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EFFECT OF HOST DEFENSE CHEMICALS ON CLONAL DISTRIBUTION AND PERFORMANCE OF DIFFERENT GENOTYPES OF THE CEREAL APHID *Sitobion avenae*

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Abstract—Five microsatellite loci were used to study the genetic variability and population structure of *Sitobion avenae* (Hemiptera: Aphididae) on some of its host plants. Individuals were collected in Chile from different cultivated and wild Poaceae. Forty-four multilocus genotypes were found among the 1052 aphids analyzed, of which four represented nearly 90% of the sample. No specialist genotypes were found, although some preferred hosts endowed with chemical defenses, *i.e.*, hydroxamic acids (Hx), while others preferred comparatively undefended hosts. Performances of some predominant and some rare genotypes were evaluated on plants differing in their Hx levels. Significant differences in performance were found among clones, the two most common genotypes showing no differences in performance among all hosts tested, and the rare genotypes showing enhanced performance on the host with highest Hx level. A hypothesis is proposed whereby the appearance of rarer genotypes is in part related to the presence of Hx.

Key Words—*Sitobion avenae*, microsatellites, genetic diversity, host structuring, chemical defenses, PCR analysis.

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INTRODUCTION

Host secondary chemistry constitutes one of the main factors guiding the evolution of plant–insect interactions (Scriber, 2002; Becerra, 2003), via the selection of phytophagous insect populations (Erlhich and Raven, 1964). Aphids, which are mostly found as clonal populations, are highly host-specific insects (Dixon, 1998); hence, genetic differentiation according to the host plant and the existence of biotypes or host races specifically adapted to some host species may occur (Via, 1991; Vanlerberghe-Masutti and Chavigny, 1998; Lushai et al., 2002; Massonnet et al., 2002; Via and Hawthorne, 2002; Miller et al., 2003; Simon et al., 2003). In cereal aphids, the host plant has a strong impact on genetic structure and clonal diversity of populations, as evidenced by several studies using molecular markers (De Barro et al., 1995a,b; Simon and Hebert, 1995; Lushai et al., 1998; Haack et al., 2000; Figueroa et al., 2002). In particular, temporal and spatial clonal structures based on different host plants have been demonstrated in *Sitobion avenae* (Fabricius) (Caillaud et al., 1995; De Barro et al., 1995a,b; Figueroa et al., 2002). However, no attempt has been made to relate host-based population structure with the occurrence of specific secondary metabolites in the plant.

Hydroxamic acids (Hx) are the main group of secondary metabolites involved in cereal resistance against aphids (Niemeyer, 1988; Niemeyer and Pérez, 1995; Sicker and Schultz, 2002). Hx exist in the intact plant as glucosides (Cambier et al., 1999), which are hydrolyzed to the more toxic aglucones by endo-β-glucosidases when the tissue is injured (Hofman and Hofmanova, 1969; Cuevas et al., 1992; Sue et al., 2000) (Figure 1). DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), the main Hx aglucone in wheat extracts (Sicker et al., 2000), produces antibiosis, feeding deterrence, and decreased performance and reproduction in aphids (Niemeyer and Pérez, 1995), and exhibits mutagenic effects (Hashimoto et al., 1979; Hashimoto and Shudo, 1996). More recently, it has been shown to affect the level of genetic polymorphism in aphid populations (Figueroa et al., 2002).

Sitobion avenae was introduced in Chile in the 1970's (Apablaza, 1974), and has invaded both cultivated and wild Poaceae, such as wheat, oat, barley, maize, cocksfoot grass, and wild *Hordeum*. In order to test whether the population genetic

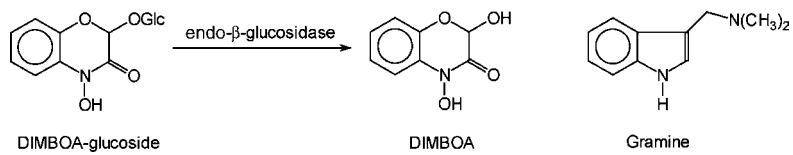

FIG. 1. Secondary metabolites in cereals.

structure of an aphid species is affected by the chemistry of its host plants, the genetic diversity and clonal distribution of *S. avenae* in Chile according to host plants differing in their chemical defenses was examined. It was hypothesized that if a differential distribution of genotypes of *S. avenae* on hosts occurs, this could be a consequence of different adaptive responses of aphid genotypes to plant chemistry. To test this hypothesis, the performance of different *S. avenae* genotypes on hosts differing in Hx level was evaluated.

