Maize and Oat Antixenosis and Antibiosis Against *Delphacodes kuscheli* (Homoptera: Delphacidae), Vector of "Mal de Rio Cuarto" of Maize in Argentina

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ABSTRACT "Mal de Río Cuarto" (MRC) is the most important virus disease of maize, *Zea mays* L., in Argentina. Several maize lines show different levels of resistance to MRC in the field; however, no studies have been conducted to investigate resistance mechanisms against its insect vector, *Delphacodes kuscheli* Fennah (Homoptera: Delphacidae). Oat, *Avena* spp., is the main overwintering host of *D. kuscheli* and main source of populations that infest maize. Although oat varieties resistant to the greenbug, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) are commercially available, their effect on *D. kuscheli* is unknown. We conducted laboratory experiments to test for the presence of antixenosis and antibiosis resistance mechanisms on six maize lines with different levels of field resistance to MRC, and seven commercial oat cultivars that include two *S. graminum*-resistant varieties. We did not find antibiotic effects of maize lines on *D. kuscheli* longevity and survivorship patterns, but we obtained antixenotic effects from the LP2 line (field moderate) due to reduced settling preference and feeding. Oat 'Bonaerense Payé' and 'Suregrain INTA' showed both antixenosis and antibiosis, with significantly less settling preference, oviposition in the no-choice test, and reduced total fecundity in comparison with the other varieties studied. The *S. graminum*-resistant 'Boyera F. A.' and 'Tambera F. A.' did not showed a consistent pattern of resistance versus *D. kuscheli* across all experiments. Our results indicate the presence of potential sources of insect resistance in the maize lines and oat cultivars tested that may be used in MRC integrated pest management programs.

KEY WORDS maize disease, planthopper vector, genetic resistance

"MAL DE RÍO CUARTO" (MRC) is the most important virus disease of maize, *Zea mays* L., in Argentina (March 1990; Ornaghi et al. 1993; March et al. 1995, 2002). MRC is spread over a large area in central Argentina and particularly in the southwest of Córdoba Province, causing losses of up to 120 million U.S. dollars per year (Velázquez et al. 2003). The disease is caused by the Fijivirus Mal de Río Cuarto Virus (MRCV), which is transmitted in a persistent manner (Nome et al. 1981, Milne et al. 1983, Uyeda and Milne 1995, Distéfano et al. 2003). *Delphacodes kuscheli* Fennah (Homoptera: Delphacidae) is the most important demonstrated vector for MRC (Remes Lenicov et al. 1985). It is a native species distributed exclusively in Argentina that develops outbreak populations in oat, *Avena sativa* L., and also breeds on wheat, *Triticum aestivum* L., and several wild grasses (Remes Lenicov and Virla 1999). Most of these hosts have been demonstrated as reservoirs of MRCV (March et al. 1997, Laguna et al. 2002). *D. kuscheli* does not breed successfully on maize, and mechanical damage has not been reported (March et al. 1997). Virus transmission occurs when macropterous adults migrate to feed on juvenile maize plants due to the senescence or harvest of oat, which is their most important winter host (Tesón et al. 1986, Remes Lenicov et al. 1991, Virla and Remes Lenicov 1991). MRCV is transmitted by nymphs, and male and female adults, and does not affect vector longevity (Arneodo et al. 2002). Two other delphacids have been shown to transmit MRCV, *Delphacodes haywardi* Muir, and *Toya propinqua* (Fieber), but their low abundance in comparison with *D. kuscheli* suggests a minor role in the epidemiology of the disease in the endemic area (Presello et al. 1997a, Velázquez et al. 2003).

Integrated pest management (IPM) of MRC includes the use of insecticides, management of planting dates, and resistant maize hybrids (March et al. 1997). Insecticides have been shown to be effective against *D. kuscheli* (March et al. 2002), but they are costly and can lead to development of insect resistance (Dent 1991). Varying planting date to avoid overlap between

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maize-susceptible stages and *D. kuscheli* peak populations also has proved to be useful, but it is constrained by environmental conditions and is not possible during all years (March et al. 1995, 1997). Finally, plant resistance also has proved to be very useful, with >20 yr of screening resulting in several maize lines and hybrids with field resistance against MRC (Presello 1991). However, most of the screening has been conducted under natural field infestations, thus not allowing distinguishing between resistance mechanisms that operate against the insect vector (i.e., antixenosis or antibiosis; Painter 1951, Panda and Khush 1995) or the virus (i.e., tolerance: Painter 1951). Presello et al. (1997b) found tolerance to MRCV multiplication in some of these maize lines.

Both antixenosis and antibiosis resistance mechanisms have been extensively used to manage planthopper pests in rice (Smith et al. 1994, Panda and Khush 1995). Survivorship of *D. kuscheli* adults and nymphs in maize is low (Virla and Remes Lenicov 1991, Costamagna 1997); thus, potential antixenosis and antibiosis resistance in maize should focus on reducing the attraction and feeding of *D. kuscheli* on the crop, to reduce the rate of virus inoculation. Within the MRC endemic area, oat is mainly used as cattle forage and despite outbreak populations of *D. kuscheli*, there are no reports of yield reductions for this crop (March et al. 1997). Therefore, *D. kuscheli* is not considered a pest in oat, and no breeding program against this insect has been conducted. There are oat-resistant varieties against another phloem-feeding Homoptera pest, the greenbug, *Schizaphis graminum* (Rondani), available (Arriaga 1954, Acciaresi and Chidichimo 1999). However, there is no information on whether this resistance also is effective against *D. kuscheli*. We conducted a series of laboratory experiments to asses the presence of antixenosis or antibiosis resistance mechanisms against *D. kuscheli* on 1) maize lines with different levels of field resistance against MRC, and 2) oat commercial cultivars with different levels of resistance to *S. graminum*.

