APHID RESISTANCE IN WHEAT VARIETIES

HENRIETT ELEK[1], PETER WERNER[1], LESLEY SMART[2], RUTH GORDON-WEEKS[2], MIKLÓS NÁDASY [3] and JOHN PICKETT[2]

[1]KWS UK Limited, Thriplow, UK
[2]Rothamsted Research, Harpenden, UK
[3]University of Pannonia Georgikon Faculty of Agriculture, Keszthely, Hungary

SUMMARY

As an environmentally compatible alternative to the use of conventional insecticides to control cereal aphids, we have investigated the possibility to exploit natural resistance to insect pests in wheat varieties. We have tested a wide range of hexaploid (*Triticum aestivum)*, tetraploid (*T. durum)* and diploid (*T. boeoticum* and *T. monococcum)* wheat lines for resistance to the bird cherry oat aphid (*Rhopalosiphum padi)*. Lines tested included Russian wheat aphid (*Diuraphis noxia)*, greenbug (*Schizaphis graminum)*, hessian fly (*Mayetiola destructor)* and orange wheat blossom midge (*Sitodiplosis mosellana)* resistant varieties. Antixenosis and antibiosis were determined in the settling and fecundity tests respectively. Since hydroxamic acids (Hx), including the most generally active, 2,4-dihidroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), are biosynthesised in many cereal plants and are implicated in resistance against insects, leaf tissue was analysed for Hx and the glucosides from which they are produced. The hexaploid varieties, which contained relatively low levels of the DIMBOA glucoside, did not deter aphid feeding or reduce nymph production significantly. Reduced settlement and nymph production were recorded on the diploid varieties, but they contained no detectable level of the glucoside or the toxic aglucone.

INTRODUCTION

Aphids are serious pests worldwide, able to cause severe damage in cereal crops, particularly wheat, *Triticum aestivum*, by direct feeding and by transmitting plant pathogenic viruses such as Barley Yellow Dwarf Virus (BYDV) (Hand 1989, Thackray et al. 2009). With climate change, the importance of cereal aphid pests is increasing because many aphids are able to feed through the winter on cereal crops without recourse to sexual reproduction. Insecticides currently give sufficient protection, but can be expensive and environmentally undesirable. Some products may ultimately be lost due to the development of insecticide resistance. Resistance by aphids to insecticide was first recognised in the 1950s and now it is a global problem (Loxdale 2008). In addition, within the EU, legislation is in place to reduce the range and quantity of pesticide applied to cereal crops. An alternative approach is the development of insect resistant wheat varieties.

Resistance breeding against cereal aphids began in the 1970s. Resistance has been reported in *Triticum turgidum conv. durum, Aegilops squarosa syn.*, *Triticum tauschii* (Castro et al. 1999) and *Aegilops speltoides* (Castro et al. 2004) against greenbug *Schizaphis graminum*. Since then a series of resistant varieties were introduced but due to the development of a new biotype of the pest which could damage those varieties, it has become more important to find different effective plant defence mechanisms against aphids. The selection pressure to evolve a biotype which is able to adapt to antixenotic and/or antibiotic plants and break host plant resistance is high because aphids are able to produce several generations a year (Basky 2005) with a potential for large population growth in a single season from a single individual. The first Russian wheat aphid, *Diuraphis noxia*, resistant cultivar 'Halt' was released in the US in 1994; since then resistance has been developed in several wheat lines. In 2003 a new biotype of *D. noxia* was discovered which is able to feed and reproduce on resistant wheat varieties (Qureshi et al. 2005, Castro et al. 2005). Currently plant resistance to *D. noxia* is largely based on antibiosis effects and Qureshi et al. (2005) have suggested that combining different categories of resistance would be more effective and durable than resistance based on individual factors.

