



Specialisation pattern of the aphid *Rhopalosiphum maidis* is not modified by experience on a novel host

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Abstract

The effect of alternative host condition on the pattern of specialisation of the aphid *Rhopalosiphum maidis* (Fitch) was studied. *R. maidis* commonly occurs in Chile on Johnson grass (*Sorghum halepense* L.) but rarely on contiguous wheat (*Triticum durum* L.) crops. The performance of 23 clones of *R. maidis* on *S. halepense* (established host) and *T. durum* (novel host) before and after rearing on wheat for 20 asexual generations was evaluated. Prior and after the period of conditioning on wheat no negative correlation of performance parameters between both hosts was found. Only 8 out of 23 clones survived the 20 asexual generations on wheat. Further, after conditioning on wheat, survival of three out of eight clones increased on wheat and three clones improved r_m on Johnson grass but not on wheat. Although some genotypes of *R. maidis* were able to withstand wheat stressing conditions, the conditioning on this alternative host did not cause a decreased capacity to use Johnson grass as host. The results suggest that *R. maidis* performance on wheat is not genetically constrained, and also that conditioning on wheat cannot substantially modify this pattern.

Introduction

The pattern of specialisation in phytophagous insects refers basically to a restricted use of host plants, regardless of their relative availability (Fox & Morrow, 1981; Rausher, 1988). This is the case with the aphid *Rhopalosiphum maidis* Fitch (Hemiptera: Aphididae), which commonly occurs in Chile on its wild host *Sorghum halepense* L. (Johnson grass) but rarely on *Triticum durum* L. (wheat) (Apablaza & Tiska, 1973; Starý et al., 1994), despite the proximity of these two species in the field during part of the year.

An aphid species may show significant intraspecific variations in host use (Via, 1990; Pilson, 1992; Guldemond & Mackenzie, 1994; De Barro et al., 1995; Sandström, 1996; Mackenzie, 1996; Douglas, 1997). This may be a consequence either of genetic differences between individuals or environmentally induced differences (Via, 1991; Douglas, 1997; Joshi & Thompson, 1997). Regardless of the causes underlying such variation, if costs are involved, optimal

performance in one host plant will lead to low performance on another host, i.e., trade-offs in host use will occur (Dethier, 1954; Thompson, 1996). This is usually visualised as negative genetic correlations between performances on two host plant species (Futuyma & Moreno, 1988; Via, 1990).

In the present work, we inquire into the pattern of distribution of *R. maidis* described above, by evaluating: (1) the presence of intraspecific variation in host use and the occurrence of negative genetic correlations for performance parameters, (2) whether its uncommon abundance on wheat is related to genetic factors, and (3) whether the pattern of host use can be altered through conditioning on wheat. Performance parameters on Johnson grass and wheat of genetically distinct lineages of *R. maidis* descending from natural populations on Johnson grass, were evaluated before and after a prolonged period of conditioning on wheat. Additionally, the reversibility of a possible effect of conditioning on wheat was evaluated.

Materials and methods

Insects and plants. *R. maidis* is an anholocyclic cereal aphid, i.e., it reproduces exclusively parthenogenetically (Etchegaray, 1975; Brown & Blackman, 1988) and, as all aphid species, shows telescoping generations, i.e., a parthenogenetic female may have within itself developing embryos which in turn have embryos within themselves (Dixon, 1998). This reproduction mechanism implies that embryos are exposed to the environment experienced by their grandmother and thus maternal effects can only be 'cleaned' after three generations. Although *R. maidis* is also found in maize and barley and occasionally in wheat, its most common wild host in Chile is Johnson grass (Apablaza & Tiska, 1973; Starý et al., 1994). Both Johnson grass and *R. maidis* occur mainly associated to orchards and cereal fields in northern and central Chile, both species showing quite similar distributions (Artigas, 1994; Matthei, 1995).

Sampling and identification of putative different clones. Between February and May 1997, 30 putatively different clones of *R. maidis* were collected from natural populations on Johnson grass in eight localities of central Chile (ca. 26 to 38°S) with a minimal distance of 5 km between sampling locations. These putatively different clones were transferred to the laboratory and colonies were started from a single parthenogenetic female. The monoclonal colonies were kept separately on 6-leaf stage Johnson grass seedlings grown from seeds collected at La Platina, INIA, Santiago. Both aphids and plants were maintained in growth chambers at 23 ± 2 °C and L16: D8 photoperiod. Putatively different clones were subsequently identified by RAPD-PCR (Welsh & McClelland, 1990; Williams et al. 1990), based on three primers (CFp1: 5'-CCG-TCG-ACG-G-3'; CFp4: 5'-TGG-ACA-CTG-A-3'; HN8: 5'-AGT-CAG-CCA-C-3'), following the procedure of Figueroa et al. (1999). A total of 20 distinguishable polymorphic bands were obtained which revealed 23 different clones.