Materials and Methods

A laboratory colony of *D. kuscheli* was maintained on oat ('Tambera F. A.' and 'Suregrain INTA') for more than two generations before trials, and experiments were conducted in a rearing room at 24.7 ± 3.6°C, 60–90% RH, and a photoperiod of 16:8 (L:D) h, unless otherwise indicated.

Experiments with Maize. Six maize lines with different levels of field resistance to MRC were used in our trials: LP116 and LP138 (MRC resistant), LP2 and P465 (moderate), and P578 and B73 (susceptible) (Presello 1991). In all experiments, corn plants in the vegetative stage coleoptile-1 leaf (Ritchie et al. 1989) were used, because this is the stage most susceptible to MRC (Ornaghi et al. 1991).

Settling Preference. A free-choice test was conducted to determine adult *D. kuscheli* settling preferences among maize lines. Maize seeds were germinated in petri dishes on moistened filter paper and after 6 d were transplanted singly to 100-ml plastic pots, filled with fertile soil. One pot for each line was placed at random in a circular pattern into a cylindrical container (23 cm in diameter). The pots with plants were put through holes in a Styrofoam floor, which generated a homogeneous background and allowed easy movement of insects among hosts. This setting was enclosed with a 35-cm-tall cylindrical wire framework covered by a fine mesh. To avoid external visual influences on insect distribution, the sides of each cage were covered with a white cardboard cylinder. Thirty macropterous females were released in a petri dish at the center of the cage, and the number that settled on different lines was recorded at 15, 20, 25, 39, 40, and 44 h after release. The experiment was replicated six times, and the proportion of females settling on each maize line was calculated for each observation.

Honeydew Excretion. Several studies have shown that planthoppers excrete less honeydew when feeding on resistant than on susceptible plant varieties, indicating nonpreference resistance in no-choice situations (Paguia et al. 1980, Khan and Saxena 1984, Padgham and Woodhead 1988, Bahagiawati et al. 1989, Mishra and Misra 1991, Smith et al. 1994). Virus inoculation occurs during *D. kuscheli* feeding (Remes Lenicov et al. 1985), and the amount of feeding can be estimated indirectly by measuring honeydew excretion (Smith et al. 1994). We conducted an experiment to test differences in the amount of feeding by 1–3-d-old macropterous females on the six maize lines and oat (Tambera F. A.) as a control host, by measuring the area of honeydew excreted. Maize and oat seeds were germinated as mentioned above and were transplanted singly into 1-kg plastic pots, filled with fertile soil. Oat was used at 1–2-leaf stage. Each pot was then covered with a plastic lid with a hole in the center through which the plant emerged. A Whatman no. 1 filter paper disk (9 cm in diameter) was placed on each lid around the base of the plants, which were then enclosed with a polyethylene terephthalate (PET) cylinder (20 cm in height, 10 cm in diameter), with a top opening covered by a fine mesh, and a lateral hole covered by a cotton plug. Three pairs of adults (one female, one male) previously starved but water satiated for 5 h were placed into the chamber through the hole at the side of the cage and allowed to feed for 24 h. The honeydew excreted dropped on to the filter paper and was readily absorbed. Filter paper disks were collected, and the spots of honeydew were visualized by applying 0.1% ninhydrin in acetone solution (Khan and Saxena 1984). The area of honeydew spots was quantified by comparison with a 1-mm^2 grid (Padgham and Woodhead 1988). The experiment was arranged in a randomized complete block design, with each treatment replicated one time within each block for a total of nine blocks. Only the cases in which at least five of the six initial insects were alive at the end of the trial were considered for analysis.

Adult Longevity and Survivorship Curves. Reduced survivorship of an insect vector also can result in lower inoculum pressure and disease transmission rate (Harpaz 1972). We conducted an experiment to compare

the longevity of newly emerged macropterous males and females on the six maize lines and oat (Tambera F. A.). The plants were prepared as described in the previous section but transplanted at a rate of two per pot. Oat plants were replaced periodically to avoid deterioration due to feeding damage during the study. Potted plants were enclosed as described for the honeydew excretion experiment, but without the plastic lid and filter paper at the base. Two pairs of newly emerged adults were enclosed in each cage. The survival and number of males and females settling on plants was recorded at 12-h intervals until the end of life (in oat the experiment was concluded 21 d after infestation, after all insects on maize were dead). The cages were arranged in a randomized complete block design, each cage serving as a replicate, and treatments were replicated seven times. Different patterns of mortality may be present with identical longevity (Rabinovich 1980); therefore, we further test for differences among lines by comparing survivorship curves obtained by pooling all data for each maize line as a single cohort. Room temperature was maintained at 25.4 ± 1.9°C, with 70–85% RH and a photoperiod of 16:8 (L:D) h.

Experiments with Oat. We conducted experiments with seven of the most commonly used commercial oat cultivars in Argentina: 'Millauquén INTA', 'Cristal INTA', 'Buck Epecuén', 'Suregrain INTA', 'Bonaerense Payé', Tambera F. A., and 'Boyera F. A'. The last two cultivars listed are resistant to *S. graminum* (Arriaga 1954, Acciaresi and Chidichimo 1999).