METHODS AND MATERIALS

Aphid Sampling. *Sitobion* aphids were sampled in Central–South Chile (from 33° to 41°S lat.) on available hosts where they were present at the time and site of collections. The survey included crops of durum wheat (*Triticum durum* L.) and oat (*Avena sativa* L.), and field margins and other surrounding areas with cocksfoot grass (*Dactylis glomerata* L.), wild oat (*Avena fatua* L.), and mouse barley (*Hordeum murinum* L.). In fields, the collection was performed throughout a linear transect, irrespective of the level of aphid infestation and host plant abundance. On field margins, the collection depended on the distribution of the plants. In both cases, an individual aphid was collected from a plant separated by at least 10 m from the last sample in order to limit the chance of resampling individuals from the same parthenogenetic mother. Samples were collected in 95% ethanol and preserved at −20°C prior to their utilization. *Sitobion* individuals were determined as *S. avenae* or *S. fragariae* according to Figueroa et al. (1999b).

DNA Extraction and PCR Amplifications. Genomic DNA was extracted from single wingless adult aphids according to Sunnucks and Hales (1996), and the DNA was precipitated in ethanol and resuspended in 20–40 μl of sterile ultrapure water depending on the aphid size. PCR amplifications of microsatellite loci (*Sm10, Sm11, Sm17, S3.R*, and *S5.L*; Sunnucks et al., 1996; Simon et al., 1999; Wilson et al., 2004) were prepared in a 15 μl reaction volume, including 0.5 units of *Taq* DNA polymerase (Invitrogen, USA), Mg^{2+}-free reaction buffer, 2 mM $MgCl_2$, 200 μM dNTPs, 10 pmol of each primer (BiosChile-IGSA, Chile), and about 10 ng of aphid DNA. PCR reactions were carried out in a Perkin-Elmer 9700 thermocycler using the following steps: an initial denaturation for 2 min at 94°C, and 40 cycles consisting of denaturation for 40 sec at 94°C, annealing for 45 sec with temperature depending on locus (Sunnucks et al., 1996; Simon et al., 1999), and elongation at 72°C for 45 sec. For the last cycle, the elongation time was extended to 4 min. The PCR reaction was mixed with 4x loading buffer (Sambrook et al., 1989), denatured for 3 min at 95°C, loaded on to a 6% polyacrylamide-urea gel, and subjected to electrophoresis in 0.5X TBE buffer at 1.0 kV. After electrophoresis, the gel was silver stained as described in Haack et al. (2000). The

size of the alleles of each locus was estimated by using a sequencing size ladder corresponding to the sequence of pGEM®-3Zf(+) vector (Promega, USA).

Genetic Analysis. Allelic polymorphism was examined at five microsatellite loci. Each multilocus genotype was characterized by its unique allelic combination at the five loci. Each host plant was considered as a population, and all subsequent analyses were performed on this sampling unit. The effect of the host plant on the distribution of genotypes was analyzed using Fisher's exact tests (Fisher, 1925).

Performance of Aphid Genotypes. Monoclonal colonies, which started from individual field-collected aphids, were maintained on oat for at least three generations. Seven genotypes were selected according to their prevalence and their host range: four very frequent and widely distributed genotypes (Sa1, Sa2, Sa3, and Sa4; Table 1 and Table 2), two genotypes with a few collections (Sa7: 14 copies; and Sa10: 5 copies), and one single-collection-genotype (Sa36). The rare genotype Sa7 was detected on oat ($N = 8$), cocksfoot grass ($N = 2$), and wheat ($N = 4$), whereas Sa10 was restricted to oat ($N = 1$) and wheat ($N = 4$). The unique genotype Sa36 was only detected on wheat. The performance of each of these genotypes was evaluated on host plants with different levels of Hx, i.e., oat (no Hx; control), and wheat cultivars differing in their Hx concentration: low Hx cv. Huayún and high Hx cv. Chagual (mean ± SE: 0.667 ± 0.097 and 3.595 ± 0.257 mmol/kg fresh wt, respectively, $N = 10$, two-leaf seedlings). Although these wheat lines are not isogenic, negative correlations between Hx level and aphid performance have been described, which include intra- and interspecific variation in cereal taxa (e.g., Argandoña et al., 1980). From each genotype (clone), one 5-day-old larva (synchronization of larvae to *ca*. 12 h) was transferred to a potted 8-day-old seedling and enclosed in a clip-cage. Larvae were observed daily, and the pre-reproductive period was recorded (T). The progeny produced in the subsequent T days was counted (Md) and removed daily. The intrinsic rate of increase (r_m) (Birch, 1948), a synthetic estimate of performance, was determined by the equation of Wyatt and

