Behavior

Rhopalosiphum padi (Hemiptera: Aphididae) Responses to Volatile Cues From Barley Yellow Dwarf Virus–Infected Wheat

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ABSTRACT In choice bioassays, Rhopalosiphum padi L. nonviruliferous apterae preferentially locate near volatile organic compounds (VOCs) emitted from Barley yellow dwarf virus (BYDV)-infected wheat plants compared with VOCs from noninfected plants. However, the specific VOCs responsible for R. padi responses are unknown. It is unclear also if R. padi responses to BYDV-infected wheat are caused by arrestment or attraction. Additionally, the responses of viruliferous apterae and nonviruliferous alate to BYDV-infected wheat have not been examined. R. padi responses were studied through emigration, immigration, and settling laboratory bioassays using BYDV-infected and noninfected wheat plants. Two wheat genotypes, virus-susceptible Lambert and virus-resistant Lambert-derived transgenic 103.1J expressing the BYDV-PAV coat protein gene, were evaluated. In a settling bioassay, alates preferentially settled on noninfected 103.1J. Responses of viruliferous and nonviruliferous R. padi to virus-infected, noninfected, and sham-inoculated (exposed to nonviruliferous aphids) Lambert and 103.1J were examined in separate bioassays. A paper leaf model served as a control. Immigration by viruliferous apterae was significantly lower toward the paper leaf model, but no significant differences were observed among plant treatments. Nonviruliferous apterae exhibited no significant differences in emigration among treatments, suggesting no arrestment occurred. Nonviruliferous apterae significantly preferred to immigrate toward BYDV-infected Lambert. Immigration toward the paper leaf model was significantly lower compared with plant treatments. Responses of R. padi to VOCs were tested by applying compounds to paper leaf models at concentrations designed to mimic those present in headspace of wheat plants. Nonviruliferous apterae immigrated in significantly greater numbers toward paper leaf models individually treated with nonanal, (Z)-3-hexenyl acetate, decanal, caryophyllene, and undecane than toward paper leaf models that served as controls and toward leaf models treated with synthetic blends made to mimic headspace of BYDV-infected compared with blends made to mimic headspace of noninfected wheat plants. Results suggest responses of R. padi to BYDV-infected plants are caused by attraction rather than arrestment.

KEY WORDS plant–insect–virus interactions, bird cherry-oat aphid, virus-induced volatiles, Barley yellow dwarf virus epidemiology, transgenic virus resistance

Rhopalosiphum padi L., the bird cherry-oat aphid, is considered among the most important insect pests of cereals worldwide (Gildow and Rochow 1983). In Idaho, R. padi is one of the most numerous and economically significant insect pests of winter and spring wheat, Triticum aestivum L. (Forster and Rochow 1985, Schotzko and Bosque-Pérez 2000). This aphid damages wheat plants both by direct feeding and as a result of virus transmission. Rhopalosiphum padi is a main vector of Barley yellow dwarf virus (BYDV) (Luteoviridae: Luteovirus) (Gildow and Rochow 1983), the causal agent of one of the most serious diseases of cereals worldwide. In Idaho, BYDV has caused yield losses in wheat of up to 70% in individual fields (Bishop and Sandvol 1984).

Virus-infected host plants have been found to affect the biology and behavior of aphid vectors (Ajayi and Dewar 1983, Eckel and Lampert 1996), including R. padi (Jiménez-Martínez and Bosque-Pérez 2004; Jiménez-Martínez et al. 2004a, b). Aphids feeding on virus-infected plants had greater nymphal survival, adult fecundity, longevity, and/or increased growth rate (Kennedy 1951, Araya and Foster 1987, Fereres et al. 1989, Jiménez-Martínez et al. 2004a, Srinivasan et al. 2008).
Studies conducted with Potato leafroll virus (PLRV) (Luteoviridae: Polerovirus) by Castle et al. (1998) showed that a main virus vector, the green peach aphid, *Myzus persicae* (Sulzer), preferentially colonized PLRV-infected potato plants (*Solanum tuberosum* L.) compared with healthy. Potato virus X (PVX) or Potato virus Y (PVY)-infected plants. Eigenbrode et al. (2002) further showed that potato plants infected with PLRV release volatile organic compounds (VOCs) that attract and arrest more aphids compared with plants infected with PVX or PVY viruses or healthy plants under laboratory conditions. More recently, Srinivasan et al. (2006) reported that PLRV-infected hairy nightshade (*Solanum sarrachoides* [Sendtner]) and potato plants were preferred by *M. persicae* over noninfected plants. Recent work has shown also that arrestment of *M. persicae* by PLRV-infected potato plants requires the blend of VOCs released by these plants and is not produced in response to a single compound (Ngumbi et al. 2007).

