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Sources of variation in the interaction between three cereal aphids (Hemiptera: Aphididae) and wheat (Poaceae)

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Abstract

The relative contributions of host plant, herbivore species and clone to variation in the interaction between cereal aphids and wheat were investigated using five clones each of three species, *Rhopalosiphum padi* (Linnaeus), *Sitobion avenae* (Fabricius) and *Schizaphis graminum* (Rondani), on seedlings of two cultivars of *Triticum aestivum* L. and one cultivar of *Triticum durum* Desf. More individuals and biomass of *R. padi* than of the other two species were produced on seedlings. The three wheat cultivars lost similar amounts of biomass as a result of infestation by aphids, with the amount lost depending on aphid species: *S. avenae* caused the lowest loss in biomass. Variation in aphid biomass production was due mostly to differences among aphid species (70%), less to the interaction between wheat type and aphid species (7%), and least to aphid clone (1%). The specific impact of the aphids on the plants ranged from 1.7 to 3.7 mg of plant biomass lost per mg of aphid biomass gained, being lowest for *R. padi* and highest for *S. graminum*. Variation in plant biomass lost to herbivory was due mostly to unknown sources of error (95%), probably phenotypic differences among individual seedlings, with 3% due to aphid species and none attributable to aphid clone. For these aphid–plant interactions, differences among aphid clones within species contributed little to variation in aphid and plant productivity; therefore, a small sample of clones was adequate for quantifying the interactions. Furthermore, one clone of each species maintained in the laboratory for about 200 parthenogenetic generations was indistinguishable from clones collected recently from the field.

Keywords: *Rhopalosiphum padi*, *Sitobion avenae*, *Schizaphis graminum*, *Triticum aestivum*, *Triticum durum*, clones, cultivars, aphid–plant interactions

Introduction

Many factors contribute to variation in the interaction between aphids and their host plants. Edwards (2001) categorized some sources of variation in the performance of aphids on their hosts based on a taxonomic hierarchy of the aphids: inter-specific variation at the top, through host races, biotypes, and parthenogenetic lineages often called clones (for brevity the term 'clone' is used here). The lowest levels in such a hierarchy would be inter-individual genetic and phenotypic variation (Simon *et al*., 1991). The genotypic and phenotypic variation of the host plant must also be considered because these sources of variation may affect aphid phenotype and, therefore, the interpretation of aphid genotype (Wool & Hales, 1996).

The importance of genetic variation within and among clones is controversial (Loxdale & Lushai, 2003). Clones are a

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central feature of aphid life histories (Dixon, 1998), and play an important role in the interactions between aphids and their host plants. Clones also provide a simple experimental tool for distinguishing genotype and phenotype, replacing the time-consuming production of inbred lines and studies of heritability required for species that are obligately sexual. Replicates of a genotype can be obtained easily if sisters or progeny of sisters are assumed to be genetically identical (MacKay *et al*., 1993; Wool & Hales, 1996). This strategy fails, however, if clones are genetically unstable in the short term (Loxdale & Lushai, 2003).

Clones appear to be phenotypically stable in long-lived laboratory cultures (van Emden, 1988). This stability causes variation to be 'very low among clone members but greater among clones' (Sunnucks *et al*., 1998). Furthermore, using a clone as the genetic individual reveals high heritabilities of many aphid life history traits (MacKay *et al*., 1993). The absence of recombination between paired chromosomes during parthenogenesis probably limits the role of mutation in generating variation (Blackman, 1985). For example, the amino acid sequences of proteins vary less within or among aphid clones than among individuals of sexual species (Simon *et al*., 1982, 1996). A single clone can be predominant and stable in a population over many generations (Steiner *et al*., 1985; Fuentes-Contreras *et al*., 2004). However, recent studies of base-pair sequences in mitochondrial DNA casts doubt on the genetic stability of clones (Loxdale & Lushai, 2003). Furthermore, defensive chemicals produced by the host plant may be mutagenic, and rapidly produce new variants which change in frequency in the population due to selection on different hosts, or as a result of differential immigration of clones (Figueroa *et al*., 2002).

