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Physiological and Biochemical Responses of Resistant and Susceptible Wheat to Injury by Russian Wheat Aphid

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ABSTRACT We examined the physiological and biochemical responses of resistant ('Halt' and 'Prairie Red') and susceptible ('TAM 107') wheat, *Triticum aestivum* L., to injury by the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). Photosynthetic capacity was evaluated by measuring assimilation/internal CO_2 (A/C_i) curves, chlorophyll fluorescence, chlorophyll, and nonstructural carbohydrate content. Total protein and peroxidase specific activity also were determined. No significant differences were detected in chlorophyll concentration between aphid-infested and control TAM 107 plants. The aphid-infested resistant cultivars had similar or significantly higher chlorophyll concentrations compared with their respective control plants. Measurements over time showed that infested Halt plants had delays in photosynthetic senescence, Prairie Red plants had photosynthetic rate changes that were similar to control plants, and TAM 107 plants displayed accelerated photosynthetic senescence patterns. The photochemical and nonphotochemical quenching coefficients were significantly higher in infested Halt plants compared with their respective control plants on day 3. Infested TAM 107 plants had significantly higher photochemical quenching compared with control plants at all times evaluated, and they had significantly higher nonphotochemical quenching on day 3. Throughout the experiment, infested Prairie Red plants exhibited photochemical and nonphotochemical quenching coefficient values that were not significantly different from control plants. Total protein content was not significantly different between aphid-infested and control plants for all cultivars. Differences between physiological responses of infested susceptible and resistant cultivars, particularly temporal changes in photosynthetic activity, imply that resistant Halt and Prairie Red wheat tolerate some impacts of aphid injury on photosynthetic integrity.

KEY WORDS plant–insect interaction, gas exchange, photosynthesis, host plant resistance

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko), has been a significant pest of wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., in the United States since its first detection in North America in 1986 (Anderson et al. 2003). Between 1987 and 1992, losses attributed to *D. noxia* feeding were estimated at $850 million, with >100 million bushels of grain lost in the western United States alone (Quisenberry and Peairs 1998). Losses from *D. noxia* resulted in $1 billion by 1993 with cumulative yield losses >106 million bushels (Shah et al. 1999).

D. noxia usually feeds at the base of the youngest leaves of the plant, which are strong sinks for phloem-mobile mineral nutrients, amino compounds, and carbohydrates (Macedo et al. 2003b). By feeding at these sites, *D. noxia* may have the potential to alter carbohydrate-partitioning patterns of wheat and alter sink–source relationships within the plant (Burd et al. 1996). *D. noxia* also can cause ultrastructural and tissue-level damage on susceptible hosts, which may affect phloem composition and create a nutritionally enhanced phloem diet (Telang et al. 1999). Feeding by *D. noxia* elicits chlorosis, which takes the form of white or yellow longitudinal bands on leaves (Kazemi et al. 2001) and leaf rolling on cereal plants. A proposed mechanism for the development of chlorosis by piercing–sucking insects (including *D. noxia*) is that chloroplast injury results from the introduction of salivary secretions, some of which may be toxic to the plant (Ni and Quisenberry 2003).

Management of *D. noxia* has largely relied on resistant wheat cultivars (Smith 1999, Webster and Kenkel 1999). Wheat cultivars containing *D. noxia*-resistant genes, which have different levels of antibiosis, tolerance, or both (Randolph et al. 2005a), have been shown to harbor fewer *D. noxia* populations and have higher yields compared with their susceptible wheat counterparts (Randolph et al. 2003, 2005b). Resistant plants also have demonstrated compatibility with other management tactics by exhibiting an absence of leaf rolling when infested with *D. noxia*, which would expose the aphids to chemical and biological management (Hawley et al. 2003).

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Resistant and susceptible plants have not only shown differences with respect to yield and aphid populations in response to *D. noxia* feeding but also with chlorophyll maintenance. Susceptible plants, infested with *D. noxia*, may experience alterations in chlorophyll (Ni et al. 2002) such as chlorosis development, reductions in chlorophyll (a and b) and carotenoids, and changes in chlorophyll fluorescence (Burd and Elliott 1996, Rafi et al. 1997). In contrast, resistant plants show minimal or no differences in chlorophyll maintenance in response to *D. noxia* infestation (Burd and Elliott 1996, Heng-Moss et al. 2003).