The behavioral ecology of *R. padi* is strongly influenced by olfactory stimuli of various types (Petterson 1994). The behavior of both apterae and alate *R. padi* is affected by volatile cues from host plants (Pickett et al. 1992, Petterson et al. 1994, 1995, Quiroz et al. 1997, Quiroz and Niemeyer 1998, Jiménez-Martínez et al. 2004b). Jiménez-Martínez et al. (2004b) studied the response of nonviruliferous apterae of *R. padi* to BYDV-infected wheat plants of both virus-resistant transgenic and nontransformed virus-susceptible genotypes. They found that volatile cues from virus-susceptible plants infected with BYDV were more attractive and/or arrestant to aphids than volatile cues from virus-infected transgenic and/or noninfected plants of either genotype. The VOCs from headspace of BYDV-infected Lambert plants were found to be higher in total concentration and to differ in relative concentrations of components compared with noninfected plants (Jiménez-Martínez et al. 2004b). However, the specific volatile cues responsible for the observed responses by *R. padi* have not been determined.

Viral pathogens are spread by both winged and wingless aphids; thus, the behavior of alate *R. padi* is important for understanding virus epidemiology. Olfactory orientation in flight has been shown in aphids (Pickett et al. 1992). *R. padi* generally is a host-alternating species, and several factors can influence its selection of host plants including light, sound, odor, and learning (Kring 1972). The abundance of secondary rhinaria on the antenna of alates suggests they are involved in location of host plants (Pickett et al. 1992) and response to plant volatiles. Recently, Srinivasan et al. (2006) showed that alate *M. persicae* preferred settling on PLRV-infected than on noninfected plants of both hairy nightshade and potato regardless of light regimen (dark or lighted).

To further understand previous findings, bioassays were conducted to determine whether responses of nonviruliferous apterae *R. padi* to BYDV-induced VOCs are caused by arrestment (reduced emigration) or attraction (greater immigration). In addition, because the behavioral responses of viruliferous apterae *R. padi* to BYDV-induced VOCs from nontransformed virus-susceptible and virus-resistant transgenic wheat genotypes have not been examined, assays were conducted to examine such responses. Further bioassays were conducted to examine responses of nonviruliferous apterae *R. padi* to individual pure VOCs and a blend of VOCs at concentrations designed to mimic those present in headspace of wheat plants. Additionally, because the influence of virus-induced volatile cues on alates has not been studied, we conducted a laboratory settling bioassay to assess the behavioral responses of nonviruliferous alate *R. padi* to BYDV-infected and noninfected transgenic and nontransformed wheat plants.

### Materials and Methods

All experiments were conducted in the Host Plant Resistance and Chemical Ecology Laboratories at the University of Idaho, Moscow, ID.

**Wheat Genotypes, Virus Isolate, and Aphids.** Third-generation transgenic soft white winter wheat (genotype 103.1J) expressing the BYDV (PAV serotype) coat protein (CP) gene, and the BYDV-susceptible cultivar Lambert were tested. The transgenic genotype 103.1J, derived from the parental cultivar Lambert, has shown low BYDV titer compared with Lambert and is considered moderately resistant to BYDV (Jiménez-Martínez et al. 2004a). Polymerase chain reaction (PCR) was performed to ensure that each transgenic plant used in bioassays carried the BYDV CP gene. The PCR protocol described by Jiménez-Martínez et al. (2004a) was followed using 20-mer primers (Invitrogen, Rockville, MD) designed to amplify a 499-base fragment of the BYDV CP gene. Plant tissue for PCR was collected after insect bioassays were conducted.

Both wheat genotypes were planted in 10.2-cm-diameter plastic pots filled with soil mixture (f:1 ratio Sunshine mix #1 [Sun-Gro Horticulture, Bellevue, WA] and sand), placing one seed per pot. Plants were grown in environmental growth chambers at 20 ± 1°C and a photoperiod of 16:8 (L:D). Plants were fertilized biweekly beginning at the two- to three-leaf stage (Zadoks 12-13) (Zadoks et al. 1974) using a soluble N-P-K fertilizer (20-20-20).