TABLE 1. SAMPLE SIZE, NUMBER OF GENOTYPES, AND FREQUENCY OF THE FOUR MOST FREQUENTLY OCCURRING MULTILOCUS GENOTYPES OF *Sitobion avenae* PER HOST PLANT

	Host plant					
	Oat	Wild-oat	Mouse barley	Cocksfoot grass	Wheat	Total
Sample size	175	27	18	70	762	1052
Number of genotypes	15	4	4	15	35	44
Sa1 (378 collections)	0.12	0.04	0.39	0.04	0.45	0.36
Sa2 (340 collections)	0.46	0.37	0.28	0.54	0.27	0.32
Sa3 (127 collections)	0.13	0.33	0.28	0.01	0.12	0.12
Sa4 (75 collections)	0.17	0.26	0	0.13	0.04	0.07
Sa5–Sa44 (132 collections)	0.12	0	0.05	0.28	0.12	0.13
Total	1.00	1.00	1.00	1.00	1.00	1.00

TABLE 2. SITES WHERE DIFFERENT CLONES OF *Sitobion avenae* WERE COLLECTED

Zone	Main focal cities	Mean latitude	Clones collected
1	Santiago-Rancagua	33° S	1-2-3-4-6-7-14-15-16-17-20-26-27-34-37-42-43
2	Talca	35° S	1-2-3-4-6-7-14-21-23-24-32-38-41
3	Chillán-Los Angeles	37° S	1-2-3-4-7-8-10-35
4	Temuco	39° S	1-2-3-4-5-7-8-9-10-11-12-13-16-18-19-20-22-25-28-29-30-31-33-39-40-44
5	Osorno	41° S	1-2-3-36

White (1977): $r_m = 0.738$ (ln Md)/T. A range of 7–16 replicates was used per genotype. Statistical significance for all performance comparisons was computed by using two-way ANOVA (factors: aphid genotype and host) with the STATISTICA package (StatSoft, 2004); multiple comparisons were performed with a LSD test in the same program. Performance data were adjusted to normal distribution by using the logarithmic transformation (Sokal and Rohlf, 1981).

RESULTS

Genetic Diversity and Structuring of Sitobion avenae *Populations.* Combining the five microsatellite loci, 44 multilocus genotypes were characterized among the 1052 sampled individuals of *S. avenae*. As a result of this clonal amplification, genotypic diversity was very low on each host plant as well as in the complete data set (Table 1). Overall, only 4% of the collections of Chilean *S. avenae* consisted of unique genotypes while the four most abundant genotypes (Sa1–Sa4, Table 1 and Table 2) represented nearly 90% of the sample. This pattern suggests that populations of *S. avenae* in Chile are mainly determined by clonal reproduction of a few genotypes, which is confirmed by strong deviations from Hardy–Weinberg equilibrium and frequent linkage disequilibrium in these populations (data not shown). Since each five-locus genotype should represent a clone, the population structure was analyzed in terms of clonal frequencies, thus studying the effects of host plants on the distribution of the clones.

Distribution of Sitobion avenae *Genotypes.* The frequency of all multilocus genotypes was compared among the five host plants. The global effect of hosts on frequency distribution of genotypes was significant (Fisher's exact test, $P < 0.01$). Differences were observed among the genotypic frequencies in most host plant comparisons (Fisher's exact test, $P < 0.05$). When only the three most-infested host plants (wheat, oat, and cocksfoot grass) were considered, differences were observed between oat and cocksfoot grass ($P < 0.03$), and highly significant differences were observed between aphid populations on wheat and cocksfoot grass ($P < 0.001$), and on wheat and oat ($P < 0.001$). Interestingly, these differences

were observed between host plants with chemical defenses against aphids (wheat) and host plants without such defenses (oat and cocksfoot).