Another area of research focus has been on understanding how aphid feeding affects plant physiology (e.g., gas-exchange responses and chlorophyll fluorescence). Haile et al. (1999) found that *D. noxia*-infested resistant and susceptible wheat plants had reduced chlorophyll fluorescence and photosynthetic rates compared with uninfested plants. After aphid removal (7 d), the tolerant cultivar showed a complete recovery of photosynthetic capacity by 7 d, whereas gradual photosynthetic recovery was not observed in the susceptible or antibiotic cultivars. Macedo et al. (2003b) also found that *D. noxia* infestation caused reductions in gas exchange and chlorophyll fluorescence for susceptible wheat, but only under continuous light. Under 72-h continuous dark, aphid infestation did not cause damage symptom formation or reductions in photosynthesis. Development of *D. noxia* feeding damage symptoms (i.e., leaf rolling and chlorotic streaks) on susceptible wheat seedlings may be a light-activated process, even though the elicitor of the plant damage symptoms is aphid feeding.

Similar reductions in gas exchange and chlorophyll fluorescence also have been found in response to greenbug, *Schizaphis graminum* (Rondani), feeding. Ryan et al. (1987) showed that a susceptible wheat cultivar had decreased photosynthetic capacity and reduced levels of chlorophyll in response to *S. graminum* feeding, whereas the resistant cultivar did not show the same reductions. The infested susceptible cultivar also exhibited decreased amounts or activity of rubisco and decreased ribulose 1,5-bisphosphate (RuBP) regeneration.

Previous work has provided insight into differential responses between resistant and susceptible plants; however, a limited number of plant parameters were typically measured. Looking at multiple parameters in combination with a time course would provide a more complete understanding of changes in the physiology of the plant (e.g., gas-exchange responses, nonstructural carbohydrates [NSC]) and offer insight into the short- and long-term changes in cellular processes.

The objectives of this research were to provide a comprehensive overview of several important plant response parameters and document the physiological and biochemical responses of resistant and susceptible wheat to *D. noxia* over a time course. The impact *D. noxia* had on resistant and susceptible plants was specifically measured by examining aphid fecundity, plant height, chlorophyll content, gas-exchange responses, chlorophyll fluorescence kinetics, peroxidase kinetics, protein content, and nonstructural carbohydrate content. Secondarily, observations were made to decipher whether plant parameters measured would be useful in identifying mechanisms of resistance.

Materials and Methods

Plant Material and Insects

Seeds of susceptible wheat 'TAM 107' and the resistant wheat 'Halt' and 'Prairie Red' were planted in SC-10 Super Cell Cone-tainers (3.8 by 21 cm) (Stuewe & Sons, Inc., Corvallis, OR). The resistant Halt and Prairie Red both carry the *Dn4* gene (Quick et al. 1996, Hawley et al. 2003). Three seeds of each cultivar were planted in a Cone-tainer to a depth of ≈2 cm in a mixture of sand–soil–peat–perlite (0.66:0.33:1:1) and placed in Cone-tainer racks. The Cone-tainer racks were then placed over a plastic tray (54 by 28 by 6 cm) filled with water, to ensure that plants were watered uniformly from the bottom. Plants were grown for 14 d in a greenhouse under 400-W high intensity lamps with a photoperiod of 16:8 (L:D) h and a temperature of 27 ± 3°C. Plants were thinned to one plant per Cone-tainer once seedlings emerged from the soil.

A colony of biotype 1 *D. noxia* was obtained from the USDA–ARS research facility in Stillwater, OK. Aphids were maintained on susceptible TAM 107 wheat, and they were kept in growth chambers (Percival Scientific, Perry, IA) at 21 ± 1°C, 40–50% RH, and a photoperiod of 16:8 (L:D) h. The experimental design was a completely randomized design, with a 3 by 2 by 3 factorial treatment design that included three wheat cultivars, two aphid infestation levels (0 and 20 *D. noxia*), and three harvest dates (3, 6, and 9 d after aphid introduction). The experimental unit was an individual plant, in a Cone-tainer with a Plexiglas cage.

Treatments were a combination of plant cultivars and aphid infestations. For infested treatments, 10 aphids were introduced onto the first and second leaf blade (total of 20 aphids) of each designated infested plant. Tubular, Plexiglas cages (4 cm in diameter by 30 cm in height) were used to confine aphids on the plants with organdy fabric securely fastened by rubber bands to the top of the cages. Uninfested treatment plants were caged just like the infested treatments. After infestation, plants were kept in the greenhouse until the experiment was completed.

Plants were evaluated for leaf chlorosis on each harvest date using a 1–9 scale, where 1 is plants look healthy and 9 is plant death or no recovery possible (Webster et al. 1991). The total number of *D. noxia* on infested plants was assessed at each harvest date by counting the removed aphids. Plant height (centimeters) was determined at the start of the experiment and also at each harvest interval to calculate the change in growth for the experimental plants.

Physiological and Biochemical Responses of Wheat to *D. noxia*

Chlorophyll Concentration. Chlorophyll levels were measured at three locations (near the base of the leaf, the middle, and toward the tip of the leaf) on the first and second leaf blade at each harvest interval by using a chlorophyll meter (model Spad-502, Minolta Camera Co., Osaka, Japan). The arithmetic mean of these measurements was used for all subsequent analyses.