Determination of performance parameters. From each clone, one 5-day old nymph (synchronisation of nymphs to ca. 18 h) was transferred to a potted seedling of each host and enclosed in a clip-cage. Nymphs were observed daily until adulthood, and the pre-reproductive period was registered (T). The new nymphs produced in the subsequent T days were counted (M_d) and removed daily. The r_m was de-

Table 1. Two-way ANOVA tables of performance parameters of *R. maidis* clones determined before the period of conditioning on wheat

Source	df	F	P-level
r_m			
Clone	22	1.60	0.04
Host	1	17.17	<0.001
Clone-host	22	1.26	0.2
M_d			
Clone	22	1.46	0.09
Host	1	31.29	<0.001
Clone-host	22	2.16	0.002
T			
Clone	22	1.57	0.049
Host	1	0.29	0.59
Clone-host	22	1.38	0.12

termined by the equation of Wyatt & White (1977): $r_m = 0.738 (\ln M_d) / T$. The daily offspring produced within the first 10 days after the first reproductive day was used to build the progeny-time curves on both hosts. These were shown not to differ significantly (Kolmogorov–Smirnov two-sample test, $D = 0.36$, $P > 0.05$) thus verifying an assumption of Wyatt and White's method for estimating the r_m . Fifteen replicates were set up per clone. In addition, survival until twice the pre-reproductive period ($2T$) was recorded.

These determinations were performed at three different occasions: (I) On both hosts (Johnson grass and wheat) for aphids sampled from Johnson grass, with the aim to assess the existence of genetic correlations between performances on both hosts. (II) On both hosts (Johnson grass and wheat) after 20 generations of conditioning on wheat, with the aim to evaluate conditioning-mediated differences of performance on both hosts. (III) On Johnson grass after three generations on Johnson grass, with the aim to evaluate reversibility of any eventual difference induced by the period of conditioning on wheat (Figure 1).

Performance before the period of conditioning on wheat. Performance parameters of each *R. maidis* clone were determined on Johnson grass or wheat as described above, before being transferred to wheat for conditioning (Figure 1).