A Washington State isolate of BYDV-PAV maintained by mass transfer of *R. padi* (Idaho clone) on barley (*Hordeum vulgare* L.) plants (cultivar Sprinter) was used to inoculate plants. A nonviruliferous colony of *R. padi* was maintained on healthy Sprinter barley plants in environmental growth chambers at 20 ± 1°C and a photoperiod of 16:8 (L:D) at the H. C. Manis Entomology Laboratory at the University of Idaho, Moscow, ID. Nonviruliferous alate aphids used for settling bioassays (see below) were produced by induced crowding (Srinivasan et al. 2006). A viruliferous colony was reared on BYDV-infected Sprinter barley plants under the same environmental conditions as the nonviruliferous colony, in the Host Plant Re-
sistance Laboratory in the Agricultural Biotechnology building.

Inoculation of Plants. Wheat plants were inoculated with BYDV at the two- to three-leaf stage. Ten viruliferous aphids (third to fourth nymphal stage) were caged per plant following the procedure of Jiménez-Martínez et al. (2004a). Aphids were undisturbed for 72 h to enhance feeding and virus inoculation, after which they were removed. Plants were kept in environmental growth chambers under the conditions described earlier for 15 d after inoculation before bioassays.

As an additional check for aphid feeding–induced changes in plants, sham-inoculated plants were produced. To obtain sham-inoculated plants, the same procedure used to produce BYDV-infected plants was followed but plants were exposed to nonviruliferous aphids rather than viruliferous ones (Jiménez-Martínez et al. 2004b). Additionally, noninfected plants exposed to neither aphids nor virus and kept in environmental growth chambers as described earlier were used in bioassays.

Bioassays. Three separate bioassays were conducted to examine behavioral responses of *R. padi* to BYDV-infected, sham-inoculated, and noninfected 103.1J and Lambert wheat plants. A paper leaf model (filter paper Whatman 42 ashless; Whatman International, Maidstone, United Kingdom) was used as an additional control to test aphid responses. Using fourth nymphal stage *R. padi* apterae, we evaluated (1) emigration (defined as aphids leaving the target area) of nonviruliferous aphids; (2) immigration (defined as aphids reaching the target area), of nonviruliferous aphids; and (3) immigration of viruliferous aphids. For purposes of our study, the terms emigration and immigration are used in an operational sense and do not imply a particular physiological state of *R. padi*. Aphids were starved for up to 1 h before bioassays. For each bioassay, each treatment was tested in an individual arena made from a 150-mm-diameter polystyrene petri dish that was fitted with a false floor of organdy cloth screen (mesh size ~1 mm) on which aphids could walk freely (Eigenbrode et al. 2002). Aphids could not probe or contact the leaves, to avoid gustatory and tactile cues, and bioassays were performed in the dark to avoid visual cues. Two leaves still attached to the plant (or paper models) were placed ~4 mm beneath the screen and offered in single-choice bioassays. The distance from leaf to screen was selected to expose aphids to headspace volatiles (volatiles near the plants) when no visual, gustatory, or contact cues were present (Eigenbrode et al. 2002, Jiménez-Martínez et al. 2004b). Experiments were conducted at room temperature (22 ± 2°C), and air flow was not controlled. The arena was covered after aphids were released. Observations were recorded every 5 min for 30 min. A red light was used for a few seconds to make observations (Jiménez-Martínez et al. 2004b). All treatments were tested simultaneously within a replication, and multiple replications were spaced over time during the same day.

Emigration Bioassay With Nonviruliferous Apterae. Thirty aphids per treatment were placed on the screen in the center of the arena directly above the headspace of leaves (or paper models). This bioassay measures the rate at which aphids move away from an odor source and was used previously to detect differential aphid emigration from virus-infected and noninfected potato plants (Eigenbrode et al. 2002, Srinivasan et al. 2006). The target area above the wheat leaves or models measured ~20 cm². Aphids leaving the target area were considered emigrants and were removed from the arena at each observation. Eight replications were evaluated per treatment for a total of 48 plants, 8 leaf-models, and 1,680 aphids tested.

Immigration Bioassay With Nonviruliferous Apterae. Thirty aphids per treatment were placed ~70 mm away from the center and on one side of the arena; leaves (or paper models) were placed under the arena on the side of the arena opposite the aphids and ~50 mm from the center of the arena. The side of the arena to be used for aphid placement was chosen randomly. Aphids found above the headspace of leaves were considered immigrants and were removed from the arena at each observation. Ten replications were evaluated per treatment, for a total of 60 plants, 10 leaf-models, and 2,100 aphids tested.

Immigration Bioassay With Viruliferous Apterae. The bioassay was conducted as the immigration assay but using viruliferous aphids obtained from the colony described previously. Ten replications were evaluated per treatment for a total of 60 plants, 10 leaf-models, and 2,100 aphids tested.