Since some rare (less than 22 individuals collected; Sa5–Sa22) or unique genotypes (Sa23–Sa44) of *S. avenae* were restricted to a single host plant (20 genotypes on wheat, seven on cocksfoot grass, and two on oat), an additional comparison was performed that included the four more frequent *S. avenae* genotypes (Sa1–Sa4). These most frequently occurring genotypes contributed to more than 80% of the total variance, the remaining 20% of the variation being explained by the bulk of rare genotypes (Sa5–Sa44). Hence, subsequent analyses were performed considering only the four most frequent genotypes (Table 1). Significant differences were found between genotypic frequencies when they were sampled from all five hosts ($P < 0.001$), and when they were sampled only from wheat, oat, and cocksfoot grass ($P < 0.001$).

Effect of the Host Plant on Performance Sitobion avenae *of* Genotypes. Performances, as measured by r_m, showed highly significant effects for the genotype (two-way ANOVA's, $P < 0.002$), the host plant ($P < 0.02$), and their interaction ($P < 0.03$, Figure 2). At an inter-host level, the frequent and widely distributed genotypes, Sa1 and Sa2, exhibited comparable performances on the three hosts tested ($P > 0.05$ on three possible host comparisons), while the remaining genotypes exhibited a higher performance on the host with highest Hx level. At an intra-host level, differences between clones were observed only when Hx were present in the host. Thus, on cv. Chagual, two groups of genotypes showed highly significant differences among their performances: genotypes Sa3, Sa4, Sa7, Sa10, and Sa36 exhibited a significantly higher performance than Sa1 and Sa2 (*a posteriori* test, $P < 0.004$), while on cv. Huayún, genotypes Sa4 and Sa10 showed a higher performance ($P < 0.03$), but only in comparison to genotype Sa2. Hence, Hx are involved in the effect of host and aphid genotype on performance, and the effect of the interaction between host and aphid genotype may also be related to the levels of Hx. This effect on performance is predominately observed for rare and unique aphid genotypes, which are mainly detected on wheat in the field.

DISCUSSION

The distribution of multilocus genotypes characterized in Chilean populations of *S. avenae* appears to be influenced by the presence of chemical defenses in their host plants. No specialized genotypes (i.e., collected on a single host plant) were found. However, some genotypes seem to be more specialized than others, as shown by their different collection frequencies on the various plant species examined (Table 1). For example, the second most frequently occurring genotype, Sa2, occurred at similar frequencies on all host plants, while genotype Sa1 showed

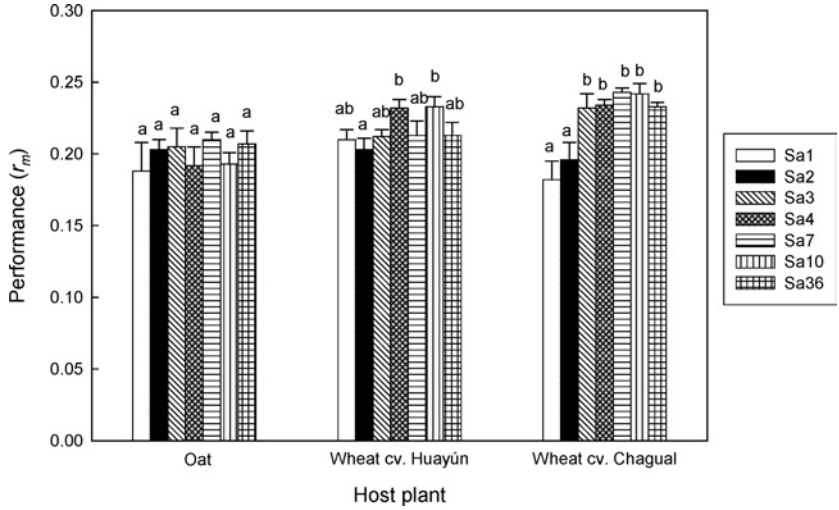

FIG. 2. Performances (r_m) of different genotypes of *Sitobion avenae* on oat (lacking hydroxamic acids—Hx; control), and wheat cultivars cv. Huayún (low-Hx) and cv. Chagual (high-Hx). Different letters indicate significant differences for intra-host comparisons (two-way ANOVA followed by LSD tests). The number of replicates performed for clones Sa1, Sa2, Sa3, Sa4, Sa7, Sa10, and Sa36 on the different hosts were as follows: oat (8, 13, 11, 9, 7, 11, 8), wheat cv. Huayún (12, 15, 12, 9, 9, 12, 10), and wheat cv. Chagual (12, 16, 10, 9, 10, 11, 10).

significant differences among several host plants (Table 1), exhibiting a higher prevalence on wheat and mouse barley than on oat, wild oat, or cocksfoot grass.