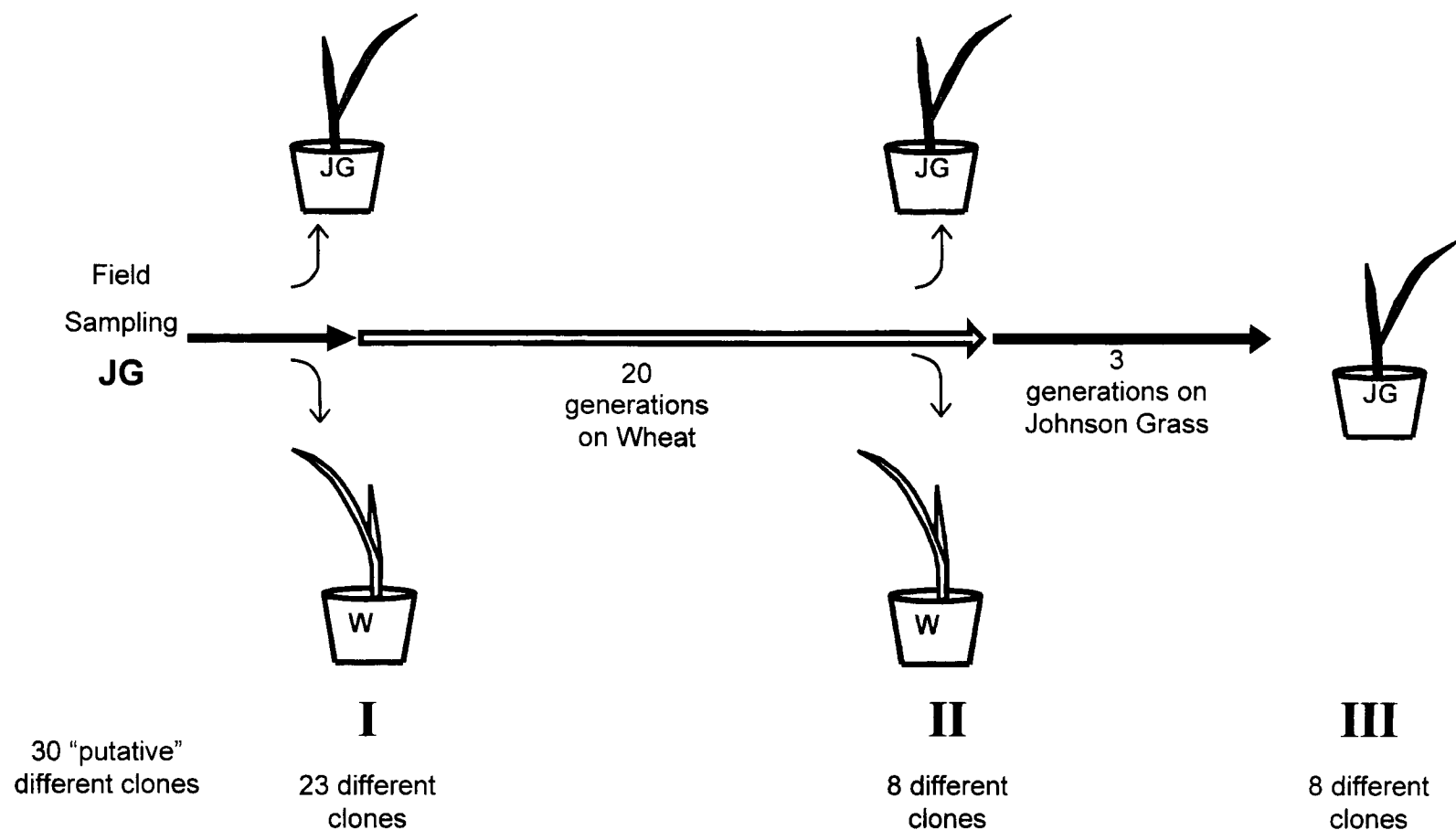


Figure 1. Design of experiment (JG = Johnson grass, W = wheat). Performance was evaluated at three different times: (I) On both hosts (JG and W) for aphids from Johnson grass, with the aim to assess the existence of genetic correlation between performances on both hosts. (II) On both hosts (JG and W) after 20 generations of rearing on wheat, with the aim to evaluate conditioning-mediated differences of performance on both hosts. (III) On Johnson grass after three generations on Johnson grass, with the aim to evaluate reversibility of any possible difference induced by the period of conditioning on wheat.

Host transference and performance after the period of conditioning. Individuals of each *R. maidis* clone maintained on Johnson grass were transferred to potted wheat seedlings in the 3- to 5-leaf-stage. Aphids were conditioned in the new host for 20 asexual generations under laboratory conditions (23 ± 2 °C and L16:D8 photoperiod). The plants were replaced weekly. Once the period of conditioning ended, performance parameters (r_m , T , M_d) and survivorship on wheat or Johnson grass of the survived clones were evaluated as described above (Figure 1).

Testing reversibility of the effect of conditioning on wheat. After conditioning for 20 asexual generations on wheat, each of the surviving clones was transferred back to Johnson grass and maintained there for three asexual generations, which is the minimal period needed to 'clean' a possible maternal effect (Dixon, 1998). At the end of that period, the three performance parameters described above were evaluated on Johnson grass (Figure 1).

Statistics. Non parametric Spearman correlations were used to evaluate genetic correlations in perfor-

mance parameters between both hosts. Furthermore, performance parameters were compared using two-way (factors: clone and host), three-way (factors: clone, host and conditioning), and two-way (factors: clone and time of the evaluation of performance parameters) ANOVA's by ranks, in order to take into account the lack of homocedasticity and normality of data (Zar, 1996). Planned comparisons (LSD test) were carried out to compare performance parameters evaluated at intra-clone level at three different times (before the period of conditioning on wheat, after the period of conditioning on wheat, and after three asexual generations back on Johnson grass) (Sokal & Rohlf, 1995). Z-test for proportions were used to compare survivorship of each clone before and after conditioning on wheat (Zar, 1996).