Although clones seem to be genetically stable in the short term (Suomalainen *et al*., 1980; Tomiuk & Wohrmann, 1982), variation in aphid–host plant interactions is widespread, even in populations reproducing largely or completely by parthenogenesis (MacKay & Lamb, 1988; Porter *et al*., 1997; Sunnucks *et al*., 1998; Edwards, 2001). Sometimes this variation is associated with chromosomal rearrangements in the aphids (Sunnucks *et al*., 1998), or strong differential selection by two crop monocultures (Via, 1991). In other cases, genetically distinct clones sort themselves out during and after migration, on different crop species (Lushai *et al*., 2002). Selection by the host, in conjunction with aphid migration between different hosts, can change the relative frequencies of clones over a season (Sandström, 1996). In still other cases, different clones colonize the same host in the same area, and the importance of the genetic variation among lineages in the interaction between aphid and plant is unknown (Weber, 1985a,b; MacKay & Lamb, 1988; Shufran *et al*., 1991; Wilhoit & Mittler, 1991). Clearly, genetically distinct clones can occur in facultatively sexual and obligately asexual populations of aphids, distinct clones can arise relatively rapidly (within a few years or decades), and the relative frequencies of clones can change dramatically through selection during interactions with host plants.

The agricultural importance of such variation has been considered mostly in terms of crop resistance where a single or small number of crop resistance genes prevent most or nearly all clones from colonizing a particular plant genotype (Porter *et al*., 1997). Variation has also been considered in relation to host specificity of aphid genotypes that are morphologically similar but differentially utilize different species of plant (Via, 1991; Sandström, 1996). The implications of the variation for other ecological processes and for pest management in agriculture are less clear. For example, determining the pest status of aphids in crops is complicated because distinct clones with different host relationships may occur in an area, the clones may occur on many genetically distinct plant cultivars or related species, and the aphid community on those crops may consist of a number of species.

This study assesses the relative importance of these three sources of variation in the interaction among cereal aphids and wheat: aphid clones within species, plant genotypes, and aphid species. The immediate objective was to assess whether variation among clones is low enough that a small number of clones can provide an adequate, representative sample to quantify the interactions between pest aphids and cereal crops in a region. The interactions of five clones of *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (Linnaeus) and *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) with seedlings of three types of wheat (Poaceae: *Triticum aestivum* L. and *Triticum durum* Desf.) were quantified by simultaneously measuring the biomass gained by aphids (a measure of antibiosis) and lost by the plants (a measure of tolerance) during the interactions (Migui & Lamb, 2003, 2004).

Materials and methods

Four clones of each species were collected from cereal fields in southern Manitoba, Canada in 1996, with each clone established from a single aphid from a different field. A fifth clone of each species came from cultures maintained for eight years in the laboratory of P.A. MacKay, Department of Entomology, University of Manitoba, and originally collected from cereal fields in southern Manitoba. Cereal fields in this area are large, often over 100 ha, and the sampled fields were widely spaced. The three species of aphids do not over-winter in this part of North America, and migrate in from the south each year. With repeated flights of aphids arriving from different areas, depending on the tracks of frontal systems that carry the immigrants, the experimental clones probably represent a small but diverse sample of the large number of clones that inhabit central North America.

A cultivar from each of three wheat classes grown in western Canada was selected to provide a diverse genetic background among hosts: *T. aestivum*, Canadian Western Red Spring (CWRS, AC Domain), *T. aestivum*, Canadian Prairie Spring (CPS, AC Foremost) and *T. durum*, Canadian Western Amber Durum (CWAD, Medora). Tests were conducted under controlled laboratory conditions to minimize phenotypic variation, so that genetic variation could be assessed as precisely as possible.