Chemical differences are known among the plant species studied. Wheat contains Hx, a family of secondary metabolites involved in deterrence and antibiosis against aphids (Niemeyer and Pérez, 1995), while *Hordeum* spp. may contain Hx (Barría et al., 1992) or gramine (Figure 1), an indolic alkaloid involved in resistance against aphids (Zúñiga and Corcuera, 1986; Gianoli and Niemeyer, 1998). These groups of metabolites are widely distributed among the Poaceae, but have not been detected in oat, wild oat, or cocksfoot grass (Niemeyer and Pérez, 1995; Niemeyer, unpublished data). Thus, plant chemical defenses and/or detoxification mechanisms in aphids may affect the clonal structure of *S. avenae* (Figueroa et al., 1999a; Loayza-Muro et al., 2000). Additionally, differences in the composition of RAPD-PCR phenotypes of *S. avenae* have been shown (Figueroa et al., 2002), with Hx affecting genetic variability in *S. avenae* populations.

These findings, in part, may explain the relative aphid distribution and abundance among hosts, as well as the nature of the most common genotypes of *S. avenae* found in Chile. Thus, genotype Sa1, the most abundant in Chile, showed

the highest frequency on hosts containing secondary chemistry that provides aphid resistance, i.e., wheat and mouse barley (Table 1). However, performance of genotype Sa1 was similar among host plants (Figure 2). The capacity of Sa1 to thrive on defended plants is a factor conferring on this genotype a particularly relevant colonizing ability. Additionally, the second most abundant genotype Sa2 was also observed with similar frequencies and performances on all host plants. These observations suggest that genotypes Sa1 and Sa2 could be the result of clonal selection promoting the evolution of general-purpose genotypes (Lynch, 1984), which would be characterized by a broad host range and a low variance for its performance on host plants with different defense chemicals levels (Figure 2).

Concerning the least common genotypes, the following model may account for their low frequency and their distribution and performance on different host plants. Introduction events of insect species have often been reported, and the low number of the individuals introduced initially leads to low genetic diversity of populations (genetic bottleneck) (Huey et al., 2000; Downie, 2002). Given the low genetic diversity and the strong clonal amplification in Chilean populations of *S. avenae*, it is likely that only a few clones were introduced into Chile some 30 years ago (Apablaza, 1974). The introduced clones were subjected, among other factors, to the selection pressure of Hx present in wheat, which occur at higher concentrations in cultivars sown in Chile than in Europe (Copaja et al., 1991; Nicol et al., 1992; Caillaud and Niemeyer, 1996), the likely region of origin of the introduced individuals (Figueroa et al., unpublished). In the absence or rarity of sexual reproduction (suggested by the predominance of a few multilocus genotypes along with departure from the Hardy–Weinberg equilibrium), emergence of new clones could occur not only as a consequence of new introductions, but also from spontaneous or induced point mutations (Dixon, 1998; Lushai et al., 2003; Wilson et al., 2003). Hydroxamic acids are mutagenic agents (Hashimoto et al., 1979; Hashimoto and Shudo, 1996), and induce the emergence of new clones within populations of *S. avenae* (Figueroa et al., 2002). New genotypes, initially at low frequencies, may have survived and even increased in relative abundance due to their enhanced performance on the best defended hosts. Indeed, some genotypes examined in this study other than the most frequent genotypes (likely, new genotypes) showed the highest performance on the host with the highest concentration of Hx (Figure 2). It is likely that these genotypes have acquired the capacity to detoxify allelochemicals such as Hx in a more efficient manner (Figueroa et al., 1999a; Loayza-Muro et al., 2000; Mukanganyama et al., 2003), or to sequester them more efficiently, as has been shown to occur in other aphid species (Wink and Romer, 1986). Based on the model presented herein, it is predicted that through time, these Hx-resistant clones will begin to prevail. Future assessments of the clonal composition of the Chilean populations of *S. avenae* will be necessary to test this prediction. Since other factors, such as climatic conditions, intraspecific competition, and reaction to natural enemies and to overwintering

refuges, can also constitute selective forces in the field, we propose Hx as an additional factor of population structuring. Further research will lead to a better understanding of the relative contributions of Hx and other factors to the genetic make-up of cereal aphid populations in the field.