Settling and Oviposition Preferences. We conducted three experiments following the methodology proposed by Webster and Inayatullah (1988) for testing cultivar preferences in aphids. Oat plants were reared in 100-ml plastic cups until they reached the two-, three-, and four- to five-leaf stage (experiment a, b, and c, respectively). Height was homogenized among cultivars by cutting the tip of the top leaves before the beginning of experiments (b and c). Plants were arranged in a completely randomized design within a 50 by 50 by 50-cm cage covered by a fine mesh (70 by 70 by 50 cm in experiment a). Pots were put through a white Styrofoam (a) or cardboard floor (b and c), with four, three, and five plants per cultivar per treatment, respectively. Macropterous adults were released by opening simultaneously vials equidistantly distributed among the plants at dark (Webster and Inayatullah 1988) at a rate of four (a), three (b), and five (c) insects per plant, for a total of (females:males) 140:0 (a), 32:31 (b), and 133: 72 (c) insects per cage. To avoid selectivity based on previous experience, insects were reared on oat cultivar Buck 152 (a and b); Bermuda grass, *Cynodon dactylon* (L.) Pers.; and Johnson grass, *Sorghum halepense* (L.) Pers. (c). In the first experiment, the external rows of plants were considered border plants and not included in the data analysis. The number of insects settled per plant was registered at 24, 48 (a–c), 72 and 96 h (a and b) after the release. In experiment a, we included a cardboard lateral cover to avoid external visual stimulus; in experiments b and c, we arranged potted plants of oat completely surrounding the cages. Oviposition was estimated indirectly by counting the nymphs emerged from each plant. In experiment a, 48 h after the release plants were enclosed using similar cages as described for the honeydew excretion experiment, whereas in b and c, plants were cut after 96 h and put individually in petri dishes with wet cotton at the base of the stem to maintain the plant fresh. Fecundity was estimated by counting and removing newly emerged nymphs until no new nymphs emerged.

Honeydew Excretion and Oviposition with No Choice. Three pairs of *D. kuscheli*, 3–5 d old, were caged using the procedure described for the experiments with maize and allowed to feed in the plant for 48 h. Insects were reared on the corresponding oat cultivar for a complete generation before the experiment to avoid confounding effects of previous feeding in different hosts (Lara 1979). Honeydew quantification was done as described for maize. Oviposition was estimated directly 4–5 d after removal of the adults by dissecting the plants and counting the endophytic eggs using a microscope at 10× magnification. The experiment was conducted with a complete randomized block design with treatments replicated five times.

Nymphal Development, Survival, Duration, and Growth Index. Two experiments were conducted to establish the effect of the different oat cultivars on nymphal development. The first one was designed to assess difference in duration among stages. Newborn nymphs were reared individually using 1.5 by 6-cm glass vials with the opening closed by a wet cotton plug to provide humidity. In each tube, an oat leaf from a three- to six-leaf plant of the corresponding cultivar was provided and renewed daily. Neonates were obtained from eggs collected from a previous generation completed on the corresponding oat cultivar to control for confounding effects of previous host. The experiment was conducted as a totally randomized design with 10–31 replicates per cultivar. Due to the small number of nymphs obtained in Cristal INTA in the previous generation, this cultivar was not included in the experiment.

A second experiment was conducted to test the effect of oat cultivars on survivorship, duration, and growth index of *D. kuscheli* by using potted plants. Plastic pots (1 kg) with two to four oat plants at the four- to six-leaf stage were caged using cylindrical PET cages (10 by 40 cm) that enclosed completely the plants and were placed 5 cm within the soil of the pot. In each pot, 10 newborn nymphs (<24 h), obtained from a rearing on Bermuda grass and oat (Buck 152), were reared. Plants were renewed once a week with greenhouse plants of the same initial stage to avoid deterioration. We obtained nymphal survivorship (NS) as proportion of the initial number that reached the adult stage, nymphal duration (ND) as the mean number of days required to reach the adult stage, and growth index as $GI = NS/ND \times 100$ (Cook et al. 1987). In addition, the sex and morph of the adults produced were recorded. The experiment was conducted as a

Table 1. Mean ± SE proportion of *D. kuscheli* macropterous females settled on six maize lines (free-choice test)

Maize lines[a]	Observation						
	15 h	20 h	25 h	39 h	44 h	49 h	Avg[b]
LP138 [R]	0.25 ± 0.05	0.34 ± 0.11	0.21 ± 0.04	0.27 ± 0.05	0.22 ± 0.04	0.25 ± 0.05	0.25 ± 0.03a
B73 [S]	0.28 ± 0.07	0.29 ± 0.09	0.17 ± 0.06	0.18 ± 0.05	0.15 ± 0.04	0.14 ± 0.06	0.20 ± 0.03ab
P465 [M]	0.14 ± 0.02	0.12 ± 0.04	0.18 ± 0.04	0.15 ± 0.04	0.20 ± 0.06	0.21 ± 0.08	0.16 ± 0.02ab
LP116 [R]	0.13 ± 0.04	0.11 ± 0.03	0.19 ± 0.06	0.14 ± 0.05	0.14 ± 0.03	0.14 ± 0.05	0.14 ± 0.02ab
P578 [S]	0.13 ± 0.04	0.09 ± 0.04	0.09 ± 0.02	0.15 ± 0.03	0.16 ± 0.03	0.18 ± 0.07	0.13 ± 0.01ab
LP2 [M]	0.07 ± 0.03	0.05 ± 0.01	0.17 ± 0.07	0.11 ± 0.05	0.13 ± 0.04	0.08 ± 0.05	0.11 ± 0.02b

Proportions based on the total number of insects settled on plants, with 30 females released per cage.

[a] Between brackets is the rank of field resistance to the disease: R, resistant; M, moderate; and S, susceptible.

[b] Observations were averaged to compare treatments due to a not significant treatment × observation effect; means followed by the same letter in a column are not significantly different at $\alpha = 0.05$ using LSM differences adjusted by Tukey–Kramer (SAS Institute 2001).

randomized complete block design with six replicates, with a total of 60 insects reared per oat cultivar.