Aphid cultures were maintained on excised pieces of the first leaf of seedling barley, *Hordeum vulgare* L. Argyle (Poaceae), at 18 : 6 L : D and 20°C, in Petri dishes (Migui & Lamb, 2003, 2004). Standard aphids were obtained by transferring a young adult (24–36 h after the adult moult) to a fresh leaf, allowing it to produce offspring for 24 h, removing the adult, and leaving the offspring to develop to the third instar (3–4 days after birth). At this instar, aphids were transferred to fresh dishes where they developed in isolation to adulthood. Wheat plants were grown under the same environmental conditions, in artificial soil in individual plastic pots, sub-irrigated with a fertilizer solution (Migui & Lamb, 2003, 2004). At the two leaf stage (growth stage 12,

Tottman & Makepeace, 1979), healthy seedlings were selected and their heights were measured. Plants were divided evenly into two groups with equal ranges of height. One group was infested with aphids; seedlings from the other group were cut at soil surface, and the aerial portion was dried to constant moisture content at 80°C for 48 h and weighed at a precision of 0.0001 g to estimate initial biomass.

Young adult aphids were selected randomly to estimate initial biomass and to infest plants. For determining aphid biomass, individual aphids were killed in a drop of 95% ethanol in pre-weighed aluminium foil dishes, dried (as above), and weighed at a precision of 0.0001 mg. Biomass was calculated by subtracting the mass of the empty foil dish from the mass of the foil dish containing a dried aphid. Each plant was infested with one adult, and covered with a transparent, perforated polyethylene cage, 22.5 cm high by 7.4 cm diameter, with its open end tightly secured around the pot. An uninfested control plant was also caged. A replicate consisted of six individual plants, five with a single aphid from a different clone from the same species, and a control plant without an aphid, assigned in random order to a plastic box containing fertilizer solution. Replicates were maintained under the same conditions as the cultures. Aphids fed and reproduced for six days, and then were collected, counted, placed in ethanol in a pre-weighed aluminium foil dish, dried, and weighed. Plants were cut at soil level and the aerial part placed in separate pre-weighed aluminium foil dishes, dried and weighed.

For practical reasons, 18 consecutive tests were conducted, each using five clones of one species and one wheat cultivar, replicated five times. Each test was repeated once. Five clones for each of three aphid species and three cultivars, with control plants and 10 replicates, resulted in 45 treatments and 540 pots. Only trivial differences and no trends were detected between replicate tests over the 18 consecutive tests, for the dry weights of pre- and post-experiment control plants and pre-experiment aphids. Analysis of variance of the biomass of control plants, using a General Linear Model (GLM) (SAS Institute Inc., 1989), detected no differences in the growth of control plants over the experiment ($P > 0.05$). Therefore, the 18 tests were considered a single experiment, and estimates of biomass for control plants were pooled by cultivar.

The performance of individual aphids from each clone on each cultivar was measured as the number of offspring produced and the difference between final and mean initial aphid biomass (aphid biomass gained). The impact of aphids on wheat seedlings was estimated as the difference in biomass between infested plants and means for control plants (plant biomass lost). The response of plants to each aphid species was estimated by a specific impact for each clone and cultivar, defined as the biomass reduction of plant tissue per unit of biomass gained by the aphid (Lamb & MacKay, 1995; Gavloski & Lamb, 2000; Migui & Lamb, 2003, 2004).

Analyses of variance of the dependent variables were conducted using General Linear Models and Mixed Model procedures (SAS Institute Inc., 1989). When a plot of variance versus mean for a dependent variable showed a strong relationship between mean and variance and/or the largest variance was greater than two times the smallest variance, data were transformed by calculating natural logarithms. Means of untransformed data are presented ± SE. Cultivar, aphid species and the interaction term for cultivar and aphid species, were considered fixed effects; clone within aphid species was considered a random effect. Tukey's multiple range test was used to discriminate means. The variances of specific impacts were estimated using the method of Cochran (1977, page 173). The largest and smallest specific impacts were compared among clones within each group of cultivars and aphid species, using a paired *t*-test. Clones did not differ from one another, except two clones of *S. graminum* on CPS wheat, and so clones were pooled and specific impacts were calculated for each cultivar-species combination. To test the hypothesis that specific impacts were equal, *t*-tests were conducted using unpaired observations and unequal variances (Steel & Torrie, 1960). Differences among the three aphid species (wheat classes pooled) or among three wheat classes (aphid species pooled) were assessed by testing all possible two-way comparisons (three comparisons each) using a *t*-test ($P = 0.005$ for rejection of equality in a two-way comparison gave an experimentwise error rate of 0.01).