Immigration Bioassay With VOCs. The bioassay was conducted as the immigration assay with nonviruliferous apterae, but using paper leaf models (as described previously) treated with VOCs rather than plant leaves. Tests were done using blends of or individual pure VOCs (see below). Controls consisted of paper leaf models treated only with 100 μl of paraffin oil (Sigma Chemical, St. Louis, MO). An immigration assay was selected to test response to VOCs because this assay was effective for detecting a differential response by *R. padi* apterae to volatile cues from BYDV-infected plants compared with controls. The bioassays using pure VOCs were conducted under normal laboratory lighting because all tested materials were visually equivalent. Tests were done at room temperature (22 ± 2°C), and air flow was not controlled. Each treatment was tested in an individual 150-mm-diameter arena as described previously. Paper leaf models were treated with 100 μl of test solution (see below), and 30 nonviruliferous apterae were released per arena. The solution was allowed to soak into the paper leaf model for 2 min before introducing the aphids into the arena. Aphids found above the treated paper leaf model were considered immigrants and were removed at each observation. Observations were recorded every 5 min for 30 min. VOCs that were elevated at least two-fold as a result of BYDV infection in studies by Jiménez-Martínez et al. (2004b) were selected for bioassays: nonanal, (Z)-3-hexenyl acetate, decanal, carophyllene, and undecane. Compounds
were obtained commercially (Aldrich, St. Louis, MO). Each compound was dissolved in paraffin oil to obtain 1,000-mg/ml solutions. Serial dilutions were used to achieve the desired concentrations for testing (Ngumbi et al. 2007). Individual VOCs were tested at concentrations of $10^{-3}$, $10^{-2}$, $10^{-1}$, 10, and 100 ng per paper leaf model. To test blends, the same VOCs tested singly were blended together in ratios that mimicked ratios at which they are present in the headspace of BYDV-infected or noninfected Lambert wheat plants based on the data by Jiménez-Martínez et al. (2004b). Synthetic blends were prepared by adding each component at a concentration that approximated that found in natural headspace collections and dissolved in paraffin oil making a total blend concentration of 100 mg/ml (Ngumbi et al. 2007). Serial dilutions were used to achieve the desired concentrations for testing. Synthetic blends were tested at 450 ng per paper leaf model (representing the concentration present in headspace of BYDV-infected Lambert plants) and 250 ng per model (representing the concentration present in headspace of noninfected Lambert plants). Tests involving blends were replicated 5 times (750 aphids tested), whereas tests involving individual VOCs were replicated 20 times (15,600 aphids tested).

**Settling Bioassay With Nonviruliferous Apterae.** A four-way choice settling bioassay was conducted following the method of Srinivasan et al. (2006), to evaluate the behavioral responses of nonviruliferous *R. padi* apterae. Twenty-five apterae were placed on a 55-mm-diameter petri dish layered with Whatman filter paper, and the dish was raised on a platform ~50 cm from the floor of each arena. Each arena consisted of a Plexiglas cage (0.5 by 0.5 by 0.75 m) where four treatments (one plant each of BYDV-infected and noninfected Lambert and 103.1J) were randomly placed in the corners of the cage (Srinivasan et al. 2006). Plants were 1 mo old at the time of testing. The assay was replicated 12 times for a total of 300 apterae tested. Tests were conducted in the dark, to avoid visual cues, at room temperature (22 ± 2°C), and airflow was not controlled. Aphids settling on plants of each treatment 24 h after initiation of the bioassay were counted and considered to have responded to that treatment.

**Virus Titer Determination.** Plants were tested for virus presence 20 d after virus inoculation using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described by Jiménez-Martínez et al. (2004a). Microtiter plates were read at A$_{405}$ nm, ~2 h after incubation or when the absorbance of the positive controls was more than two times higher than the negative controls. Virus titer data are presented as mean ± SE of nanograms of virus per milliliter of plant sap. A set of four purified virus standards described by Jiménez-Martínez et al. (2004a) allowed conversion of absorbance values to virus titer concentrations.

**Data Analysis.** Data on emigration and immigration of nonviruliferous apterae and on immigration of viruliferous apterae were analyzed by analysis of variance (ANOVA) using a generalized linear model (PROC GLM) followed by planned contrasts for means comparison (SAS Institute 2004) when significant differences were found. For each bioassay, the cumulative number of apterae responding to each treatment after 30 min was compared. Numbers of apterae settling on plants of different treatments after 24 h were tested by ANOVA using PROC GLM followed by planned contrasts for comparison of means (SAS Institute 2004). The cumulative number of apterae responding to individual VOCs after 30 min and to blends of VOCs after 5, 10, 15, 20, 25, and 30 min were tested by ANOVA using PROC GLM with least significant difference (LSD; $\alpha = 0.05$) for means separation (SAS Institute 2004). For virus data, absorbance values and virus titer were tested by ANOVA (PROC GLM) in SAS, followed by LSD mean comparison among treatments.