Hydroxamic acids (Hx) (1,4-benzoxazin-3-ones) are the main group of secondary metabolites involved in the resistance of certain cereals against bacteria, fungi and several insects including aphids (Thackray et al. 1991, Gianoli et al. 2000). Hydroxamic acids (Hx) are absent in the seed and increase following germination; the concentration peak is in the young seedlings (Gianoli et al. 2000) and mainly located in the mesophyll protoplasts, the vascular bundles (Givovich and Niemeyer 1995) and in the sieve elements (Givovich et al. 1994). After the seedling stage the Hx levels decrease in mature plants but the youngest tissues still show high concentrations of Hx (Gianoli et al. 2000). The maximum recorded Hx level in cultivated wheat is between 1.4–10.9 mmol/kg fresh weight, but nearly 40 mmol/kg fresh weight was found in wild varieties (Nicol et al. 1993). Hx compounds are present in the plant as 2-β-O-D-glucopyranosides (Gianoli et al. 2000), which are enzymatically hydrolyzed by endo-β-glucosides to DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3- one) when the tissue is injured (Givovich et al. 1994). DIMBOA, the main Hx aglucone in wheat extracts produces antibiosis, feeding deterrence, decreased performance and reduced reproduction in aphids (Figueroa et al. 2004).

In this study we investigate the differences between elite wheat varieties and some ancestral wheat species with the objective to help plant breeders to develope marketable aphid resistant wheat varieties in the future.

METHODS AND MATERIALS

Plant material

A series of 6x (hexaploid), 4x (tetraploid) and 2x (diploid) wheat species/cultivars was assembled from collections supplied by institutes and companies listed in the table 1.We included Russian wheat aphid (RWA), greenbug, hessian fly and orange wheat blossom midge resistant hexaploid and tetraploid varieties in our experiments.

Table 1. Origins of tested varieties

No	Accession	Name	Supplier	Resistance against	Gene	Species	Included in settling test	Included in fecundity test
1	PI137739	Gandium-I-Fasai	USDA	*Diuraphis noxia*	Dn1	Triticum aestivum	x	
2	PI262660	Turtsikum	USDA	*Diuraphis noxia*	Dn2	Triticum aestivum	x	x

3	PI294994	Strelinskaja Mestnaja	USDA	*Diuraphis noxia*	Dn8	Triticum aestivum	x	x
4	PI372129	Turcikum 57	USDA	*Diuraphis noxia*	Dn4	Triticum aestivum	x	
5	PI378921	Fiorello	USDA	*Diuraphis noxia*		Triticum aestivum	x	
6	PI435095	MV4	USDA	*Rhopalosiphum padi ?*		Triticum aestivum	x	x
7		Robigus	KWS UK	*Sitodiplosis mosellana*		Triticum aestivum	x	x
8		Istabraq	LIMAGRAIN			Triticum aestivum	x	x
9		Napier	MONSANTO			Triticum aestivum	x	x
10		Tasman	Australian Wheat Board			Triticum aestivum	x	x
11		Solstice	LIMAGRAIN			Triticum aestivum	x	x
12		Humber	KWS UK			Triticum aestivum	x	x
13		Welford	ELSOMS	*Sitodiplosis mosellana*		Triticum aestivum	x	x
14	CLTR17895	Largo	USDA	*Schizaphis graminum biotype C,E,H,I*	Gb3	Triticum aestivum	x	x
15		Svilena	KWS UK			Triticum aestivum	x	x
16		102	RRes			Triticum boeoticum	x	x
17		8116	RRes			Triticum boeoticum	x	x
18		8150	RRes			Triticum boeoticum	x	x
19		8404	RRes			Triticum boeoticum	x	x
20		MDR 298	RRes			Triticum boeoticum		x
21		Alifen	Chile (Niemeyer H.M.)			Triticum durum	x	x
22		920/1	ICARDA			Triticum durum	x	x
23		920/2	ICARDA			Triticum durum	x	x
24		920/3	ICARDA			Triticum durum	x	x
25		920/4	ICARDA			Triticum durum	x	x
26		920/9	ICARDA			Triticum durum	x	x
27		920/10	ICARDA			Triticum durum	x	x
28		920/11	ICARDA			Triticum durum	x	x
29		MDR 002	RRes			Triticum monococcum	x	
30		MDR 037	RRes			Triticum monococcum	x	
31		MDR 040	RRes			Triticum monococcum	x	
32		MDR 043	RRes			Triticum monococcum	x	x
33		MDR 044	RRes			Triticum monococcum	x	x
34		MDR 049	RRes			Triticum monococcum	x	x
35		MDR 050	RRes			Triticum monococcum	x	x
36	PI355520		RRes			Triticum monococcum	x	