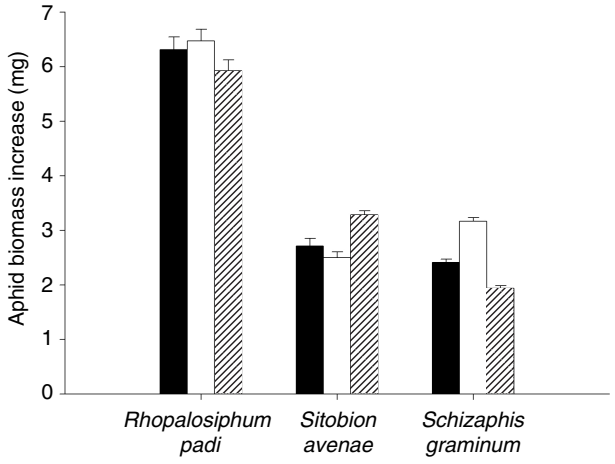

Fig. 1. Mean biomass (±SE) of aphids produced by a young, wingless adult aphid during six days on wheat seedlings (■, Canadian Western Red Spring; □, Canadian Prairie Spring; ▨, Canadian Western Amber Durum).

Results

The three aphid species fed and reproduced on all wheat seedlings. Wingless adult aphids of *S. graminum*, at 0.190 ± 0.003 mg ($n = 80$), had a lower dry weight than adults of either *S. avenae* (0.329 ± 0.010 mg, $n = 50$) or *R. padi* (0.316 ± 0.004 mg, $n = 145$). The number of offspring produced by females during six days of infestation (wheat classes pooled) was highest for *R. padi* (71 ± 1.8, $n = 149$), intermediate for *S. graminum* (62 ± 1.6, $n = 149$), and lowest for *S. avenae* (29 ± 0.4, $n = 149$). *Rhopalosiphum padi* produced about twice as much biomass as *S. graminum* or *S. avenae* (fig. 1). Biomass production by *S. avenae* was similar to that of *S. graminum*: the former produced half as many offspring, which were on average twice as large.

Wheat class, aphid species, aphid clone and the interaction between wheat class and aphid species all had statistically significant effects on aphid numbers and biomass, although variance components revealed that the major contributor to total variance, 70% or more, was aphid species

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Table 1. Analysis of variance of the effects of wheat class, aphid species and clone within aphid species on aphid numbers, aphid biomass increase and plant biomass lost after six days of seedling infestation.

Source of variation[1]	df	MSE	*F*	Significance level[2]	Variance component, %[3]
Log_e (aphid numbers)					
CLASS	2	1.8	54.6	***	0
AP	2	29.85	196.7	***	69.5
CL(AP)	12	0.15	4.6	***	1.5
CLASS*AP	4	2.44	74.0	***	16.6
ERROR	426	0.03			12.4
Log_e (aphid biomass increase)					
CLASS	2	0.2	5.6	**	0
AP	2	23.06	227.2	***	74.1
CL(AP)	12	0.1	2.8	***	1.1
CLASS*AP	4	1.01	28.1	***	7.0
ERROR	426	0.04			17.8
Plant biomass loss					
CLASS	2	350.83	2.9	ns	0.8
AP	2	819.74	19.1	***	3.3
CL(AP)	12	42.95	0.4	ns	0
CLASS*AP	4	192.97	1.6	ns	1.2
ERROR	429	121.81			94.7

[1] AP, aphid species; CL, aphid clone.
[2] **, ***significant, *P*#0.01 and *P*#0.001, respectively; ns, not significant, $P > 0.05$.
[3] Estimates of variance component based on a random effect ANOVA (mixed model with all effects random).