Adult Longevity and Fecundity. Adults obtained from rearing on the corresponding oat cultivar were randomly placed in pairs on single three- to four-leaf oat plants inside a glass tube (3 by 24 cm) directly placed in the soil and with the top closed by a fine mesh. Adults were held on the same plant for 7 d to ensure sexual maturity and after that they were moved to different plants daily until 15 d, to obtain daily fecundity. After that they were kept on the same plant until death, because no signs of deterioration of the plants were observed. After adult removal, plants were cut and placed in petri dishes to estimate fecundity using the methodology described for the settling and oviposition preference trials b and c.

Data Analysis. The number of insects settled and progeny produced on each host in the settling preference trials were analyzed by two-way analysis of variance (ANOVA) with treatment and block as fixed factors and observation as a repeated factor (PROC MIXED, SAS Institute 2001). Honeydew excretion, adult longevity, nymphal survivorship, nymphal duration, and growth index were analyzed by two-way ANOVA, with block and host as fixed effects (PROC MIXED, SAS Institute 2001). Means were compared using the least square mean (LSM) difference adjusted by the Tukey–Kramer method for multiple comparisons (PROC MIXED, SAS Institute 2001). Data were transformed to square root before analysis to achieve homogeneity of variances and normality of the residuals. In the adult longevity experiment with maize, sex was incorporated as a nested factor within lines to detect differences between males and females, and the interactions of block × maize line, and block × sex were estimated (Zar 1999). Survivorship curves in maize were compared using χ^2 contingency table test, and the number of insects settling on plants was compared using single classification χ^2 goodness-of-fit test, with numbers of males and females pooled in both analyses (Zar 1999). Duration of stages of nymphal development was analyzed using the nonparametric one-way ANOVA test of Kruskal–Wallis because the absence of a transformation that stabilized the variance (PROC NONPAR1WAY, SAS Institute 2001).

Results

Experiments with Maize. *Settling Preference.* Females of *D. kuscheli* showed distinct differences in their settling preferences among treatments ($F = 2.56$; df = 5, 30; $P = 0.0484$) with no significant time of observation ($F = 0.01$; df = 5, 138; $P = 1.0000$) or time of observation × treatment interaction effects ($F = 1.36$; df = 25, 139; $P = 0.1366$) (Table 1). The most preferred line was the field-resistant LP138, that differed significantly from the least preferred LP2 (field moderate), whereas the rest of the lines were intermediate. After the two initial observations that showed this trend, the settling response of *D. kuscheli* females tend to be homogeneous among lines (Table 1).

Honeydew Excretion. Due to insect mortality, 54 of 63 cases were used for data analysis. Honeydew production by *D. kuscheli* adults differed significantly between some of the maize lines and oat ($F = 6.09$; df = 6, 39; $P < 0.0001$), and there was not a significant block effect ($F = 0.78$; df = 8, 39; $P = 0.6224$). As expected, excretion on oat was higher than on most maize lines, but it did not differ significantly from LP116 (field resistant) (Table 2). The lowest honeydew production was obtained on LP2 (field moderate), but no significant differences among maize lines were obtained (Table 2).

Adult Longevity and Survivorship Curves. The longevity of *D. kuscheli* adults reared on the six maize lines

Table 2. Mean ± SE honeydew excretion and longevity of *D. kuscheli* adults reared on six maize lines and oat as control

Host[a]	Honeydew excretion[b]	Longevity[c]	
		Females	Males
LP116 [R]	58.83 ± 12.21ab	4.4 ± 0.7	4.1 ± 0.7
B73 [S]	43.67 ± 8.56b	2.7 ± 0.5	3.8 ± 0.4
P465 [M]	39.50 ± 5.80b	3.9 ± 0.3	3.4 ± 0.6
P578 [S]	37.25 ± 4.60b	3.8 ± 0.3	3.3 ± 0.4
LP138 [R]	27.11 ± 7.12b	3.6 ± 0.6	3.8 ± 0.6
LP2 [M]	17.50 ± 2.05b	2.6 ± 0.5	4.1 ± 0.5
Oat	113.56 ± 27.95a		

[a] See references for maize field resistance levels to the disease in Table 1.

[b] Values are in square millimeters per six adults per 48 h; means followed by the same letter are not significantly different (LSM adjusted by Tukey–Kramer, $\alpha = 0.05$; SAS Institute 2001).

[c] Values are in days; the experiment was stopped at day 21, when nine individuals (30%) were still alive on oat (control).

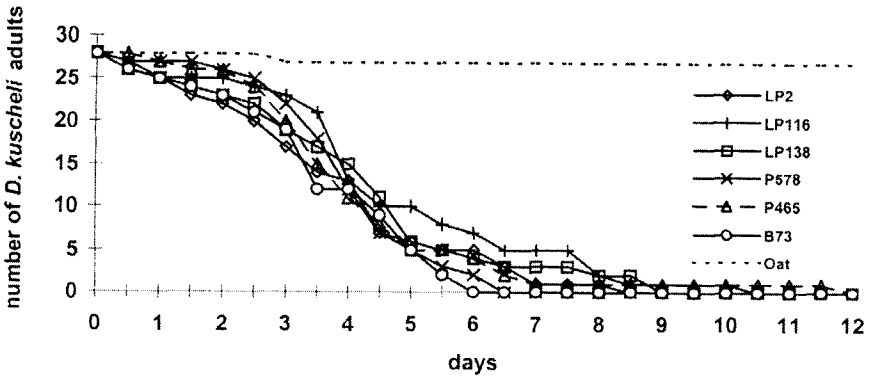

Fig. 1. Survivorship curves of *D. kuscheli* adults on six maize lines. The experiment was stopped at day 21, when nine individuals (30%) were still alive on oat (control).

