converts to a sphere of 3.00 µl, only four times greater than the applied drop, and with only about one-half the surface area of an uncrowded aphid. It is therefore possible that some run-off may have occurred.

The results of the present study, as far as insecticides and plant resistance are concerned, confirm a phenomenon that may have practical implications for aphid management. Both are important tools in cereal aphid IPM and synergistic interactions

between the two are a desirable outcome. Our findings suggest that aphids stressed either by crowding or plant resistance can be killed with significantly lower concentrations of malathion than those growing under more favourable conditions, an effect that could be exploited to diminish pesticide impact on aphid natural enemies. In contrast to crowding that only affects aphids in high density colonies, the beneficial effects of plant resistance can be expected to act during all stages of aphid development and in colonies of all sizes. Thus, the antibiosis in Rapier wheat is not merely compatible with the use of organophosphate insecticides for control of *M. dirhodum*, but has the potential to enhance their efficacy.

However, perhaps the most interesting result of these experiments is the contrast in apparent 'physiological' sensitivity to malathion of aphids subjected to the two stresses of plant resistance and crowding, both of which result in a reduction in aphid size. Although some run-off of insecticide may have occurred from the very much smaller crowded aphids, we cannot say how far this accounts for the large increase in LD_{50} compared with uncrowded ones. Further research is clearly warranted.

Acknowledgements

This research was conducting at the University of Reading, Reading, Berkshire, and was partially funded by a postgraduate studentship to PJC from the Ministry of Agriculture Fisheries and Food, U.K. We thank Ms. Elizabeth Wild for her dedication and assistance with laboratory cultures.

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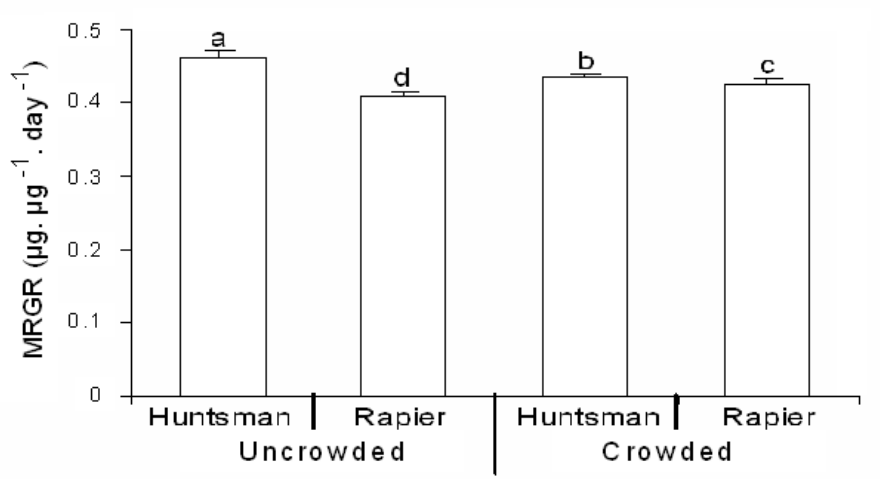

Fgure 1. Mean + SE (n=15) MRGR (Mean Relative Growth Rate) of uncrowded and crowded *Metopolophium dirhodum* reared on two wheat cultivars (Hunstman and Rapier). Columns with the same letter so not differ significantly at P = 0.05).