Results

Performance of clones on Johnson grass and wheat before the period of conditioning. No significant correlation between both host plants were found for any of performance parameters (Figure 2a-c), thus ex-

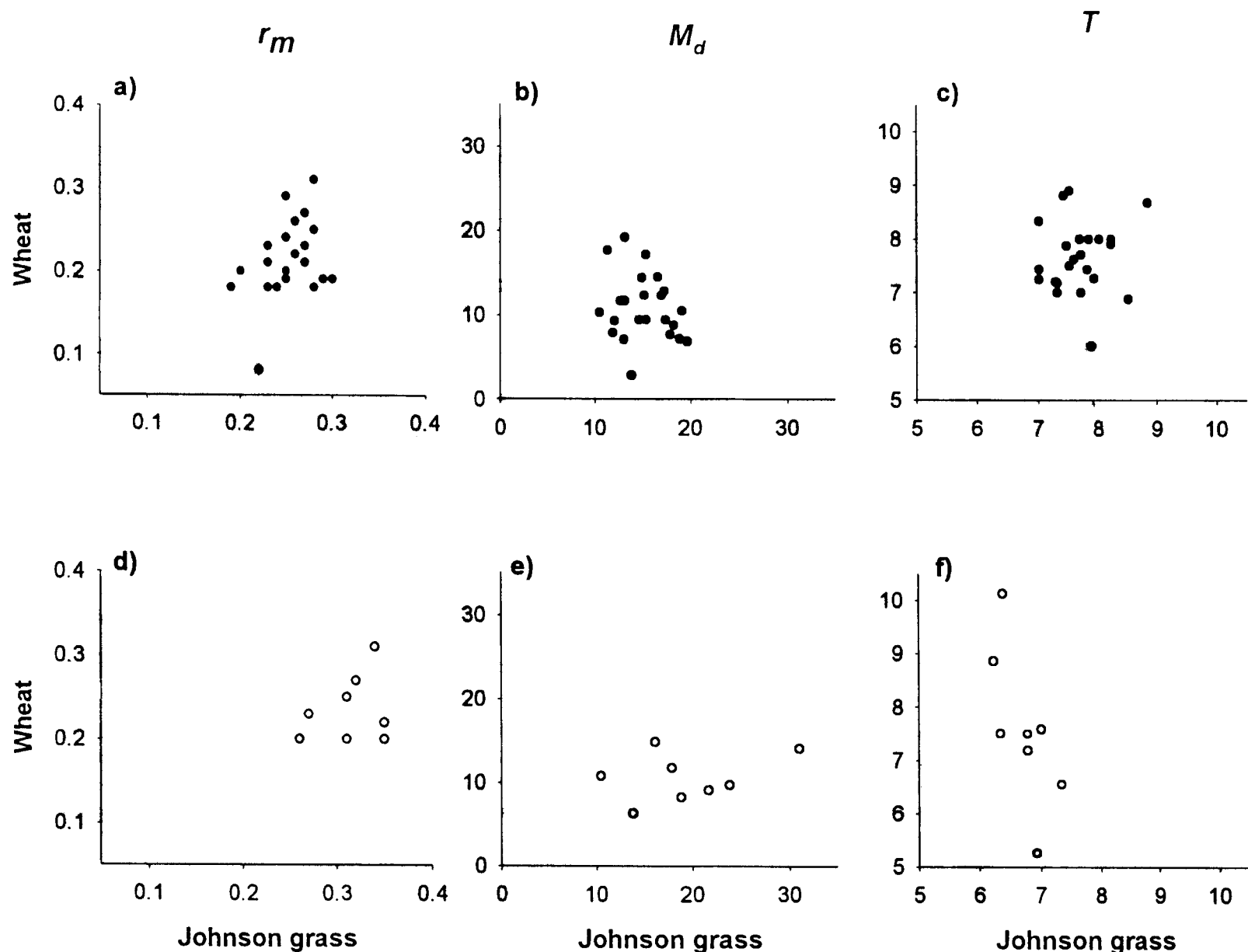


Figure 2. Correlations of performance parameters of *R. maidis* clones between Johnson grass and wheat. Three different performance parameters are shown: (a) and (d) intrinsic rate of population increase (r_m); (b) and (e) number of nymphs produced in a period equal to T (M_d); (c) and (f) time to first reproduction (T). Plots (a), (b) and (c) refer to values from 23 clones before conditioning on wheat; plots (d), (e), and (f) refer to values from eight clones which survived conditioning on wheat.

cluding negative genetic correlations of performance parameters between the two host plants (r_m : $R = 0.24$, $P = 0.26$; M_d : $R = 0.09$, $P = 0.66$; and T : $R = -0.19$, $P = 0.38$).