did not differ significantly ($F = 0.95$; df = 5, 30; $P = 0.4643$), nor was there a significant sex effect ($F = 1.59$; df = 1, 36; $P = 0.4491$; Table 2) or block effect ($F = 1.17$; df = 6, 30; $P = 0.3483$), and none of the interaction terms were significant (block × lines: $F = 0.86$; df = 30, 36; $P = 0.6650$; block × sex: $F = 1.30$; df = 36, 84; $P = 0.1629$). On oat, longevity was higher, and the control was stopped at day 21, when nine individuals (30%) remained alive (longevity: 16.2 ± 2.5 d, mean ± SE). Therefore, no statistical comparison was performed between maize lines and oat for this parameter. Survivorship curves did not differ among maize lines, indicating a similar pattern of mortality on the different maize lines ($\chi^2 = 76.01$, df = 138, $P = 0.9999$) (Fig. 1). No significant differences in the number of insects settling on plants were found among maize lines, providing no evidence of repellence (χ^2 tests, all $P > 0.10$).

Experiments with Oat. *Settling and Oviposition Preferences.* In all trials, there were no significant effects of time of observation (all $P > 0.98$) or the interaction between time of observation and treatment (all $P > 0.40$); therefore, we compared settling responses averaged across all observation times. Experiments a and b showed no significant differences among treatments ($F = 1.64$; df = 6, 21; $P = 0.1846$; and $F = 0.43$; df = 6, 14; $P = 0.8480$, respectively), but treatments differed significantly in experiment c) ($F = 3.31$; df = 6, 28; $P = 0.0138$) in which the most preferred Boyera F. A. differed significantly from Bonaerense Payé INTA (LSM adjusted by Tukey–Kramer at $\alpha = 0.07$; Table 3). Oviposition preferences differed significantly at the 10% level in experiments a and c ($F = 2.30$; df = 6, 21; $P = 0.0731$ and $F = 2.05$; df = 6, 28; $P = 0.0915$, respectively), but they did not differ in experiment b ($F = 0.78$; df = 6, 14; $P = 0.5968$). In all cases, oviposition preferences reflected the trend of settling observed for the adults (Table 3).

Honeydew Excretion and Oviposition with No Choice. The amount of honeydew excreted did not differ significantly among oat cultivars ($F = 1.14$; df = 6, 28; $P = 0.3655$) and ranged on average between 149 and 90 mm^2 for six adults in 48 h (Table 4). However, 10 times more eggs were obtained on Millauquén INTA, which differed significantly from Suregrain INTA, Tambera F. A., Bonaerense Payé, and Boyera F. A. ($F = 6.84$; df = 6, 28; $P = 0.0002$; Table 4). High oviposition on Millauquén INTA also was obtained in free-choice trials.

Nymphal Development, Survival, Duration, and Growth Index. We obtained significant differences in the duration of the first and the last nymphal instar

Table 3. Mean ± SE proportion of *D. kuscheli* adults settled and progeny allocated on seven oat cultivars in free-choice tests

Oat[a]	Exp. a		Exp. b		Exp. c	
	Settling[b]	Progeny[c]	Settling[b]	Progeny[c]	Settling[b,d]	Progeny[c]
Bo	0.039 ± 0.006	22.0 ± 6.1	0.039 ± 0.005	8.0 ± 2.6	0.045 ± 0.006a	21.0 ± 6.8
Ta	0.020 ± 0.005	8.8 ± 3.1	0.036 ± 0.006	4.3 ± 1.3	0.041 ± 0.006ab	13.6 ± 4.6
Mi	0.051 ± 0.009	26.3 ± 13.2	0.054 ± 0.009	4.0 ± 1.6	0.039 ± 0.009ab	21.6 ± 7.1
Cr	0.020 ± 0.003	11.3 ± 6.3	0.049 ± 0.014	11.3 ± 10.8	0.020 ± 0.004ab	4.6 ± 1.4
Su	0.040 ± 0.007	41.3 ± 13.4	0.064 ± 0.006	2.0 ± 0.6	0.020 ± 0.004ab	6.2 ± 1.5
BE	0.047 ± 0.005	35.8 ± 6.5	0.049 ± 0.011	1.7 ± 1.2	0.019 ± 0.004ab	12.2 ± 3.0
BP	0.035 ± 0.005	15.3 ± 4.0	0.042 ± 0.007	2.3 ± 1.9	0.015 ± 0.006b	10.0 ± 4.2

[a] Oat cultivars: Mi, Millauquén INTA; Bo, Boyera F. A.; Ta, Tambera F. A.; BE, Buck Epecuén; BP, Bonaerense Payé; Su, Suregrain INTA; and Cr, Cristal INTA.

[b] Proportions based on the total number of adults settled on plants averaged across all observations periods (a: $n = 4$, 140 insects; b: $n = 3$, 63 insects; and c: $n = 5$, 205 insects).

[c] Nymphs emerged per plant.

[d] Means followed by the same letter in a column are not significantly different (LSM adjusted by Tukey–Kramer, $\alpha = 0.07$, SAS Institute 2001).

Table 4. Mean ± SE honeydew excreted and oviposition with no choice of *D. kuscheli* adults on seven oat cultivars during 48 h

Oat	Honeydew excretion[a]	Oviposition[b]
Millauquén INTA	130.4 ± 22.8	40.4 ± 9.8a
Cristal INTA	149.0 ± 14.9	18.6 ± 4.8ab
Buck Epecuén	90.0 ± 18.1	14.8 ± 4.2ab
Suregrain INTA	135.6 ± 28.4	12.6 ± 2.9b
Tambera F. A.	122.2 ± 23.9	10.4 ± 3.4b
Bonaerense Payé	121.6 ± 11.3	4.6 ± 3.1b
Boyera F. A.	96.6 ± 11.3	3.6 ± 2.5b

[a] Values are in square millimeters per six adults per 48 h.
[b] Number of eggs per plant; means followed by the same letter are not significantly different (LSM adjusted by Tukey–Kramer, $\alpha = 0.05$, SAS Institute 2001).