(table 1). Wheat class contributed least to the variation, but the interaction between wheat class and aphid species was an appreciable source of variation: the number of offspring produced by *R. padi* on CWAD was reduced to fewer than 60, and increased for *S. graminum* on CPS to more than 80. This effect was less pronounced for aphid biomass, although still evident (table 1, fig. 1). Clones within aphid species contributed less than 2% to the variation.

The heights and biomasses of pre-experiment plants showed linear relationships (AC Domain, $R^2 = 0.66$, $P \leq 0.0001$, $n = 84$; AC Foremost, $R^2 = 0.78$, $P \leq 0.0001$, $n = 147$; Medora, $R^2 = 0.79$, $P \leq 0.0001$, $n = 77$). The initial biomasses of infested plants were estimated from their initial heights using the linear regression model derived from pre-experimental plants. Initial heights and final biomasses of control plants were also found to be linearly related (AC Domain, $R^2 = 0.59$, $P \leq 0.0001$, $n = 30$; AC Foremost, $R^2 = 0.66$, $P \leq 0.0001$, $n = 30$; Medora, $R^2 = 0.40$, $P \leq 0.0001$, $n = 30$), and the respective linear equations were used to estimate the expected final biomasses of a control plant with initial heights that matched infested plants. Plant biomass loss was estimated by taking the difference between the expected final biomass of a control and actual final biomass of a matching infested plant.

For the three wheats, uninfested seedlings of CWAD (Medora) had an above-ground biomass of 83.5 ± 3.1 mg ($n = 30$), compared with 79.3 ± 2.3 mg ($n = 30$) for CPS (AC Foremost) and 67.9 ± 2.1 mg ($n = 30$) for CWRS (AC Domain) after six days. All infested seedlings had reduced biomass compared to controls, with reductions ranging from 3–17% (fig. 2). The aphid species caused differential reductions in biomass among wheat classes, although aphid species accounted for only about 3% of the variation, almost 95% of which was error (table 1). *Rhopalosiphum padi* and *S. graminum* caused higher losses than *S. avenae*, particularly on CWRS and CWAD (fig. 2). Neither wheat class nor aphid clone affected the amount of seedling biomass that was lost, and no interaction between wheat class and aphid species was detected. Losses in seedling biomass were positively

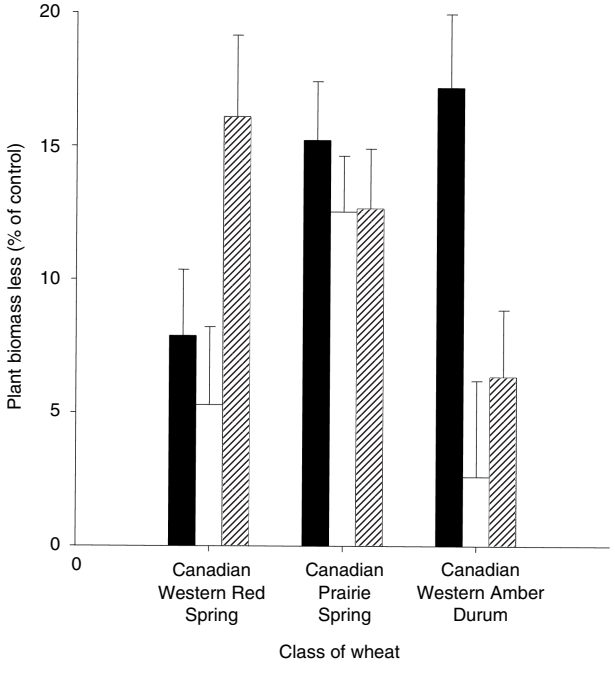

Fig. 2. Mean percentage of aerial biomass lost (±SE) by wheat seedlings after six days of infestation by cereal aphids (■, *Rhopalosiphum padi*; □, *Sitobion avenae*; ▨, *Schizaphis graminum*) in a controlled environment.