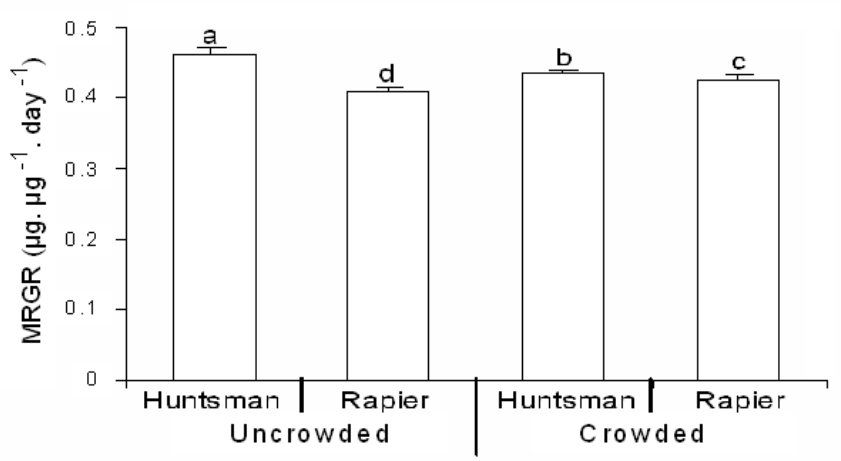

Figure 2. Mean + SE (n=3 batches of 15 aphids) dry weight of uncrowded and crowded adult *Metopolophium dirhodum* reared on two wheat cultivars (Hunstman and Rapier). Columns with the same letter so not differ significantly at P = 0.05).

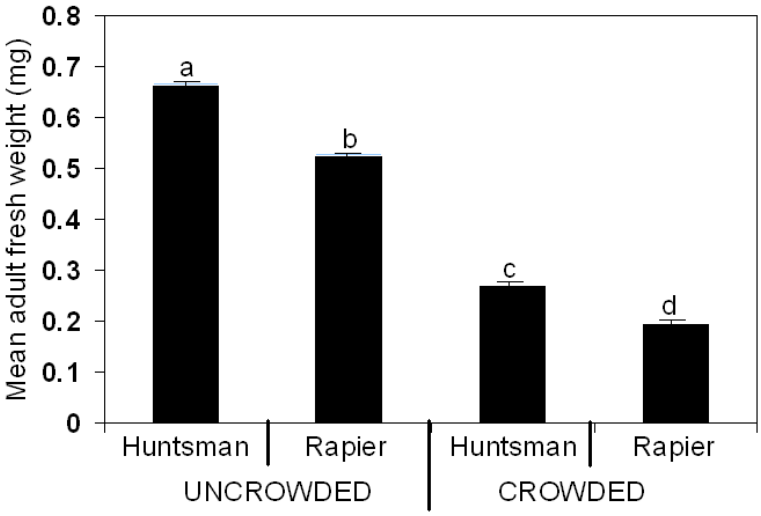

Figure 3. Mean + SE (n=3 batches of 15 aphids) fresh weight of uncrowded and crowded adult *Metopolophium dirhodum* reared on two wheat cultivars (Hunstman and Rapier). Columns with the same letter so not differ significantly at P = 0.05).

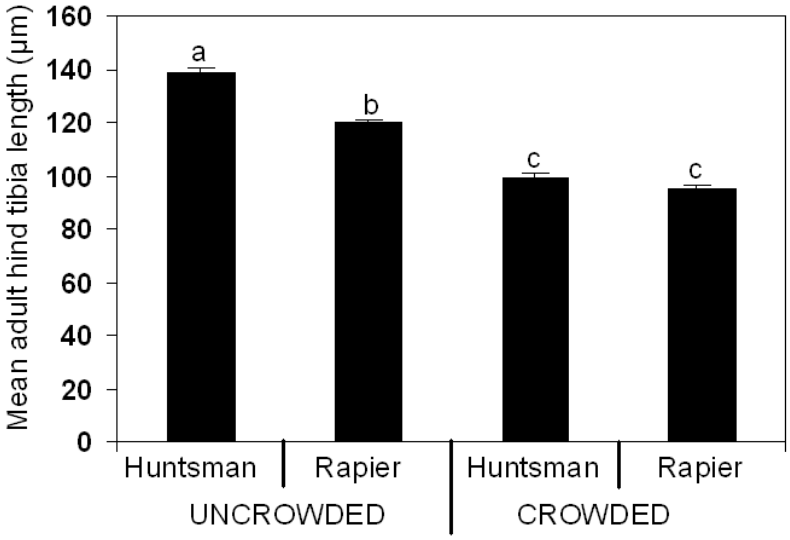

Figure 4. Mean + SE (n=3 averages of 100 aphids) hind tibia length of uncrowded and crowded adult *Metopolophium dirhodum* reared on two wheat cultivars (Hunstman and Rapier). Columns with the same letter so not differ significantly at P = 0.05).