Aphids

Rhopalosiphum padi was collected from volunteer wheat plants from the field in Thriplow, Herts, UK in August 2006, in September 2007 and again (refreshing the colony) in 2008. The colony used in this study was established from one aphid using the mildew resistant spring wheat variety Tybalt (from Limagrain) as the culture plant. The colony was kept in a glasshouse in a temperature range of 12-25C and light 16:8 L:D.

Settling test

This was a choice test between the test plant and the control plant which was the hexaploid wheat variety Solstice. The settling preference of alatae was tested on 7 days old seedlings, which were at the same growth stage. We used alatae because their olfactory receptors are more developed than in apterous aphids and alatae are the primary colonising morph. The experiment was set up on a wet sand tray to keep the humidity high in a 12 cm diameter by 20 cm high cage. One test and one control plant were placed into the cage with 20 *R.padi* alatae which were collected with an electric pouter from the top of the aphid colony cage. Alatae which had settled on each of the two plants were counted and recorded after 2, 5 and 24 hours from the beginning of the experiment. The test was conducted in the glasshouse at 20C, 16:8 L:D.

Fecundity test

This test was used to determine the intrinsic rate of population increase by recording how long it takes an aphid from birth to produce the first nymph and how many nymphs were produced over an equivalent time on the test varieties.

Seven alatae were put in a cage with one plant of the test variety for 24 hours to produce pre-conditioned nymphs for the experiment after which the alatae were removed. Nymphs were allowed to develop on those plants for 3-4 days until they reached a reasonable size making them easier to transfer onto the 7 days old test plants. One experimental 3 day old nymph was placed on the middle part of the first leaf in a 2cm diameter clip cage. The developing aphids were monitored daily at the same time each day. From the first day of nymph production the new nymphs were removed and recorded daily. The experiment was carried out in a glasshouse at ≈20C and 16:8 L:D.
From the data the intrinsic rate of population increase was calculated using the formula by Wyatt and White (1977).

intrinsic rate of population increase $r_m = c (\log_e Md) / d$
c = 0.74
d = pre-productive period (days)
Md = number of nymphs produced in the reproductive period equal to d

Hydroxamic acid analysis by high pressure liquid chromatography (HPLC)

Leaf samples were taken from 7 days old plants and frozen immediately in liquid nitrogen. Samples were ground in a pestle in liquid nitrogen, ensuring the sample remained frozen during preparation. 25mg of frozen sample was weighed into an Eppendorf tube containing 0.5ml Methanol and Acetic acid mixture. It was sonicated for 10 minutes and centrifuged for 10 minutes at 4C. Supernatant was transferred into a glass vial and run on the HPLC machine. Standard graphs of DIMBOA and DIMBOA-glucoside concentrations were used to work out the level of these compounds in the plant tissue in mmol /kg fresh weight.
A thermal hypersil C-18 column was used, mobile phase (A) water HPLC grade water (B) Methanol/Isopropanol(95/5) + 0.025% acetic acid. The gradient profile of solvent A and B was 0-2 min, 10% B; 2-11min, 10-50% B; 16-17min, 50 to 10% B. Injection volume 20µl, the flow rate was 1ml/min and the run time 17 minutes.

RESULTS

Settling test

Over 50 varieties were tested with the settling test assay (Figure 1.); we detected some differences in preference of aphids between the tested varieties.