Gas Exchange. Photosynthetic responses of resistant and susceptible wheat were recorded at 3, 6, and 9 d after aphid introduction by using a portable photosynthesis system (model LI-6400, LI-Cor, Lincoln, NE). Although plants were maintained in a greenhouse, all measurements were taken outdoors after plants had acclimatized for >1 h. Photosynthetic measurements included stomatal conductance (moles of H_2O per square meter per second) versus intercellular CO_2 concentration (C_i) (parts per million) and an assimilation rate (A) versus intercellular CO_2 concentration (C_i) (A/C_i curve), where rates were measured at 1,400 μmol photons $m^{-2}s^{-1}$ light intensity and CO_2 concentrations ranging from 50 to 1,000 ppm. A/C_i response curves and stomatal conductance were determined by the automated programs of the LI-6400.

Calculations of the stomatal and nonstomatal components of photosynthesis were made using the methods described by Farquhar and Sharkey (1982). By comparing A at a C_i of 400 μl $liter^{-1}$ CO_2 to A at the C_i corresponding to a C_a (intracellular CO_2) of 400 μl $liter^{-1}$ CO_2, the stomatal limitation to photosynthesis can be calculated (Ryan et al. 1987). In equation form, stomatal limitation (SL) can be defined as follows:

$$SL = A(C_i = 400\ \mu l\ liter^{-1}) - A(C_a = 400\ \mu l\ liter^{-1})/A(C_i = 400\ \mu l\ liter^{-1})$$

The A versus C_i response curves also can be used to find the CO_2 compensation point (the C_i value where $A = 0$, given in Pa), changes in net CO_2 assimilation at saturating (A_{max}), and carboxylation efficiency (CE, the slope of the linear portion of the A versus C_i response curve). CE is the region of the A/C_i curve where rubisco function is CO_2 limited or RuBP saturated and corresponds to the CE of rubisco (von Caemmerer and Farquhar 1981, Farquhar and Sharkey 1982). Analyses of the A/C_i curves also allowed determination of the maximum rate of rubisco-mediated carboxylation (V_{cmax} determined from the linear portion of the curve, micromoles of CO_2 per square meter per second) and the maximum potential rate of electron transport contributing to RuBP regeneration (J_{max} micromoles of electrons per square meter per second) (Manter and Kerrigan 2004). These values were calculated using the Photosyn Assistant Software (Dundee Scientific, Dundee, Scotland). For each treatment, response curves from leaves of three different plants (replications) were measured and estimated for SL, CE, CO_2 compensation point, A_{max}, V_{cmax}, and J_{max}.

Chlorophyll Fluorescence. Chlorophyll fluorescence can convey the use of energy absorbed by chlorophyll via photosystem II (PSII) and the degree to which PSII is being damaged by excess light. Chlorophyll fluorescence was measured at each harvest interval using an OS5-FL modulated chlorophyll fluorometer (Opti-Sciences, Tyngsboro, MA). Leaves were dark adapted with clips for at least 20 min before measurements. Determinations were made of minimum fluorescence for dark-adapted leaves (F_o), maximum fluorescence for dark-adapted leaves (F_m), fluorescence under steady-state conditions (F_s), maximal fluorescence under steady-state conditions (F_{ms}), quantum yield [$Y = (F_{ms} - F_s)/F_{ms}$)], photochemical quenching [$qP = (F_{ms} - F_s)/(F_{ms} - F_o)$], and nonphotochemical quenching [$qN = (F_m - F_{ms})/(F_m - [F_o])$] (see OS5-FL Manual for additional details).

Protein and Enzyme Assays. Plant biomass measurements of control and infested plants were recorded on each harvest date. Plant material was ground in liquid nitrogen and prepared for protein analyses by using a modified protocol from Hildebrand et al. (1986) and Heng-Moss et al. (2004). Proteins were extracted with 1.5 ml of HEPES buffer, pH 7.2, 1% (wt:vol) final concentration of polyvinylpolypyrrolidone, and 1 ml of plant cocktail (Sigma-Aldrich, St. Louis, MO) per 30 g of plant material by grinding the plant material with a mortar and pestle. This plant mixture was centrifuged at 4°C for 10 min, and the supernatant was used for protein assays. A colorimetric protein binding assay (Pierce Chemical, Rockford IL) by using bovine serum albumin as a standard was performed to determine the protein content of the plant extracts (Bradford 1976).