($\chi^2 = 14.87$; df = 5; $P = 0.0109$ and $\chi^2 = 13.67$; df = 5; $P = 0.0179$, respectively), but both were in opposite directions, and there was no difference among different oat cultivars for overall nymphal development (17.4 ± 0.12 d, $n = 109$; $\chi^2 = 6.46$; df = 5; $P = 0.2637$). Nymphal survivorship fluctuated between 60 and 80% in this test, and all adults obtained were macropterous. No significant differences were obtained on the survivorship ($F = 0.74$; df = 6, 32; $P = 0.6196$), duration ($F = 0.65$; df = 6, 28; $P = 0.6933$), and growth index ($F = 0.70$; df = 6, 32; $P = 0.6489$) of nymphs reared on potted plant (Table 5). The rate of braquipterous: macropterous obtained did not differ significantly among cultivars ($\chi^2 = 8.29$, df = 6, $P = 0.22$), further confirming the absence of differences among the oat cultivars tested as hosts for the development of *D. kuscheli*. To remove variability introduced by the different morphs, we conducted separate ANOVAs for the duration of the nymphal stage for each morph, and they also showed no significant effect of oat cultivar (data not shown).

Adult Longevity and Fecundity. Adults lived on average 21.6 ± 1.0 d ($n = 35$), and longevity did not differ significantly among oat cultivars ($F = 1.46$; df = 6, 13; $P = 0.2300$; Table 5). Longevity was independent of adult sex and variety ($\chi^2 = 3.53$, df = 6, $P = 0.7399$). However, significantly more nymphs were produced on Tambera F. A. and Boyera F. A., than on Suregrain INTA, being the rest of the varieties intermediate ($F = 3.14$; df = 6, 13; $P = 0.0396$; Table 5).

Discussion

Our results showed no antibiotic effects of maize lines on the longevity and feeding of adult *D. kuscheli*, but some degree of antixenosis for settling. The line LP2 (field moderate resistance) showed consistently lower number of adults settling in the free choice experiment and yielded low amounts of honeydew in the no-choice test. Using slightly different methods, Costamagna (1997) obtained a low but significant reduction on *D. kuscheli* longevity reared on LP2. Therefore, our results confirmed that line LP2 has some degree of resistance to *D. kuscheli*, and thus it may be potentially exposed to a lower rate of virus inoculation than the other lines.

We did not obtain correspondence between field resistance (measured as disease incidence) and maize antixenosis or antibiosis effects. Field resistant lines LP138 and LP116 showed more susceptibility to *D. kuscheli* than field moderate and susceptible lines. Presello et al. (1997b) demonstrated that juvenile plants of lines LP116 and LP2 show lower initial increase in virus concentration than susceptible B73, and this was reflected later in the symptoms expressed by each line, with B73 producing the most severe symptoms of the disease. Therefore, resistance to virus multiplication could explain the levels of field resistance expressed in these three lines, suggesting a minor role of resistance to the vector in this system.

Oat cultivars resistant to *S. graminum* did not show a consistent pattern of resistance across all experiments. Both Boyera F. A. and Tambera F. A. received significantly fewer eggs in the no-choice experiment, but they were significantly preferred in free-choice tests for settling and had high growth index, adult longevity, and significantly higher fecundity than other cultivars. By contrast, Bonaerense Payé and Suregrain INTA showed some degree of antixenosis and antibiosis to *D. kuscheli*, with significantly lower settling preference in one of three experiments, oviposition in the no-choice experiment, and total fecundity. Both Buck Epecuén and Cristal INTA showed a consistent intermediate response pattern across all experiments. Finally, Millauquén INTA received significantly more eggs in no-choice experiments and

Table 5. Mean ± SE nymphal survivorship (proportion), duration (ND, in days), growth index (GI), relative number of braquipterous: macropterous (B: M), adult longevity (in days), and progeny of *D. kuscheli* reared on seven oat cultivars

Oat	Survivorship	ND	GI	B: M	Adult longevity	Progeny[a]
Tambera F. A.	0.68 ± 0.07	16.20 ± 0.16	5.06 ± 0.48	1.56	20.0 ± 2.3	128.0 ± 17.0a
Boyera F. A.	0.68 ± 0.06	16.46 ± 0.18	4.12 ± 0.45	1.56	26.7 ± 2.4	113.0 ± 21.6a
Millauquén INTA	0.76 ± 0.05	16.24 ± 0.20	4.70 ± 0.33	2.46	25.0 ± 1.1	114.0 ± 3.0ab
Bonaerense Payé	0.73 ± 0.09	16.48 ± 0.20	4.19 ± 0.59	1.44	19.2 ± 3.0	102.7 ± 33.2ab
Buck Epecuén	0.60 ± 0.05	16.00 ± 0.19	3.77 ± 0.34	3.71	19.8 ± 2.6	88.3 ± 19.2ab
Cristal INTA	0.58 ± 0.10	16.17 ± 0.16	4.33 ± 0.66	1.69	20.6 ± 1.6	49.0 ± 3.0ab
Suregrain INTA	0.70 ± 0.09	16.38 ± 0.20	4.09 ± 0.67	1.62	19.3 ± 4.2	28.3 ± 23b

[a] Total number of nymphs produced per female. Means followed by the same letter are not significantly different (LSM adjusted by Tukey–Kramer, $\alpha = 0.05$, SAS Institute 2001).

allowed high rates of *D. kuscheli* settling, oviposition, fecundity, growth, and adult longevity.