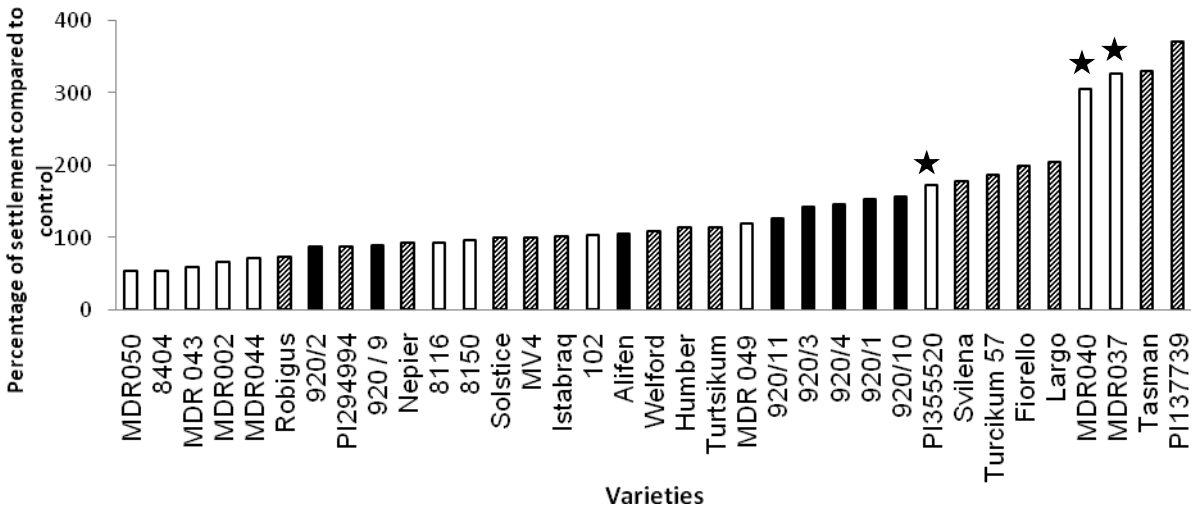

Figure 1. Settlement on the varieties in percentage compared to Solstice (control). 100%= the number of aphids settled on the control, ▨ hexaploid varieties, ■ tetraploid varieties, □ diploid varieties, ★ seedlings touched the top of the cage

A very diverse set of hexaploid wheats was tested and while a few showed some reduction in aphid preference several were more preferred than Solstice. The varieties carrying resistance genes to other insect pests did not show negative effects on *R. padi* settling behaviour except PI 294994 which contains the Dn8 resistance gene against RWA. This showed reduced settling but the difference was not significant compared to the control. The least preferred hexaploid variety was the orange wheat blossom midge resistant variety Robigus, where the settlement was lower by 27%, but not significant compared to Solstice. The variety PI 137739 (Dn1 resistance gene against RWA) had the greatest number of alatae settled, 3.7 times more than on the control. Some of the tetraploid accessions were less preferred by aphids such as 920/2 and 920/9, but these differences were not significant. The diploid varieties of *Triticum monococcum* (MDR 002, MDR 043, MDR 044, MDR 050) and *T. boeoticum* (8404) showed significantly reduced attraction for *R. padi* alatae. However, the diploids MDR 040 and MDR 037 had three times more alatae settled on the plants compared to the control but this result could be because of the growth habit of these lines. These diploid varieties grew faster and taller than the other test varieties and the tip of the leaf reached the top of the cage during the experiment. This probably allowed the alatae to walk onto the leaf instead of flying to their preferred variety. Diploid and hexaploid species are morphologically different, which could be one of the reasons why alatae found them less attractive. The tested diploid accessions have narrower, darker green leaves and in most cases strongly pigmented coleoptiles.

Fecundity test

In the fecundity test, nymphs were forced to feed on the test variety only and from the beginning of the experiment the test aphid was observed daily to determine the time taken to become adult and to the start of nymph production. Nymphs were counted, recorded and removed daily over an equivalent time scale. From the data we calculated the intrinsic rate of population increase (r_m) which showed differences in aphid development rate between the varieties (Figure 2.).