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REFERENCES

ARGANDOÑA, V. H., LUZA, J. G., NIEMEYER, H. M., and CORCUERA, L. J. 1980. Role of hydroxamic acids in the resistance of cereals to aphids. *Phytochemistry* 19:1665–1668.

APABLAZA, J. 1974. Presencia de *Macrosiphum* (*Sitobion*) *avenae* (F.) (Homoptera, Aphididae) en sementeras de trigo en Chile. *Ciencia e Invest. Agr.* 1:69–70.

BARRÍA, B. N., COPAJA, S. V., and NIEMEYER, H. M. 1992. Occurrence of DIBOA in wild *Hordeum* species and relation to aphid resistance. *Phytochemistry* 31:89–91.

BECERRA, J. X. 2003. Synchronous coadaptation in an ancient case of herbivory. *Proc. Natl Acad. Sci. USA* 100:12804–12807.

BIRCH, L. C. 1948. The intrinsic rate of natural increase of an insect population. *J. Anim. Ecol.* 17:15–26.

CAILLAUD, C. M. and NIEMEYER, H. M. 1996. Possible involvement of phloem sealing system in the acceptance of a plant as host by an aphid. *Experientia* 52:927–931.

CAILLAUD, C. M., DEDRYVER, C. A., DI PIETRO, J. P., SIMON, J. C., FIMA, F., and CHAUBET, B. 1995. Clonal variability in the response of *Sitobion avenae* (Homoptera: Aphididae) to resistant and susceptible wheat. *Bull. Entomol. Res.* 85:189–195.

CAMBIER, V., HANCE, T., and DE HOFFMANN, E. 1999. Non-injured maize contains several 1,4-benzoxazin-3-one related compounds but only as glucoconjugates. *Phytochem. Anal.* 10:119–126.

COPAJA, S. V., NIEMEYER, H. M., and WRATTEN, S. D. 1991. Hydroxamic acid levels in Chilean and British wheat seedlings. *Ann. Appl. Biol.* 118:223–227.

CUEVAS, L., NIEMEYER, H. M., and JONSSON, L. M. V. 1992. Partial purification and characterization of a hydroxamic acid glucoside β-D-glucosidase from maize. *Phytochemistry* 31:2609–2612.

DE BARRO, P. J., SHERRATT, T. N., DAVID, O., and MACLEAN, N. 1995a. An investigation of the differential performance of clones of the aphid *Sitobion avenae* on two host species. *Oecologia* 104:379–385.

DE BARRO, P. J., SHERRATT, T. N., BROOKES, C. P., DAVID, O., and MACLEAN, N. 1995b. Spatial and temporal genetic variation in British field populations of the grain aphid *Sitobion avenae* (F.) (Hemiptera: Aphididae) studied using RAPD-PCR. *Proc. R. Soc. Lond. B* 262:321–327.

DIXON, A. F. G. 1998. Aphid Ecology. Chapman&Hall, London.

DOWNIE, D. A. 2002. Locating the sources of an invasive pest, grape phylloxera, using a mitochondrial DNA gene genealogy. *Mol. Ecol.* 11:2013–2026.

ERLHICH, P. R. and RAVEN, P. H. 1964. Butterflies and plants: A study in plant coevolution. *Evolution* 18:586–608.

FIGUEROA, C. C., KOENIG, C., ARAYA, C., SANTOS, M. J., and NIEMEYER, H. M. 1999a. Effect of DIMBOA, a hydroxamic acid from cereals, on peroxisomal and mitochondrial enzymes from aphids: evidence for the presence of peroxisomes in aphids. *J. Chem. Ecol.* 25:2465–2475.

FIGUEROA, C. C., SIMON, J. C., LE GALLIC, J. F., and NIEMEYER, H. M. 1999b. Molecular markers to differentiate two morphologically-close species of the genus *Sitobion* (Homoptera: Aphidoidea). *Ent. Exp. Appl.* 92:217–225.