### Results

**Emigration Bioassay With Nonviruliferous Apterae.** There were no significant differences in emigration of nonviruliferous apterae among treatments by the end of the 30-min bioassay ($F =$ 0.65; $P = 0.6892$; Table 1).

**Immigration Bioassay With Nonviruliferous Apterae.** Immigration assays showed significant differences among treatments ($F =$ 3.89; $P = 0.0027$; Table 2; Fig. 1). Nonviruliferous apterae immigration toward BYDV-infected Lambert was significantly greater than toward sham-inoculated Lambert plants (contrast: $F =$ 9.55; $P =$ 0.0032; Table 2). Immigration did not differ toward BYDV-infected transgenic 103.1J compared with sham-inoculated 103.1J plants ($P >$ 0.05; Table 2). Regardless of genotype, immigration of apterae toward sham-inoculated and noninfected wheat plants was not significantly different ($P >$ 0.05; Table 2). Immigration toward the paper leaf model was significantly lower compared with all plant treatments (contrast: $F =$ 10.66; $P =$ 0.0019; Table 2).

**Immigration Bioassay With Viruliferous Apterae.** Immigration tests showed significant differences among treatments ($F =$ 2.75; $P =$ 0.0208; Table 3;
Fig. 1. Cumulative immigration response of nonviruliferous apterous *R. padi* toward headspace of BYDV-infected, noninfected, and sham-inoculated leaves from Lambert and transgenic 103.1J wheat plants and a paper leaf model. Error bars are SEM of the cumulative number of aphids responding to each treatment.

Fig. 2. Cumulative immigration response of viruliferous apterous *R. padi* toward headspace of BYDV-infected, noninfected, and sham-inoculated leaves from Lambert and transgenic 103.1J wheat plants and a paper leaf model. Error bars are SEM of the cumulative number of aphids responding to each treatment.

Table 2. No. of nonviruliferous apterous *R. padi* immigrating toward target areas above BYDV-infected, noninfected, and sham-inoculated leaves from Lambert and transgenic 103.1J wheat plants and a paper leaf model

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Cumulative no. aphids immigrating at 30 min (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambert</td>
<td>BYDV-infected (I)</td>
<td>7.9 ± 1.19</td>
</tr>
<tr>
<td>Lambert</td>
<td>Non-infected (NI)</td>
<td>4.4 ± 0.91</td>
</tr>
<tr>
<td>Lambert</td>
<td>Sham-inoculated (SI)</td>
<td>4.1 ± 0.97</td>
</tr>
<tr>
<td>103.1J</td>
<td>BYDV-infected (I)</td>
<td>4.9 ± 0.95</td>
</tr>
<tr>
<td>103.1J</td>
<td>Non-infected (NI)</td>
<td>4.6 ± 0.64</td>
</tr>
<tr>
<td>103.1J</td>
<td>Sham-inoculated (SI)</td>
<td>5.1 ± 0.67</td>
</tr>
<tr>
<td>Control</td>
<td>Paper leaf model</td>
<td>2.1 ± 0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Planned contrasts</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control versus plants</td>
<td>10.66</td>
<td>0.0019</td>
</tr>
<tr>
<td>Lambert I versus SI</td>
<td>9.55</td>
<td>0.0032</td>
</tr>
<tr>
<td>Lambert SI versus NI</td>
<td>0.06</td>
<td>0.8082</td>
</tr>
<tr>
<td>103.1J I versus SI</td>
<td>0.03</td>
<td>0.8714</td>
</tr>
<tr>
<td>103.1J SI versus NI</td>
<td>1.25</td>
<td>0.6859</td>
</tr>
</tbody>
</table>

Means of 30 aphids per treatment per replication and 10 replications. * Sham-inoculated plants were exposed to non-viruliferous aphids 15 d before bioassays.

Fig. 2). Immigration of viruliferous apteretae toward the paper leaf model was significantly lower compared with other treatments (contrast: \( F = 12.92; \ P = 0.0007 \); Table 3). There were no differences in immigration toward the different plant treatments (contrasts for sham-inoculated versus BYDV-infected and sham-inoculated versus noninfected for each genotype, \( P > 0.05 \); Table 3).