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Table 2. Specific impacts (mg/mg) of aphids on wheat seedlings (biomass reduction in plant per unit biomass gained by aphid) six days after infestation.

Factor	Specific impact	
	n	Mean ± SE
Aphid species		
Rhopalosiphum padi	149	1.7 ± 0.13a[2]
Sitobion avenae	149	2.5 ± 0.23b
Schizaphis graminum	149	3.7 ± 0.30c
Wheat class[1]		
CWRS	150	2.1 ± 0.19a
CPS	148	2.4 ± 0.19b
CWAD	149	2.4 ± 0.22b

[1]CWRS, Canadian Western Red Spring; CPS, Canadian Prairie Spring; CWAD, Canadian Western Amber Durum.
[2]Mean specific impacts from the same factor within a column followed by the same letter(s) do not differ significantly. Differences among three aphid species (wheat classes pooled) or among three wheat classes (aphids species pooled) were assessed by testing all possible two-way comparisons (three comparisons each) using a *t*-test ($P = 0.005$ for rejection of equality in a two-way comparison gave an experiment-wise error rate of 0.01).

correlated with the biomass gained by aphids, although the relationship explained little of the variation (aphid species pooled, $r_P = 0.16$, $P = 0.0008$, $n = 447$). Inter-individual variation in the growth of seedlings, or the reaction of individual seedlings to aphid herbivory, was by far the largest cause of overall variation in the response of the plants.

The specific impact, a measure of the effect of an aphid species on plant growth corrected for differences in aphid biomass production among species, was lowest for *R. padi* and highest for *S. graminum* (table 2). Aphid herbivory caused a loss of seedling production of between 1.7 and 3.7 mg for each mg of aphid biomass gained. For wheat classes, the specific impact of aphids on CWRS was slightly less than for the other two classes (table 2). The different aphid clones had no differential effects on plant growth and so no clone-specific impacts were detected.

Clones within each aphid species showed similar increase in biomass and numbers on wheat during the six-day feeding period, with no significant differences between the long-term laboratory clone and four other clones collected from the field just prior to the study. The mean biomass of aphids on CPS wheat were 6.5 ± 0.5 mg ($n = 10$) for the *R. padi* laboratory clone and 6.8 ± 0.4 mg ($n = 40$) for other clones; 3.0 ± 0.2 mg ($n = 10$) for the *S. avenae* laboratory clone and 2.8 ± 0.2 mg ($n = 40$) for other clones; and 3.3 ± 0.1 mg ($n = 10$) for the *S. graminum* laboratory clone and 3.4 ± 0.2 mg ($n = 40$) for other clones.

Discussion

Each clone of the three aphid species increased in number and biomass on three types of wheat, and infested plants of the three cultivars produced less biomass than uninfested plants. The three aphid species differed in their production and caused differential losses in plant production. Specific impacts ranged from 1.7 mg of plant production lost for each mg of production by *R. padi*, to 2.5 mg per mg of *S. avenae*, and 3.7 mg per mg of *S. graminum*. These values are similar to the specific impacts of 2 to 4 mg per mg for Australian populations of five species of aphids feeding on barley seedlings (Lamb & MacKay, 1995; MacKay & Lamb, 1996), but substantially lower than the specific impacts of 12 and 16 mg per mg, respectively, for the aphids *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer), feeding on *Brassica napus* L. (Cruciferae) (Gavloski & Lamb, 2000). Considering that specific impacts are unlikely to be less than 1, corresponding to a loss of plant tissue equal to the gain in insect tissue, the specific impacts of these cereal aphids on wheat are surprisingly low. Specific impacts for other guilds of insect herbivores are often much higher and may exceed 100 mg per mg (Gavloski & Lamb, 2000; Lamb *et al*., 2000a).