ANOVA showed that r_m was significantly affected by host and clone, M_d was significantly affected by host and by the [clone \times host] interaction, and T was significantly affected by clone (Table 1).

Performance of clones on Johnson grass and wheat after the period of conditioning. Since only eight out of the initial 23 clones survived the period of conditioning on wheat, the following results refer to those eight clones. No significant correlation between both hosts were found for any of the performance parameters (r_m : $R = 0.19$, $P = 0.65$; M_d : $R = 0.12$, $P = 0.87$; and T : $R = -0.58$, $P = 0.13$) (Figure 2d-f).

The three-way ANOVA by ranks showed that [host-conditioning] interaction was significant only for r_m , and the [clone-host-conditioning] interactions were significant for all performance parameters (Table 2). Planned comparisons showed that after conditioning on wheat, performance on Johnson grass increased in several clones (CH5, I5, JGM4, and LS2 clones improved in r_m , CH5 and JGM4 in M_d , and I5 and LS2 in T). Performance on wheat increased in two clones (JGM4 improved in r_m and LA2 in T), and decreased in 3 clones (CH5 performed worse in r_m and in M_d , and I5 performed worse in T) (Figure 3).

Three clones showed a significant increase in survivorship on wheat after the period of conditioning on wheat (CH4, I32, and LS1), whereas survivorship on Johnson grass showed no significant changes after conditioning on wheat in all clones (Table 3).