FIGUEROA, C. C., LOAYZA-MURO, R., and NIEMEYER, H. M. 2002. Temporal variation of RAPD-PCR phenotype composition of the grain aphid *Sitobion avenae* (Hemiptera: Aphididae) on wheat: The role of hydroxamic acids.*Bull. Entomol. Res.* 92:25–33.

FISHER, R. A. 1925. Statistical Methods for Research Workers. Oliver and Boyd, Edinburgh.

GIANOLI, E. and NIEMEYER, H. M. 1998. DIBOA in wild Poaceae: sources of resistance to the Russian wheat aphid (*Diuraphis noxia*) and the greenbug (*Schizaphis graminum*). *Euphytica* 102:317–321.

HAACK, L., SIMON, J. C., GAUTHIER, J. P., PLANTEGENEST, M., and DEDRYVER, C. A. 2000. Evidence for predominant clones in a cyclically parthenogenetic organism provided by combined demographic and genetic analysis. *Mol. Ecol.* 9:2055–2066.

HASHIMOTO, Y. and SHUDO, K. 1996. Chemistry of biologically active benzoxazinoids. *Phytochemistry* 43:551–559.

HASHIMOTO, Y., SHUDO, K., OKAMOTO, T., NAGAO, M., TAKAHASHI, Y., and SUGIMURA, T. 1979. Mutagenicities of 4-hydroxy-1,4-benzoxazinones naturally occurring in maize plants and of related compounds. *Mutat. Res.* 66:191–194.

HOFMAN, J. and HOFMANOVA, O. 1969. 1,4-Benzoxazine derivatives in plants. Sephadex fractionation and identification of a new glucoside. *Eur. J. Biochem.* 8:109–112.

HUEY, R. B., GILCHRIST, G. W., CARLSON, M. L., BERRIGAN, D., and SERRA, L. 2000. Rapid evolution of a geographic cline in size in an introduced fly. *Science* 287:308–309.

LOAYZA-MURO, R., FIGUEROA, C. C., and NIEMEYER, H. M. 2000. Effect of two wheat cultivars differing in hydroxamic acid concentration on detoxifying and oxidative metabolism in the aphid *Sitobion avenae*. *J. Chem. Ecol.* 26:2725–2736.

LUSHAI, G., SHERRATT, T. N., DAVID, O., DE BARRO, P. J., and MACLEAN, N. 1998. Host selection by winged summer females of the aphid *Sitobion avenae*. *Ent. Exp. Appl.* 85:199–209.

LUSHAI, G., MARKOVITCH, O., and LOXDALE, H. D. 2002. Host-based genotype variation in insects revisited. *Bull. Entomol. Res.* 92:159–164.

LUSHAI, G., LOXDALE, H. D., and ALLEN, J. A. 2003. The dynamic clonal genome and its adaptive potential. *Biol. J. Linn. Soc.* 79:193–208.

LYNCH, M. 1984. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. *Q. Rev. Biol.* 59:257–290.

MASSONNET, B., SIMON, J. C., and WEISSER, W. W. 2002. Metapopulation structure of the specialized herbivore *Macrosiphoniella tanacetaria* (Homoptera, Aphididae). *Mol. Ecol.* 11:2511–2521.

MILLER, N. J., BIRLEY, A. J., OVERALL, A. D. J., and TATCHELL, G. M. 2003. Population genetic structure of the lettuce root aphid, *Pemphigus bursarius* (L.), in relation to geographic distance, gene flow and host plant usage. *Heredity* 91:217–223.

MUKANGANYAMA, S., FIGUEROA, C. C., HASLER, J. A., and NIEMEYER, H. M. 2003. Effects of DIMBOA on detoxification enzymes of the aphid *Rhopalosiphum padi* (Hemiptera:Aphididae). *J. Insect Physiol.* 49:223–229.

NICOL, D., COPAJA, S. V., WRATTEN, S. D., and NIEMEYER, H. M. 1992. A screen of worldwide wheat cultivars for hydroxamic acid levels and aphid antixenosis. *Ann. Appl. Biol.* 121:11–18.

NIEMEYER, H. M. 1988. Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. *Phytochemistry* 27:3349–3358.

NIEMEYER, H. M. and PÉREZ, F. J. 1995. Potential of hydroxamic acids in the control of cereal pests, diseases and weeds, pp. 260–270, *in* Inderjit, M. M. Dakshini, and F. A. Einhellig (eds.). Allelopathy: Organisms, Processes, and Applications. ACS Symposium Series, American Chemical Society, Washington, DC.