Immigration Bioassay With VOCs. Results of bioassays with individual compounds showed that VOCs produced by BYDV-infected wheat plants are attractive to nonviruliferous apterae *R. padi*. Aphids immigrated in significantly greater numbers to paper leaf models individually treated with nonanal, \((Z)\)-3-hexenyl acetate, decanal, Caryophyllene, or undecane than to paper leaf models that served as controls (\( F = 3.90; \ P < 0.0001 \); Fig. 2). There were no significant differences in immigration by nonviruliferous apterae among the different concentrations tested for any of the VOCs (\( P > 0.05 \)).

In bioassays with VOC blends, nonviruliferous apterae immigrated in significantly greater numbers toward paper leaf models treated with synthetic blends made to mimic headspace of BYDV-infected Lambert plants compared with blends made to mimic headspace of noninfected plants (Fig. 4). These differences were recorded 10 (\( F = 2.69; \ P = 0.0345 \)), 15 (\( F = 3.00; \ P = 0.0264 \)), and 20 min (\( F = 2.55; \ P = 0.0375 \)) after the initiation of the experiments (Fig. 4).

Settling Bioassay With Nonviruliferous Alates. *Rhopalosiphum padi* alates showed a significant (\( F = 4.19; \ P = 0.0129 \)) preference for noninfected 103.1J plants compared with other treatments (Table 4). Significantly more nonviruliferous alates settled on noninfected 103.1J compared with BYDV-infected 103.1J
plants (contrast: $F = 10.54; P = 0.0027$). None of the other contrasts were significant ($P > 0.05$), including the comparison between BYDV-infected Lambert and noninfected Lambert. In general, the mean number of alates that settled on plants was small and ranged between 0.6 and 3.4 insects per plant (of a total of 25 aphids tested per replication). Alates not on plants were found settling on the surface of Plexiglas cages.

**Virus Titer on BYDV-Infected Plants.** ELISA tests were conducted to determine absorbance values and virus titer on wheat plants of both genotypes infected with BYDV. Lambert plants had a significantly higher virus titer compared with 103.1J plants ($P = 0.05; Table 5$).

**Discussion**

In a bioassay designed to compare emigration rates, cumulative emigration at 30 min did not differ among plants (contrast: $F = 10.54; P = 0.0027$). None of the other contrasts were significant ($P > 0.05$), including the comparison between BYDV-infected Lambert and noninfected Lambert. In general, the mean number of alates that settled on plants was small and ranged between 0.6 and 3.4 insects per plant (of a total of 25 aphids tested per replication). Alates not on plants were found settling on the surface of Plexiglas cages.

**Virus Titer on BYDV-Infected Plants.** ELISA tests were conducted to determine absorbance values and virus titer on wheat plants of both genotypes infected with BYDV. Lambert plants had a significantly higher virus titer compared with 103.1J plants ($P < 0.05; Table 5$).

**Table 4.** No. of nonviruliferous alate *R. padi* settling on BYDV-infected and noninfected Lambert and transgenic 103.1J wheat plants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Total aphids settling after 24 h (mean ± SEM)</th>
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</thead>
<tbody>
<tr>
<td>Lambert</td>
<td>BYDV-infected (I)</td>
<td>1.08 ± 0.20</td>
</tr>
<tr>
<td>Lambert</td>
<td>Noninfected (NI)</td>
<td>1.17 ± 0.42</td>
</tr>
<tr>
<td>103.1J</td>
<td>BYDV-infected (I)</td>
<td>0.58 ± 0.26</td>
</tr>
<tr>
<td>103.1J</td>
<td>Noninfected (NI)</td>
<td>3.42 ± 1.00</td>
</tr>
<tr>
<td>Planned contrasts</td>
<td>$F$</td>
<td>$P$</td>
</tr>
<tr>
<td>Lambert I versus NI</td>
<td>0.01</td>
<td>0.9245</td>
</tr>
<tr>
<td>103.1J I versus NI</td>
<td>10.54</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