Resistance mechanisms such as antibiosis and antixenosis have been successfully used in rice to control delphacid pests in Southeast Asia (Smith et al. 1994, Panda and Khush 1995, Cohen et al. 1997). Planthopper pests of rice increase their numbers within the same crop, whereas *D. kuscheli* population increase occurs outside maize, thus limiting the development of resistance toward the insect vector in this crop. However, we observed some level of resistance on maize line LP2, in agreement with previous reports, suggesting that further testing of this line is necessary. Moreover, by combining maize lines that have resistance to the virus with those that have resistance to the vector, further reduction in MRC incidence may be achieved. In addition, our experiments showed an absence of resistance mechanisms against *D. kuscheli* in commercially available *S. graminum*-resistant oat cultivars, but indicated low levels of resistance in Bonaerense Payé and Suregrain INTA. Our results showed that both experimental maize lines and oat cultivars present sources of resistance against *D. kuscheli*. More research is required to further explore the potential of these resistance sources for breeding resistant lines and cultivars to improve MRC IPM.

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References Cited

Acciaresi, H. A., and H. O. Chidichimo. 1999. Interacción genotipo-ambiente en *Avena sativa* L. utilizando los modelos AMMI y factorial de correspondencias. Pesq. Agrop. Bras. 34: 1823–1830.

Arneodo, J. D., F. A. Guzmán, L. R. Conci, I. G. Laguna, and G. A. Truol. 2002. Transmission features of *Mal de Río Cuarto virus* in wheat by its planthopper vector *Delphacodes kuscheli*. Ann. Appl. Biol 141: 195–200.

Arriaga, H. O. 1954. Resistencia a la toxemia de *Schizaphis graminum* (Rondani) en cereales finos. Rev. Fac. Agron. 30: 65–101.

Bahagiawati, A. H., E. A. Heinrichs, and F. G. Medrano. 1989. Effect of host plant on the level of virulence of *Nilaparvata lugens* (Homoptera: Delphacidae) on rice cultivars. Environ. Entomol. 18: 489–493.

Cohen, M. B., S. N. Alam, E. B. Medina, and C. C. Bernal. 1997. Brown planthopper, *Nilaparvata lugens*, resistance in rice cultivar IR64: mechanism and role in successful *N. lugens* management in Central Luzon, Philippines. Entomol. Exp. Appl. 85: 221–229.

Cook, A. G., S. Woodhead, V. F. Magalit, and E. A. Heinrichs. 1987. Variation in feeding behaviour of *Nilaparvata lugens* on resistant and susceptible rice varieties. Entomol. Exp. Appl. 43: 227–235.

Costamagna, A. C. 1997. Mal de Río Cuarto del Maíz. Estudios de resistencia de 6 líneas de maíz en relación al vector: *Delphacodes kuscheli* (Homoptera: Delphacidae), pp. I.73–I.78, vol. I. *In* Proceedings, VI Congreso Nacional de Maíz, 12–15 November 1997, Pergamino, Buenos Aires, Argentina.

Dent, D. 1991. Insect pest management. C.A.B. International, Wallingford, Oxon, United Kingdom.

Distéfano, A. J., L. R. Conci, M. M. Hidalgo, F. A. Guzman, H. E. Hopp, and M. del Vas. 2003. Sequence and phylogenetic analysis of genome segments S1, S2, S3 and S6 of Mal de Rio Cuarto virus, a newly accepted Fijivirus species. Virus Res. 92: 113–121.

Harpaz, I. 1972. Maize rough dwarf. A planthopper virus disease affecting maize, rice, small grains and grasses. Israel University Press, Jerusalem, Israel.

Khan, Z. R., and R. C. Saxena. 1984. Technique for demonstrating phloem or xylem feeding by leafhoppers (Homoptera: Cicadellidae) and planthoppers (Homoptera: Delphacidae) in rice plant. J. Econ. Entomol. 77: 550–552.

Laguna, I. G., A. M. Remes Lenicov, E. Virla, A. Avila, M. P. Giménez Pecci, P. Herrera, J. Garay, D. Ploper, and R. Mariani. 2002. Difusión del virus del Mal de Río Cuarto, su vector, delfácidos asociados y hospedantes alternativos en Argentina. Rev. Soc. Arg. Entomol. 61: 87–97.

Lara, F. M. 1979. Principios de resistencia de plantas a insetos. Livroceres Ltda., Piracicaba, S. P., Brazil.

March, G. J. 1990. Mal de Río Cuarto. Rev. Invest. Agrop. 12: 147–156.

March, G. J., M. Balzarini, J. A. Ornaghi, J. E. Beviacqua, and J. Marinelli. 1995. Predictive model for 'Mal de Río Cuarto' disease intensity. Plant Dis. 79: 199–201.

March, G. J., J. A. Ornaghi, J. E. Beviacqua, and S. L. Lenardon. 1997. Manual técnico del Mal de Río Cuarto, 1st ed. Morgan, Tecnología Mycogen, Buenos Aires, Argentina.

March, G. J., J. A. Ornaghi, J. E. Beviacqua, J. Giuggia, A. Rago, and S. L. Lenardon. 2002. Systemic insecticides for control of *Delphacodes kuscheli* and the Mal de Rio Cuarto virus on maize. Int. J. Pest Manag. 48: 127–132.

Milne, R. G., G. Boccardo, E. Dalbo, and F. Nome. 1983. Association of maize rough dwarf virus with "Mal de Río Cuarto" in Argentina. Phytopathology 73: 1290–1292.

Mishra, N. C., and B. C. Misra. 1991. Preference of white-backed planthopper, *Sogatella furcifera* to different rice varieties. Entomol. Exp. Appl. 59: 87–92.