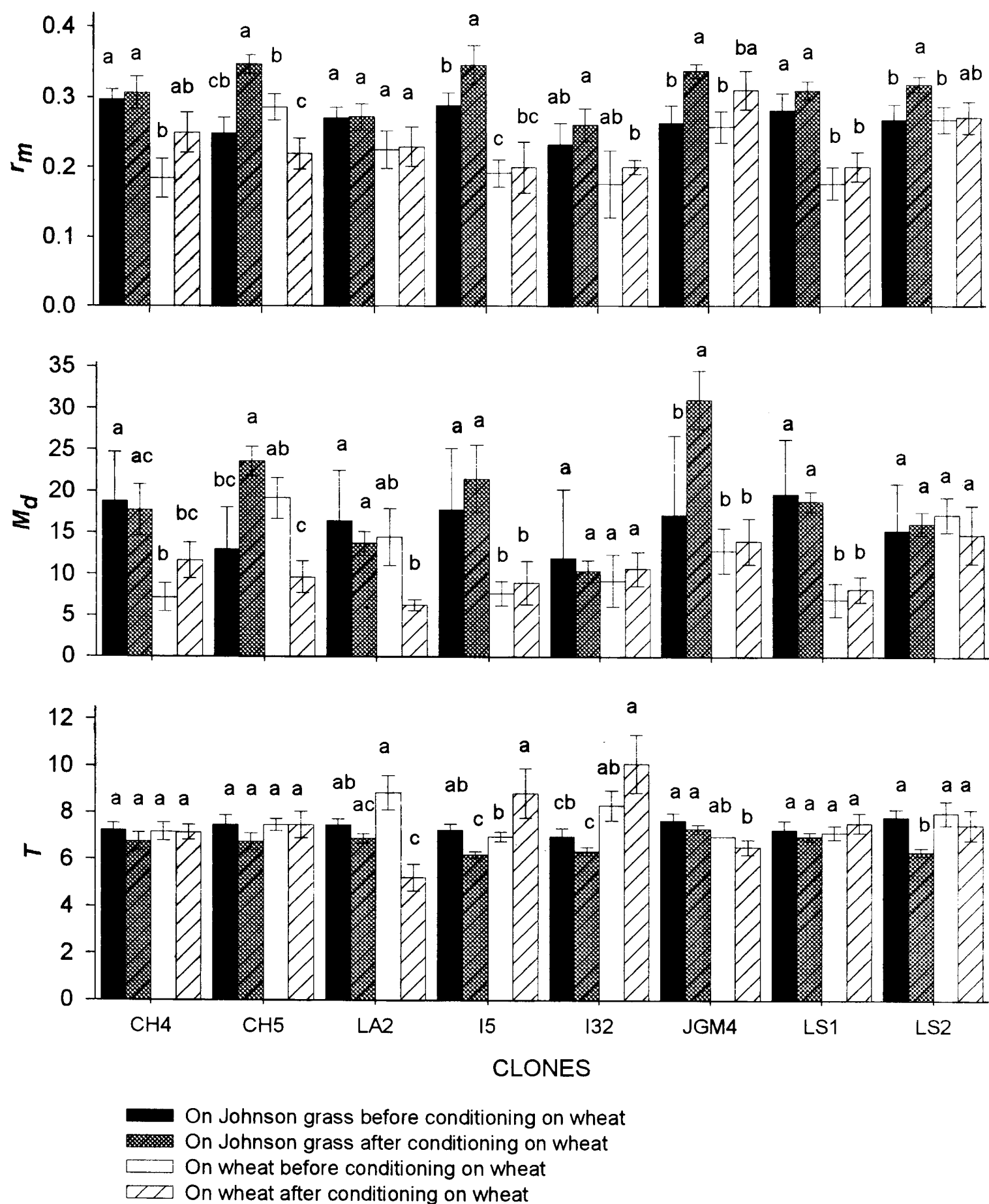


Figure 3. Histograms of performance of *R. maidis* on Johnson grass and wheat before and after the period of conditioning on wheat. Performance parameters are as in Figure 2. Lines on the top of the bars represent the standard error. Different letters within each clone indicate significant differences ($P < 0.05$).

Table 2. Three-way ANOVA table for performance parameters of *R. maidis* clones determined before and after the period of conditioning on wheat

Source	df	F	P-level
<i>r_m</i>			
Clone	7	4.02	<0.001
Host	1	58.93	<0.001
Conditioning	1	10.61	<0.01
Clone-host	7	1.97	0.06
Clone-conditioning	7	0.93	0.48
Host-conditioning	1	6.83	<0.05
Clone-host-conditioning	7	2.31	<0.05
<i>M_d</i>			
Clone	7	3.43	<0.01
Host	1	59.21	<0.001
Conditioning	1	0.10	0.75
Clone-host	7	3.77	<0.01
Clone-conditioning	7	1.37	0.22
Host-conditioning	1	3.56	0.06
Clone-host-conditioning	7	3.54	<0.01
<i>T</i>			
Clone	7	0.46	0.86
Host	1	17.53	<0.001
Conditioning	1	5.36	<0.05
Clone-host	7	1.51	0.16
Clone-conditioning	7	4.120	<0.001
Host-conditioning	1	2.939	0.09
Clone-host-conditioning	7	2.929	<0.01

Reversibility of conditioning effect. Two-way ANOVA tables for the comparison of performance on Johnson grass before the period of conditioning on wheat, after period of conditioning on wheat, and after three generations back on Johnson grass, showed that clone and evaluation time significantly affected the three performance parameters studied (r_m , T , and M_d) (Table 4). Planned comparisons showed that performance parameters of clones which improved on Johnson grass after the conditioning period, returned to values that did not differ significantly from those before the period of conditioning on wheat, with the exception of LS1 and LS2 (Figure 4).

Discussion

No negative genetic correlations between performance parameters of *R. maidis* on wheat and Johnson grass were observed before the period of conditioning on wheat. Thus, no evidence of genetic trade-off was found, hence performance on wheat is not genetically constrained. However, ANOVA results showed that M_d was affected significantly by [host-clone] interaction, suggesting some intraspecific (inter-clonal) variation in the reproductive capacity of *R. maidis* on both host plants. In other words, this result suggests some degree of genetical constraint of M_d on both host plants.

Similarly, after conditioning on wheat, the eight surviving clones showed non-significant genetic correlation between the two host plants for all performance parameters. However, values of r_m , T , and M_d showed significant [clone-host-conditioning] interaction in the ANOVA analysis, suggesting a conditioning-mediated change in the performance of some clones in either host plant. Unexpectedly, performance on wheat decreased in three clones after conditioning on wheat. These later results differed from other studies which have shown that conditioning on the novel host may increase performance on it (Via, 1991; Douglas, 1997).

Since conditioning on wheat improved the performance parameters of some clones of *R. maidis* on Johnson grass, and also of some (fewer) clones on wheat, conditioning for a prolonged period in a novel host (wheat) may produce phenotypic changes that alter the pattern of use of the established host (Johnson grass) and, less frequently, on the novel host (wheat). Increased performance on wheat after conditioning on wheat is to be expected (Via, 1991; Douglas, 1997). More intriguing is the fact that some clones improved performance on Johnson grass after conditioning on wheat. This may be ascribed to differences in quality as hosts of Johnson grass and wheat. Wheat is a very adverse host to *R. maidis*, on account of the presence of secondary metabolites with antibiotic and antifeeding effects on cereal aphids (Leszczynski et al., 1989; Thackray et al., 1990; Givovich & Niemeyer, 1995); this is in fact reflected in the incapacity of 15 out of 23 clones to persist during the period of conditioning on wheat. Long exposure of some clones to an adverse host may have induced compensation mechanisms which enable those clones to perform better in a non-demanding host such as Johnson grass.