Means of 25 aphids per replication and 12 replications.
treatments, although slightly more nonviruliferous apterae *R. padi* emigrated from the paper leaf model compared with most plant treatments. In contrast, in an immigration bioassay, more nonviruliferous apterae *R. padi* moved toward BYDV-infected plants of nontransformed cultivar Lambert than toward sham-inoculated Lambert plants. These results are consistent with previous findings of Jimeñas-Martínez et al. (2004b), who showed nonviruliferous apterae preferentially settled over BYDV-infected Lambert compared with noninfected Lambert when aphids could not contact leaves. These results also suggest that differential immigration rather than emigration is taking place and that responses of nonviruliferous apterae *R. padi* to BYDV-infected plants are caused by attraction rather than arrestment. Furthermore, because all bioassays were conducted in darkness over a screen that prevented aphids from contacting the plants, the differential responses strongly suggest nonviruliferous apterae *R. padi* respond to volatile cues from BYDV-infected plants. In bioassays with VOCs that are produced at higher concentrations by BYDV-infected wheat plants compared with noninfected ones, more nonviruliferous apterae *R. padi* immigrated toward paper leaf models treated with individual VOCs than to paper leaf models that served as controls. This confirms that individual VOCs produced by BYDV-infected wheat plants are attractive to nonviruliferous apterae, in a bioassay setting identical to that used with intact plants. There were, however, no significant differences in immigration rates by nonviruliferous apterae among the different concentrations of individual VOCs tested; thus, the basis of differential responses of aphids to BYDV-infected plants requires further research. It is possible that tests with a wider range of concentrations of individual VOCs would detect expected concentration-dependent responses. Nonetheless, the materials were tested at concentrations to approximate those in plant headspace and thereby confirm activity at these ecologically relevant concentrations. Bioassays with synthetic blends of VOCs that mimic headspace of BYDV-infected wheat showed that nonviruliferous apterae *R. padi* preferentially responded to such blends compared with blends made to mimic headspace of noninfected wheat plants. This further shows that nonviruliferous apterae *R. padi* are attracted to VOCs produced by BYDV-infected wheat.

Jimeñas-Martínez et al. (2004b) suggested that *R. padi* might be responding to *(Z)-3-hexenyl acetate*, because the concentrations of this compound in Lambert plants infected with BYDV were up to three-fold higher than on noninfected Lambert. Results from this study confirm that *(Z)-3-hexenyl acetate* is an attractant for nonviruliferous apterae *R. padi*. Additionally, the other compounds tested, nonanal, decanal, caryophyllene, and undecane, were also found to attract nonviruliferous apterae. Such volatile compounds potentially could be used as baits for attracting and controlling aphids under field conditions, with the ultimate goal of reducing BYDV spread. Further studies need to address the implications of attractant volatiles from BYDV-infected wheat plants on the epidemiology of BYD disease.

Neither emigration nor immigration bioassays showed differential responses by nonviruliferous apterae *R. padi* for BYDV-infected transgenic 103.1J plants compared with sham-inoculated plants of this genotype, consistent with the previous report by Jimeñas-Martínez et al. (2004b) working with this transgenic line. Also consistent with Jimeñas-Martínez et al. (2004b), virus titer was found to be lower in BYDV-infected 103.1J than in infected Lambert. Jimeñas-Martínez et al. (2004b) reported a positive relationship between virus titer and the response of *R. padi* to BYDV-infected 103.1J, in contrast to BYDV-infected Lambert plants, which consistently had high virus titer and where no such relationship was detected. In their studies, the proportion of *R. padi* that responded to individual BYDV-infected 103.1J plants increased significantly as virus titer in the plants increased. The lower virus titer in 103.1J plants observed in this study and reduced impact of the virus on the plants could have influenced VOC emissions, accounting for the lack of responsiveness by nonviruliferous apterae to these plants. The lack of attraction to virus-infected 103.1J plants could reduce secondary transmission and virus incidence in the field (Irwin and Thresh 1990), if transgenic genotypes such as this were to be deployed (Jimeñas-Martínez et al. 2004b).

Viruliferous apterae *R. padi* showed no differential responses in immigration bioassays to any of the plant treatments examined, although they were more responsive to plants than to the paper leaf model. Thus, behavioral responses of viruliferous apterae were different compared with responses of nonviruliferous apterae tested under similar conditions. Three general types of mechanisms could account for the lack of discrimination by viruliferous aphids, in contrast to nonviruliferous ones. First, because viruliferous aphids in our tests were exposed to virus-infected plants before the bioassay for virus acquisition to oc-

### Table 5. BYDV-PAV absorbance (OD) and virus titer (ng/ml) on Lambert and 103.1J wheat genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>Absorbance (OD&lt;sub&gt;405 nm&lt;/sub&gt;)&lt;sup&gt;a&lt;/sup&gt; (mean ± SEM)</th>
<th>Virus titer (ng/ml) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambert</td>
<td>Infected</td>
<td>0.672 ± 0.099 a</td>
<td>467.5 ± 84.7 a</td>
</tr>
<tr>
<td>Lambert</td>
<td>Noninfected</td>
<td>0.200 ± 0.011 b</td>
<td>135.3 ± 20.45 b</td>
</tr>
<tr>
<td>103.1J</td>
<td>Infected</td>
<td>0.284 ± 0.024 b</td>
<td>135.3 ± 20.45 b</td>
</tr>
<tr>
<td>103.1J</td>
<td>Noninfected</td>
<td>0.185 ± 0.018 b</td>
<td>135.3 ± 20.45 b</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.0007</td>
<td>0.0186</td>
</tr>
</tbody>
</table>

Absorbance means are based on an avg of 28 plants for Lambert and 18 plants for 103.1J. ELISA conducted 20 d after inoculation. Means on the same column followed by the same letter are not significantly different at P < 0.05.