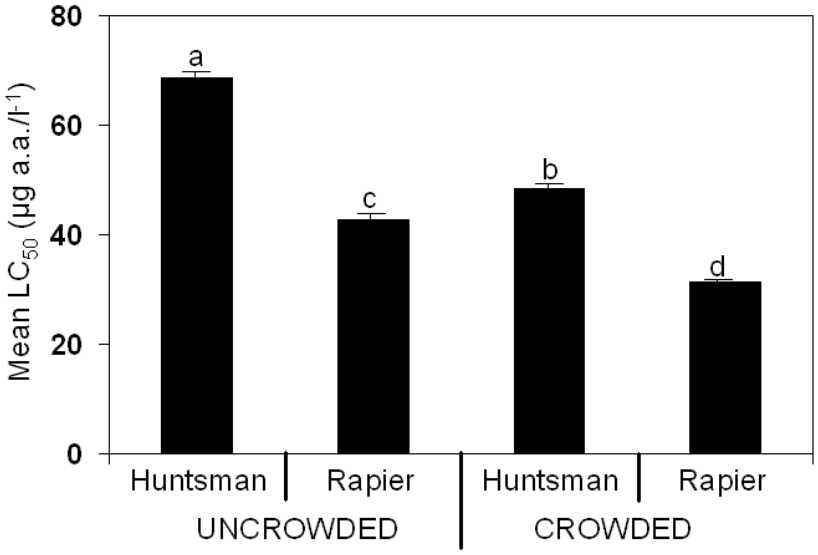

Figure 5. Mean + SE (n=3 batches of 100 aphids) LC_{50} to malathion of uncrowded and crowded adult *Metopolophium dirhodum* reared on two wheat cultivars (Hunstman and Rapier). Columns with the same letter so not differ significantly at P = 0.05).

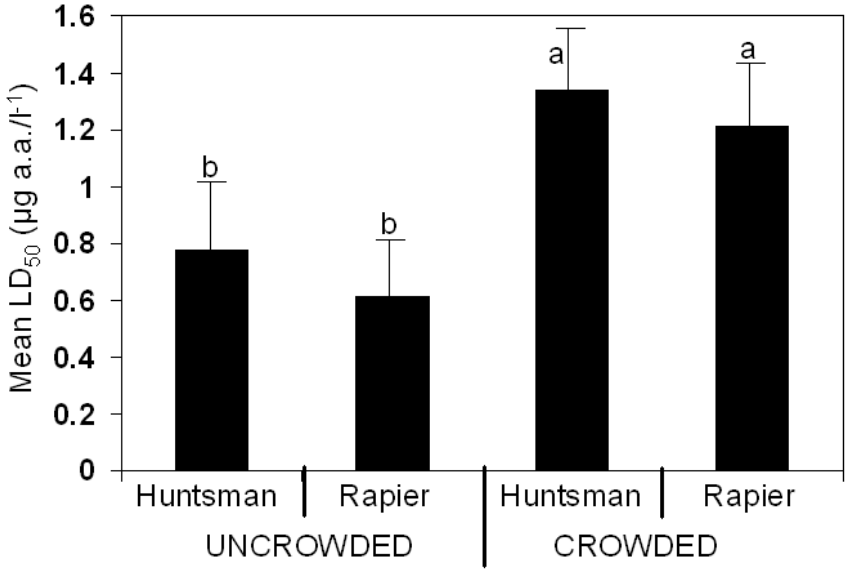

Figure 6. Mean + SE (n=3 batches of 100 aphids) LD_{50} to malathion of uncrowded and crowded adult *Metopolophium dirhodum* reared on two wheat cultivars (Hunstman and Rapier).

Columns with the same letter so not differ significantly at P = 0.05).