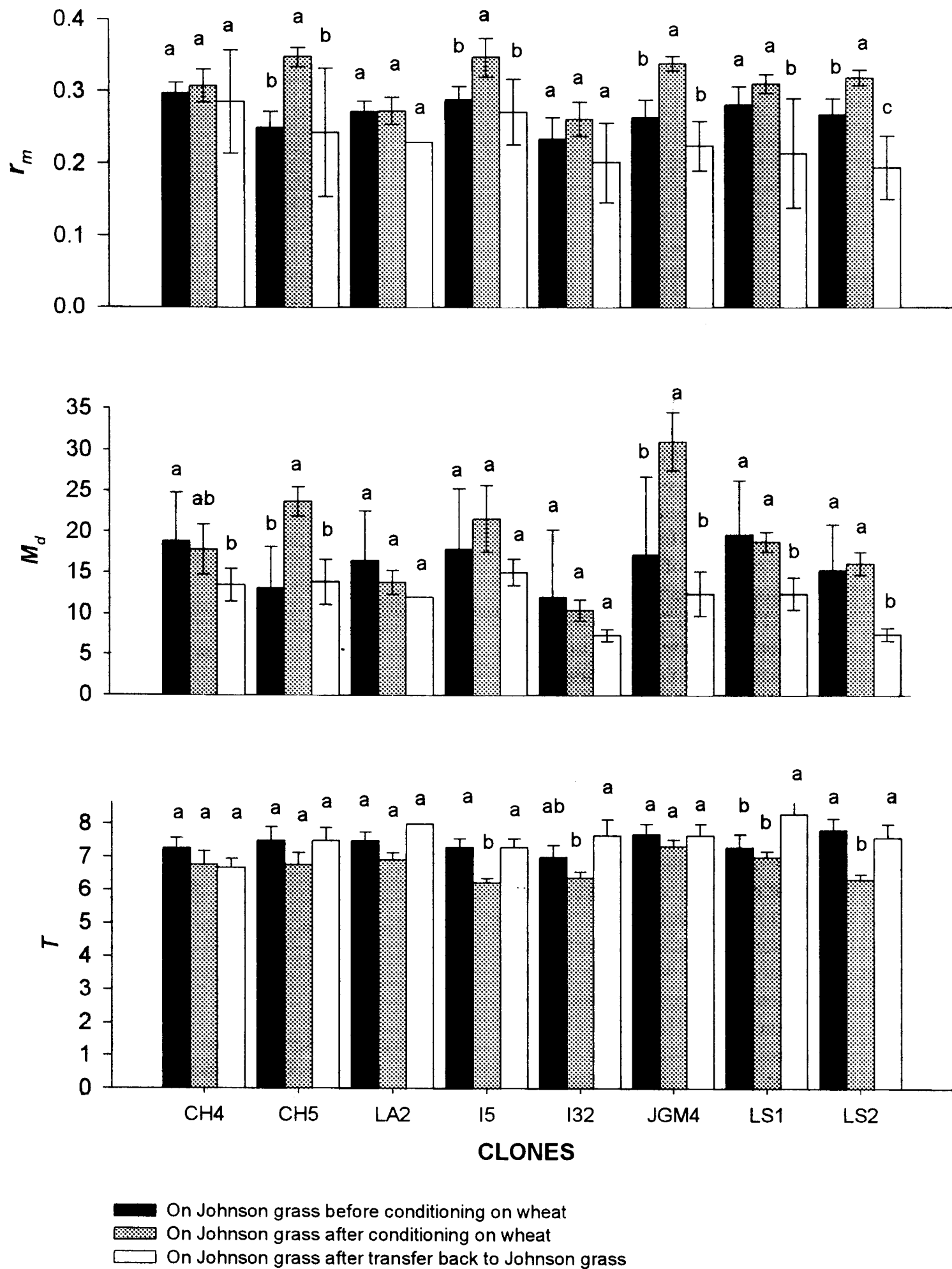


Figure 4. Histograms of performance of *R. maidis* on Johnson grass before 20 asexual generations of conditioning in wheat, after the conditioning period on wheat, and after three generations back on Johnson grass. Performance parameters are as in Figure 2. Lines on top of the bars represent the standard error. Different letters within each clone mean significant differences ($P < 0.05$).