SAMBROOK, J., FRITSCH, E. F., and MANIATIS, T. 1989. Molecular Cloning: a Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

SCRIBER, J. M. 2002. Evolution of insect-plant relationships: chemical constraints, coadaptation, and concordance of insect/plant traits *Ent. Exp. Appl.* 104:217–235.

SICKER, D. and SCHULTZ, M. 2002. Benzoxazinones in plants: occurrence, synthetic access, and biological activity, pp. 185–232, *in* Atta-ur-Rahman (ed.). Studies in Natural Products Chemistry, Vol. 27. Bioactive Natural Products (Part H). Elsevier, Amsterdam.

SICKER, D., FREY, M., SCHULTZ, M., and GIERL, A. 2000. Role of natural benzoxazinones in the survival strategy of plants. *Int. Rev. Cytol.* 198:319–346.

SIMON, J. C. and HEBERT, P. D. N. 1995. Patterns of genetic variation among Canadian populations of the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Homoptera: Aphididae). *Heredity* 74:346–353.

SIMON, J. C., BAUMANN, S., SUNNUCKS, P., HEBERT, P. D. N., PIERRE, J. S., LE GALLIC, J. F., and DEDRYVER, C. A. 1999. Reproductive mode and population genetic structure of the cereal aphid *Sitobion avenae* studied using phenotypic and microsatellite markers. *Mol. Ecol.* 8:531–545.

SIMON, J. C., CARRE, S., BOUTIN, M., PRUNIER-LETERME, N., SABATER-MUÑOZ, B., LATORRE, A., and BOURNOVILLE, R. 2003. Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. *Proc. R. Soc. Lond. B.* 270:1703–1712.

SOKAL, R. R. and ROHLF, F. J. 1981. Biometry, 2nd edn. W.H. Freeman and Company, San Francisco, USA.

StatSoft. 2004. STATISTICA for Windows. Computer Program Manual. StatSoft Inc., Tulsa, OK.

SUE, M., ISHIHARA, A., and IWAMURA, H. 2000. Purification and characterization of a hydroxamic acid glucoside β-glucosidase from wheat (*Triticum aestivum* L.) seedlings. *Planta* 210:432–438.

SUNNUCKS, P. and HALES, D. F. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Mol. Biol. Evol.* 13:510–524.

SUNNUCKS, P., ENGLAND, P. R., TAYLOR, A. C., and HALES, D. F. 1996. Microsatellite and chromosome evolution of parthenogenetic *Sitobion* aphids in Australia. *Genetics* 144:747–756.

VANLERBERGHE-MASUTTI, F. and CHAVIGNY, P. 1998. Host-based genetic differentiation in the aphid *Aphis gossypii* Glover, evidenced from RAPD fingerprints. *Mol. Ecol.* 7:905–914.

VIA, S. 1991. The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution* 45:827–852.

VIA, S. and HAWTHORNE, D. J. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. *Am. Nat.* 159:S76–S78.

WILSON, A. C. C., SUNNUCKS, P., and HALES, D. F. 2003. Heritable genetic variation and potential for adaptive evolution in asexual aphids (Aphidoidea). *Biol. J. Linn. Soc.* 79:115–135.

WILSON, A. C. C., MASSONNET, B., SIMON, J. C., PRUNIER-LETERME, N., DOLATTI, L., LLEWELLYN, K. S., FIGUEROA, C. C., RAMÍREZ, C. C., BLACKMAN, R. L., ESTOUP, A., and SUNNUCKS, P. 2004. Cross-species amplification of microsatellite loci in aphids:assessment and application. *Mol. Ecol. Notes* 4:104–109.

WINK, M. and ROMER, P. 1986. Acquired toxicity—the advantage of specializing on alkaloid-rich lupins to *Macrosiphon albifrons* (Aphididae). *Naturwissenschaften* 73:210–212.

WYATT, I. J. and WHITE, P. F. 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *J. Appl. Ecol.* 14:757–766.

ZÚÑIGA, G. E. and CORCUERA, L. J. 1986. Effect of gramine in the resistance of barley seedlings to the aphid *Rhopalosiphum padi*. *Ent. Exp. Appl.* 40:259–262.