Aphid production, either numbers or biomass, differed among clones for each of the three aphid species, confirming that clones differ genetically. Clone, however, explained only 1–2% of the variation. Aphid species explained most of the variation in aphid production, 70% or more, compared with less than 20% for the wheat types.

For plant production under herbivory, phenotypic variation among seedlings was by far the largest source of variation, even though uniform seedlings were grown under controlled conditions, from single seed lots selected for uniformity by plant breeders. Differences in the effects of the aphid species explained about 3% of the variation in lost plant production, and neither wheat class nor aphid clone explained an appreciable or statistically significant amount of the variation.

For these three aphid species feeding on wheat seedlings, variation among clones contributed little to our understanding of the insect–plant interaction. Interestingly, the one clone of each species that had been reared in the laboratory for about 8 years and over 200 parthenogenetic generations showed no appreciable difference from clones established from field collections a few months prior to the experiment. Although our methods allowed for detection of small wheat-class and clone effects on aphid performance, only the difference between *R. padi* and the other two aphid species was substantial. *Rhopalosiphum padi* produces about twice as much aphid biomass on seedlings of these three diverse wheats as *S. avenae* or *S. graminum*. Because the impact of *R. padi* on these wheats is relatively low, the effect of this aphid on seedlings is similar to that of *S. graminum*, which has about twice the specific impact but is half as productive. Overall, these interactions between the three aphid species and three diverse wheat cultivars resulted in similar effects on the growth of seedlings.

Aphid populations in summer cereals exist as an assortment of parthenogenetic clones, which are genetically diverse. Nevertheless, a small number of clones may be adequate to assess the interaction between aphid and plant, because the contributions of different clones to variation in the interactions between aphids and plants may be low. How many clones should be included in research on the interaction between an aphid and plant? The answer depends on the research question. If the objective is to find a rare clone expressing a virulence allele that can overcome crop resistance, the sample size should be determined by the frequency of the allele. Such virulent clones occur in aphid–crop systems, and have overcome antibiotic resistance (Porter *et al*., 1997), but the frequency of virulence has rarely been measured prior to the breakdown of resistance. The frequency of wheat midge larvae, *Sitodiplosis mosellana*

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(Géhin) (Diptera: Cecidomyiidae), that survive on resistant wheat and may be virulent, is between 3 and 5 per 1000 individuals (Lamb *et al*., 2000b). This frequency is likely to be high enough to cause a rapid breakdown in resistance, but low enough to be difficult to detect. If a virulent aphid clone occurred at this frequency, at least 500 clones would need to be assessed even to detect its presence, and thousands of clones would need to be assessed to estimate the frequency of the clone with much precision. Assessing such large numbers of clones is impractical using the kinds of bioassays described in the present study, or using current molecular methods, because markers for virulence alleles are not usually available until after a virulence allele has increased sufficiently in frequency to make plant resistance ineffective. In asexual field populations of aphids, however, a virulent genotype shows itself quickly in resistant wheat, because clones have such high rates of increase, and individual aphid clones are often widely distributed (Shufran *et al*., 1991).

To quantify the interaction between aphid and host plant, either in terms of aphid production or plant losses, a small sample of clones may be adequate. For the three species of cereal aphid in this study, five clones were sufficient because inter-clonal variation was low. Nevertheless, researchers must be alert to the possibility that morphologically indistinguishable and uncommon clones may be present in a population, clones that interact with host genotypes in ways that differ from the majority of clones that commonly occur in a crop. Therefore, a single clone should not be used to characterize the interaction, unless the clone is known to interact with the host plant in a way that is typical for the aphid species, host-adapted population of clones, or regional population of clones under investigation. Based on the occurrence of intra-specific variation, the argument has been made that a large number of clones must be studied to assess the susceptibility of crops to aphids (MacKay & Lamb, 1988; Edwards, 2001). This conclusion can be tempered, however, because although intra-specific variation is common, differences among clones may be small and contribute little to the overall variation in the interaction between aphid and host plant.

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