Nome, S. F., S. L. Lenardón, I. G. Laguna, S. K. Lowe, and D. M. Docampo. 1981. Partículas de virus (Reovirus) asociados al Mal de Río Cuarto en cultivos de maíz. Serie Didáctica. Universidad Nacional de Córdoba, Facultad de Ciencias Agrarias.

Ornaghi, J., G. Boito, G. Sánchez, and A. Marinelli. 1991. Incidencia del maize rough dwarf virus-Río Cuarto según su transmisión en diferentes estados fenológicos del maíz y tiempos de inoculación, pp. 45–46. *In* Proceedings "Taller de actualización sobre Mal de Río Cuarto", INTA-CIMMYT, 30–31 May 1991, Pergamino, Buenos Aires, Argentina.

Ornaghi, J., G. Boito, G. Sánchez, G. March, and J. Beviacqua. 1993. Studies on the populations of *Delphacodes kuscheli* Fennah in different years and agricultural areas. J. Genet. Breed. 47: 277–282.

Padgham, D. E., and S. Woodhead. 1988. Variety-related feeding patterns in the brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae), on its host, the rice plant. Bull. Entomol. Res. 78: 339–349.

Paguia, P., M. D. Pathak, and E. A. Heinrichs. 1980. Honeydew excretion measurement techniques for determining differential feeding activity of biotypes of *Nilaparvata lugens* on rice varieties. J. Econ. Entomol. 73: 35–40.

Painter, R. H. 1951. Insect resistance in crop plants. Macmillan Co., New York.

Panda, N., and G. S. Khush. 1995. Host plant resistance to insects. C.A.B. International. Wallingford, Oxon, United Kingdom.

Presello, D. A. 1991. Incidencia del Mal de Río Cuarto en Pergamino durante la campaña 1990 /91, pp. 73–77. *In* Proceedings "Taller de actualización sobre Mal de Río Cuarto", INTA-CIMMYT, 30–31 May 1991, Pergamino, Buenos Aires, Argentina.

Presello, D. A., A. C. Costamagna, L. Conci, A.M.M. de Remes Lenicov, F. A. Guzmán, and P. Herrera. 1997a. Mal de Río Cuarto del maíz. Estudio de la capacidad vectora de las poblaciones de *T. propinqua* presentes en el área de Pergamino, pp. II.1–II.5, vol. II. *In* Proceedings, VI Congreso Nacional de Maíz, 12–15 November 1997, Pergamino, Buenos Aires, Argentina.

Presello, D. A., P. E. Rodríguez Pardina, A. E. Céliz, I. G. Laguna, and P. S. Herrera. 1997b. Concentración viral en raíces de líneas de maíz con diferente nivel de resistencia al Mal de Río Cuarto Fijivirus, pp. I.5–I.13, vol. I. *In* Proceedings, VI Congreso Nacional de Maíz, 12–15 November 1997, Pergamino, Buenos Aires, Argentina.

Rabinobich, J. E. 1980. Introducción a la ecología de poblaciones animales. C.E.C.S.A., México.

Remes Lenicov, A.M.M. de, and E. G. Virla. 1999. Delfácidos asociados al cultivo de maíz en la República Argentina (Insecta–Homoptera–Delphacidae). Rev. Fac. Agron. 104: 1–15.

Remes Lenicov, A.M.M. de, A. Tesón, E. Dagoberto, and N. Huguet. 1985. Hallazgo de uno de los vectores del "Mal de Río Cuarto" del maíz. Gac. Agron. 5: 251–258.

Remes Lenicov, A.M.M. de, E. G. Virla, and E. Dagoberto. 1991. Cambios estacionales en la población del vector del "Mal de Río Cuarto" del maíz (*Delphacodes kuscheli* Fennah, 1935) en cultivos de avena y sus malezas circundantes en Sampacho, Córdoba. (Insecta, Homoptera-Fulgoroidea), pp. 116–129. *In* Proceedings "Taller de actualización sobre Mal de Río Cuarto", INTA-CIMMYT, 30–31 May 1991, Pergamino, Buenos Aires, Argentina.

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1989. How a corn plant develops. Iowa State University Special Report No. 48, Ames.

SAS Institute. 2001. SAS/STAT user's manual, version 8.2. SAS Institute, Cary, NC.

Smith, C. M., Z. R. Khan, and M. D. Pathak. 1994. Techniques for evaluating insect resistance in crop plants. CRC, Boca Raton, FL.

Tesón, A., A.M.M. de Remes Lenicov, E. Dagoberto, and S. Paradell. 1986. Estudio de las poblaciones de delfácidos sobre maíz, avena y maleza circundante. Gac. Agron. 6: 507–517.

Uyeda, I., and R. G. Milne. 1995. Introduction: genomic organization, diversity and evolution of plant reoviruses. Semin. Virol. 6: 85–88.

Velázquez, P. D., J. D. Arneodo, F. A. Guzman, L. R. Conci, and G. A. Truol. 2003. *Delphacodes haywardi* Muir, a new natural vector of Mal de Río Cuarto virus in Argentina. J. Phytopathol. 151: 669–672.

Virla, E., and A.M.M. de Remes Lenicov. 1991. Ciclo de vida de *Delphacodes kuscheli* criado sobre diferentes hospedantes en condiciones de laboratorio, pp. 104–115. *In* Proceedings "Taller de actualización sobre Mal de Río Cuarto", INTA-CIMMYT, 30–31 May 1991, Pergamino, Buenos Aires, Argentina.

Webster, J. A., and C. Inayatullah. 1988. Assessment of experimental designs for greenbug (Homoptera: Aphididae) antixenosis tests. J. Econ. Entomol. 81: 1246–1250.

Zar, J. H. 1999. Biostatistical analysis, 3rd ed. Prentice-Hall, Englewood Cliffs, NJ.

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