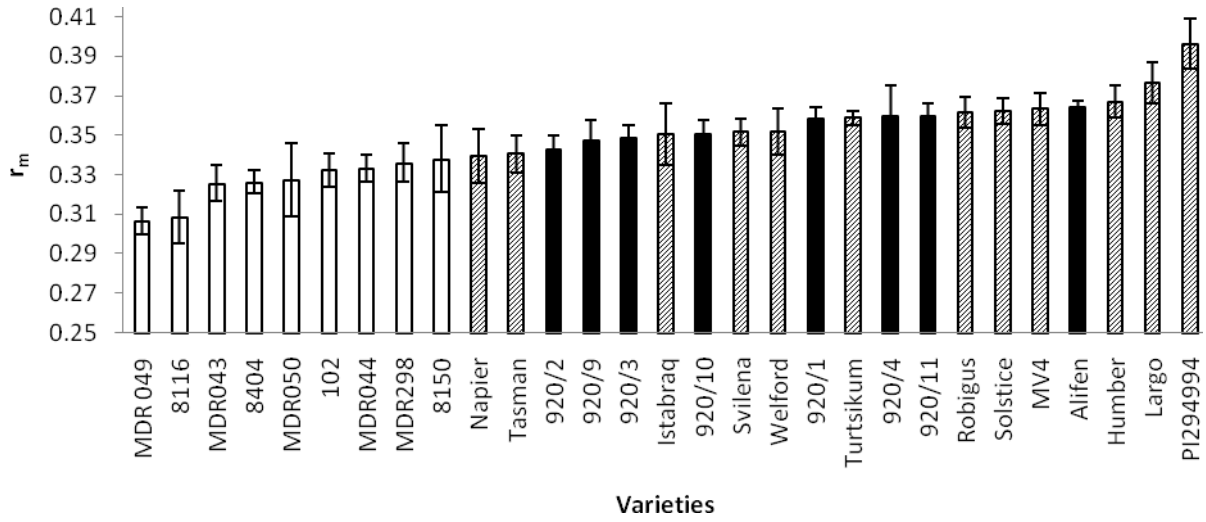

Figure 2. Intrinsic rate of population increase (r_m), ▨ hexaploid varieties, ■ tetraploid varieties, □ diploid varieties

When grouped by ploidy, the slowest nymph maturation was recorded on diploid varieties where *R. padi* took 9-10 days to become an adult. However, nymphs feeding on hexaploid and tetraploid varieties only took 7-9 days from birth. This difference showed in the size as well. On the diploid varieties aphids were smaller and produced fewer offspring than on the hexaploid varieties.

The average of total nymph production was 53.8 on the hexaploid varieties, 57.1 on the tetraploid and we found the lowest nymphs production on the diploid varieties where the average was 42.3. In the fecundity test we included some of the RWA and greenbug resistant varieties, in particular PI 294994, which was less preferred in the settling test then Solstice. These had no negative effects on the nymph production of *R. padi* and moreover, PI 294994 had the highest intrinsic rate of population increase (Figure 2). Robigus, the orange wheat blossom midge resistant variety showed similar results to PI294994, in the settling test it was the least attractive hexaploid wheat but in the fecundity test did not reduce nymph production. Tasman, an Australian hexaploid variety, which was reported by Wu et al, 2001, to have high constitutive levels of DIMBOA, was highly attractive to *R. padi* alatae in the settling test (Figure 1) but nymph production was reduced compared to Solstice in the fecundity test (Figure 2). Aphids on the diploid species *T. monococcum* and *T. boeoticum* reduced nymph production significantly compared to the tetraploid and hexaploid varieties. These results indicate that the diploid varieties may contain attributes that could be important in the resistance breeding against aphids in the future.