Table 3. Survival of *R. maidis* clones on Johnson grass and wheat before and after conditioning on wheat

Clones	Survival on Johnson grass				Survival on wheat			
	Before	After	Z	P	Before	After	Z	P
CH4	0.9	0.82	-0.09	ns	0.17	0.70	2.39	*
CH5	0.5	0.82	1.08	ns	0.75	0.67	-0.2	ns
LA2	0.54	0.64	0.07	ns	0.50	0.30	0.46	ns
I5	0.80	1.00	0.67	ns	0.21	0.36	0.4	ns
I32	0.38	0.31	-0.16	ns	0.17	0.70	2.08	*
JGM	0.82	1.00	0.8	ns	0.50	0.27	0.53	ns
LS1	1.00	0.60	1.68	ns	0.20	0.70	2.08	*
LS2	0.82	0.67	0.26	ns	0.78	0.33	1.43	ns

*: < 0.05, ns = not significant.

When comparing performance parameters for the eight clones surviving conditioning on wheat, the factor evaluation time significantly affected r_m , T , and M_d (Table 4), indicating a change of performance of aphids due to conditioning. Planned comparisons showed that for most clones the performance on Johnson grass after three generations of rearing was not significantly different from the performance before conditioning on wheat (Figure 4). Thus, only three generations were sufficient for these clones to return to their original performance on Johnson grass. This result implies that: (1) the phenotypic changes produced by the period of conditioning on wheat were short-term reversible effects; (2) the eight clones that survived the period of conditioning on wheat did not modify their ability to use Johnson grass (their established host), even though they were able to survive and use wheat as a host; and (3) the capacity to use and survive on wheat did not imply costs leading to a decrease in the capacity to use Johnson grass.

As discussed above, some clones of *R. maidis* seem to have the capacity to incorporate wheat into its host plant range. The question that remains is why does not *R. maidis* develop successful colonies on wheat. In this sense, one intrinsic factor such as the number of chromosomes may be playing an important role. Karyotype biotypes of *R. maidis* ($2n = 8$, $2n = 9$, and $2n = 10$) have been reported, explaining differences in host plant fidelities (Brown & Blackman, 1988). However, in Chile only the $2n = 8$ karyotype biotype has been found (P. A. Brown, pers. comm.), a factor that could explain the reduced number of host plants (maize, sorghum, Johnson grass, barley) of *R. maidis* reported in Chile (Zúñiga, 1969; Apablaza & Tiska, 1973; Starý et al. 1994).

Table 4. Two-way ANOVA tables for performance parameters on Johnson grass of *R. maidis* clones, comparing three evaluation times: before the period of conditioning on wheat, after the period of conditioning on wheat, and after three asexual generations on Johnson grass

Source	Df	F	P-level
r_m			
Clone	7	3.04	<0.01
Evaluation time	2	25.99	<0.001
Host-evaluation time	14	1.41	0.15
M_d			
Clone	7	4.92	<0.001
Evaluation time	2	14.01	<0.001
Host-evaluation time	14	1.84	0.04
T			
Clone	7	2.02	<0.05
Evaluation time	2	14.78	<0.001
Host-evaluation time	14	1.17	0.30

Given that some clones were able to persist on wheat, and indeed even increase survival on wheat, it is likely that the low occurrence of *R. maidis* on wheat under natural conditions is not exclusively due to the intrinsic limitations studied herein. Ecological extrinsic factors affecting local populations of *R. maidis*, such as predation, parasitism, interspecific competition, and plant architecture may also be involved. Indeed, the consistent periodic disappearance of wheat, which usually remains in the field for less than 6 months (a time roughly corresponding to 20 generations of *R. maidis*), coupled to the reversibility

of the patterns produced by conditioning, resets the system every season, avoiding a possible adaptation to wheat. On the other hand, restricted use of wheat by *R. maidis* may be a consequence of this host not being selected during the host selection process performed by winged morphs (Blackman, 1988). Alternatively, dispersal between adjacent hosts may occur, as has been reported for pea aphid *Acyrtosiphon pisum* (Via, 1999), in which case performance differences between both hosts would be the main factor accounting for the lack of wheat use by *R. maidis*. Further studies evaluating limitations of host use due to extrinsic factors are needed to understand the specialisation pattern of *R. maidis*.

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