*a* Genotype 103.1J is a third-generation Lambert-derived transgenic soft white winter wheat expressing the coat protein gene of the BYDV-PAV serotype.
cur, their behavioral response might have been influenced by learning or habituation (Leibrecht and Askew 1980), in which the response to a stimulus decreases with repeated exposure. If habituation to olfactory cues from BYDV-infected plants occurred in our bioassays, viruliferous apterae *R. padi* would have been less likely to show a preference for BYDV-infected wheat plants compared with nonviruliferous apterae. However, because *R. padi* were reared on BYDV-infected barley plants, rather than on BYDV-infected wheat plants before bioassays, the cues to which they could have habituated are unclear. Second, before bioassays, viruliferous apterae were exposed to virus-infected plants, which are better hosts for *R. padi* (Fereres et al. 1989, Jiménez-Martínez et al. 2004a). Infection with BYDV increases amino acid (Markkula and Laurema 1964, Ajayi 1986) and soluble carbohydrate (Jensen 1972) content in leaves, and this is believed to enhance aphid life history on virus-infected plants (Fereres et al. 1989). Feeding on the superior BYDV-infected host could have altered behavioral responsiveness of apterae to volatile cues, perhaps as a result of nutrient satiation (Miller and Strickler 1984). Third, the presence of virus particles in apterae may somehow alter their behavior. This latter possibility, if shown, would be surprising because there is no published evidence to our knowledge for direct effects of plant virus particles on vector behavior. Additional studies are required to examine these mechanisms. Further work should include tests in which responses of nonviruliferous and viruliferous apterae are examined simultaneously in the same type of immigration or emigration bioassay.

Our prior studies examining the behavioral response of *R. padi* to VOCs from BYDV-infected wheat involved apterae only (Jiménez-Martínez et al. 2004b). Thus, these studies were expanded to assess the response of alates. In a four-way choice test, nonviruliferous *R. padi* alates preferentially settled on noninfected 103.1J compared with BYDV-infected 103.1J plants. Olfactory orientation in flight has been shown in aphids (Pickett et al. 1992), but other factors in the four-way choice test such as light regimen or gustatory cues could have influenced the results reported here. It is inconclusive if the preference observed for noninfected 103.1J by alates was caused by volatiles cues, because aphids could contact the plants and be exposed to tactile and gustatory cues. Although previous studies have reported aphid takeoff in the dark, this behavior has not been shown in a conclusive fashion, because sufficient light is apparently necessary for takeoff by most aphid species (Halgren 1970). Because bioassays were conducted in the dark to avoid visual clues, this might help explain the low response by alates to the treatments. However, following the same method used in the current study, Srinivasan et al. (2006) showed no effect of light regimen on *M. persicae* response. In their studies, *M. persicae* alates preferentially settled on PLRV-infected nightshade and potato plants compared with noninfected plants. It is possible that the attraction to and settling response on virus-infected plants differ among pathogen spread. Neither McElhany et al. (1995) nor Sisterson (2008) included changes in vector preference for infected plants after acquisition of the virus in their models, although McElhany et al. (1995) discussed the possibility. If *R. padi* apterae prefer virus-infected plants until they become viruliferous, as our data suggest, this would have the effect of increasing initial acquisition while not impairing subsequent transmission to noninfected plants. In addition, if volatile cues from virus-infected plants attract *R. padi* apterae, greater aphid colonization of a field with a high initial proportion of infected plants would be predicted. After becoming viruliferous, apterae would show no preference between virus-infected and noninfected plants, and given the higher number of aphids in the field, greater virus spread would be predicted. Wheat plants infected with BYDV are superior hosts for aphids, and this results in a greater intrinsic rate of increase for the insects (Fereres et al. 1989, Jiménez-Martínez et al. 2004a). Subsequent crowding and plant quality deterioration would result in aphid dispersal (De Barro 1992), two factors that should increase virus spread after colonization of virus-infected plants. Thus, volatile-mediated preference for virus-infected plants could contribute to a syndrome that accelerates BYDV spread relative to a pathogen with no behav-
ioral or physiological effects on its vector. Further studies are required to fully understand the potential impact of these responses on the epidemiology of this important disease.

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