Hydroxamic acid analysis

The level of hydroxamic acids was measured in the leaf tissue of a small number of the test varieties (Table 2). In the hexaploid varieties we were able to detect DIMBOA, the main aglucone of the hydroxamic acid pathway, for which the peak appeared at 14.2-14.3 minutes on the HPLC and its non toxic form, DIMBOA-glucoside, which ran at 13.1 minutes. Of the hexaploid varieties tested, the highest level of DIMBOA was recorded in the variety Tybalt, whose leaf tissue contained 6.9 mmol DIMBOA per kilogram fresh weight in the 7 days old plant. The lowest level of DIMBOA (1 mmol per kilogram fresh weight) was found in

Solstice. We could not detect either of these compounds in the leaf tissue of the diploid varieties, but in all the AA genome diploid species accessions from *T. monococcum* and *T. boeoticum* we recorded an unknown peak at 13.3-13.4 minutes. Analysis of these peaks is not yet complete but it is considered likely that these may be related to hydroxamic acids.

Table 2. DIMBOA glucoside and DIMBOA level in the leaf tissue of the tested varieties

Variety	DIMBOA glucoside mmol/kg fresh weight	DIMBOA mmol/kg fresh weight
Tybalt	1.91	6.91
Solstice	0.5	1.01
Napier	0.81	4.02
MDR 050	0	0
MDR 298	0	0
8404	0	0

DISCUSSION

The settling test is a choice test based on physical and chemical differences between the test plant and the control plant (the hexaploid wheat variety Solstice). A very diverse set of hexaploid wheats were tested and while a few showed some reduction in aphid preference (not significantly) several were significantly more preferred than Solstice. The varieties carrying resistance genes to other insect pests did not have negative effects on aphid settling behaviour. The greatest number of alatae was noted on the RWA resistant hexaploid variety PI 137739 which has the Dn1 resistance gene. The least preferred varieties belong to the *T. monococcum* and *T. boeoticum* species, both AA genome diploid species. Significantly lower numbers of aphids settled on these plants than on the control.

In the fecundity test, aphids had no choice but to feed on the test plants and substantial differences in the rate of reproduction of *R. padi* between varieties was seen. All the A genome diploid species varieties tested showed significantly reduced intrinsic rate of population increase, both in the development time and in the size of the aphids. As with the settling test the insect resistant plants showed the same effect as the ordinary hexaploid varieties and did not reduce the reproduction of the *R. padi*. Thus these results have been unable to show any antixenosis or antibiosis efficacy of the Russian wheat aphid, greenbug or orange blossom midge resistance sources against *R. padi.*

Leaf tissue was tested for secondary metabolites, which may play an important role in resistance against bacteria, insects and nematodes. DIMBOA, one of the main hydroxamic acids, was found in the hexaploid varieties. The highest level 6.9 mmol/kg fresh weight of DIMBOA was recorded in Tybalt. In none of the leaves of the diploid varieties could we detect any DIMBOA or DIMBOA glucoside, but in all the AA genome diploid species we recorded an unknown peak at 13.3-13.4 minutes.

In artificial feeding studies (Niemeyer and Givovich 2000.) it was suggested that a single relationship between the level of DIMBOA in the host plant and the survival of feeding aphids would exist. However, our results show the level of hydroxamic acids that we found in the hexaploid varieties did not reduce reproduction or have a feeding deterrent effect, although equivalent amounts would be expected to have an effect in artificial feeding tests. This could indicate that aphids feeding *in situ* may be able to sequester or detoxify the toxic aglucone or alternatively the free DIMBOA may not be present in the phloem and therefore is not ingested by phloem feeding aphids. Furthermore, on the diploid varieties where reduced reproduction and settling was detected, no DIMBOA (or DIBOA) was present in the

leaf tissue. The cause of the reduced fecundity on these diploid varieties is at present unknown, but we may speculate that this could be effected by a generally reduced nutritional value of diploid plants to the aphid or could be caused by the unidentified compound detected on the HPLC.

Acknowledgement

This work was funded by Biotechnology and Biological Sciences Research Council and KWS UK Limited with the academic support from Rothamsted Research and the University of Pannonia Georgikon Faculty of Agriculture.

References

BASKY, ZS. (2005): Levéltetvek. Mezőgazda Kiadó p14-18

CASTRO, M. A., VASICEK, A., ELLERBROOK, C., GIMÉNEZ, D., TOCHO, E., TACALITI, S. M., CLÚA, A. A. AND SNAPE W. J. (2004): Mapping quantitative trait loci in wheat for resistance against greenbug and Russian wheat aphid. Plant Breeding, **123.** p361-365

CASTRO, M. A., VASICEK, A., MANIFIESTO, M., GIMÉNEZ, O. D., TACALITI, M. S., DOBROVOLSKAYA, O., RÖDER, M. S., SNAPE W. J. AND BÖRNER A. (2005): Mapping antixenosis genes on chromosome 6A of wheat to greenbug and to a new biotype of Russian wheat aphid. Plant Breeding, **124.** p229-233

FIGUEROA, C.C., SIMON, J., GALLIC, J., PRUNIER – LETERME, N., BRIONES, L. M., DEDRYVER, C. AND NIEMEYER, H. M. (2004): Effect of host defense chemicals on clonal distribution and performance of different genotypes of the cereal aphid *Sitobion avenae.* Journal of Chemical Ecology. **30.** (12) p2515-2525

GIANOLI E., RÍOS, J.M. & NIEMEYER H.M. (2000) Allocation of a hydroxamic acid and biomass during vegetative development in rye. Acta Agriculturae Scandinavica, Section B. Soil and Plant Science, **50.** p35-39.

GIVOVICH, A. & NIEMEYER, H.M. (1994): Effect of hydroxamic acids on feeding behavior and performance of cereal aphids on wheat. European Journal of Entomology **91** p371-374.

GIVOVICH, A. AND NIEMEYER, H. M. (1995): Comparison of the effect of hydroxamic acids from wheat on five species of cereal aphids. Entomologia Experimentalis et Applicata **74** p115-119

NIEMEYER, H.M. AND GIVOVICH, A. (2000) Use of electropenetration graphs and phloem sap chemical analysis in studies of the effects of hydroxamic acids in cereals on aphid (Homoptera: Aphididae) feeding behavior. In Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior, Walker, G.P. & Backus, E.A., eds. Thomas Say Publications in Entomology: Proceedings of the Entomological Society of America, Lanham, MD, USA, p237-244.

HAND, C.S. (1989): The overwintering of cereal aphids on Gramineae in southern England, 1977-1980. Annual Applied Biology, **115.** p17-29

LOXDALE, H.D. (2008): The nature and reality of the aphid clone: genetic variation, adaptation and evolution. Agricutural and Forest Entomology, **10.** p81-90

NICOL, D., WRATTEN, S.D., EATON, N., COPAJA, S.V. AND NIEMEYER, H.M. (1993): Hydroxamic acids in wheat: antibiosis, antixenosis and effects upon aphid susceptibility to an insecticide. IOBC/WPRS Bulletin **16.** p130-139

THACKRAY, D.J., WRATTEN, S.D., EDWARDS, P.J. & NIEMEYER, H.M. (1991): Hydroxamic acids - potential resistance factors in wheat against the cereal aphids *Sitobion avenae* and *Rhopalosiphum padi*. Proceedings of 1990 Brighton Pest Control Conference-Pests and Diseases-1990. p215-220

THACKRAY, D.J., DIGGLE, A.J. AND JONES, R.A.C. (2009): BYDV predictor: a simulation model to predict aphid arrival, epidemics of Barley yellow dwarf virus and yield losses in wheat crops in a Mediterranean – type environment. Plant Pathology, **58.** p186-202

QURESHI, A. J., JYOTI, L. J. AND MICHAUD P. J. (2005): Differential colonization of wheat cultivars by two biotypes of Russian wheat aphid (Homoptera: Aphididae). Insect Science, **12.** p341-349

WU, H., HAIG, T., PRATLEY, J., LEMERLE, D. AND AN, M. (2001) Allelochemicals in wheat (*triticum aestivum* l.): production and exudation of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. Journal of Chemical Ecology, **27,** p1691-1700.

WYATT, J.I. AND WHITE, F.P. (1977): Simple estimation of intrinsic rates for aphids and tetranychid mites. Journal of Applied Ecology, **14.** p757-766