Peroxidase activity was measured by monitoring the increase in absorbance at 470 nm for 2 min by using a protocol modified from Hildebrand et al. (1986), Hori et al. (1997), and Heng-Moss et al. (2004). The enzymatic reaction was started by adding 10 μl of 30% hydrogen peroxide to a cuvet containing 300 μl of 18 mM guaiacol, 100 μl of 200 mM HEPES, pH 7.0, 585 μl of distilled water, and 5 μl of plant extract. The specific activity of peroxidase was determined using the molar absorptivity of guaiacol at 470 nm ($26.6 \times 10^3\ M^{-1}\ cm^{-1}$).

Nonstructural Carbohydrate Determination. Nonstructural carbohydrates were quantified by determining reducing sugar concentrations as glucose equivalents present in the plant material. After storage at −80°C, plant material was dried at 70°C in a draft oven for 48 h, and then it was mechanically ground. Twenty-five milliliters of 0.02 M benzoic acid was added to a Folin-Wu tube containing a 10-mg sample of the plant material. The tubes were covered loosely with aluminum foil and placed in an autoclave for 20 min on the liquid cycle and slow cooled. The autoclaving step was repeated one additional time to ensure nonstarch oligosaccharides and polysaccharides were hydrolyzed. Approximately 500-μl aliquots were then diluted to 750 μl with 50 mM potassium acetate, pH 5, and 0.02 M benzoic acid. Two-hundred fifty

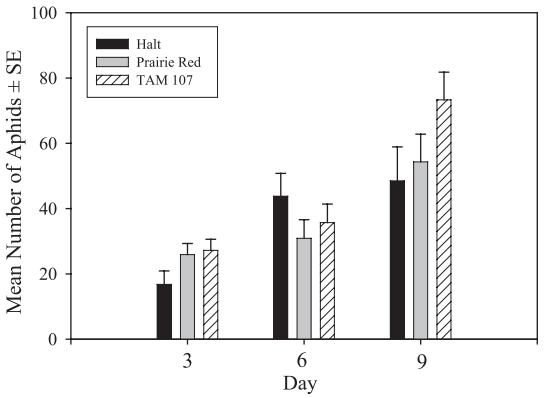

Fig. 1. Mean number of Russian wheat aphids (RWA) produced on each wheat cultivar at 3, 6, and 9 d. No significant differences were detected at $P \leq 0.05$ by LSD.

microliters of an amylase/amyloglucosidase solution was added to the sample, which was then incubated at 42°C for 15 h. The solution was then assayed for total extractable carbohydrate according to Nelson–Somogyi copper reducing method (Somogyi 1952). The specific amount of glucose equivalents was quantified by measuring the absorbency of Cu_2O at 600 nm by using a spectrophotometer. Known concentrations of glucose were used to generate a standard curve.

Data Analysis. Mixed model analysis (PROC MIXED, SAS Institute 2002) was conducted for each measurement to detect differences in aphid numbers, plant height, protein content, peroxidase specific activity, chlorophyll levels, gas-exchange responses, nonstructural carbohydrate content, and chlorophyll fluorescence measurements. When appropriate, the means were separated according to Fisher least significant difference (LSD) method. Statistical significance was assumed when $P \leq 0.05$.

Results and Discussion

Physiological Responses of Resistant and Susceptible Wheat to *D. noxia*

Leaf Chlorosis. Aphid-infested TAM 107 plants showed limited visual damage on day 3 and day 6 (day 3 = 1.1 ± 0.13; day 6 = 1.1 ± 0.13). Although not statistically significant, on day 9 aphid-infested TAM 107 plants had increased chlorosis (chlorosis rating = 1.6 ± 0.13) compared with resistant Prairie Red and Halt plants (chlorosis rating = 1.1 ± 0.13). Visual evidence of leaf chlorosis was not observed on infested Halt (day 3 = 1.0; day 6 = 1.0) or Prairie Red (day 3 = 1.0; day 6 = 1.0) plants throughout the experiment.

Aphid Number. No significant differences were detected in numbers of nymphs among the three wheat cultivars at 3, 6, or 9 d after aphid introduction (F = 1.8; df = 2, 63; P = 0.18) (Fig. 1). The greatest number of aphids was recorded on TAM 107 wheat. The resistant Prairie Red and Halt supported similar numbers of aphids throughout the experiment, demonstrating that the two resistant cultivars display tolerance to *D. noxia*. Studies by Randolph et al. (2005a) and Hawley et al. (2003) have previously characterized Prairie Red and Halt as conveying tolerance in the seedling stage and antibiotic properties at later stages of development.

Plant Height. Overall, *D. noxia* did not have a significant impact on plant growth for any of the cultivars evaluated. All experimental plants grew significantly taller over the course of the experiment (F = 27.2; df = 2, 121; P = 0.0001). Hawley et al. (2003) also found no significant differences in plant height between *D. noxia*-infested resistant (Halt) and susceptible (TAM 107) plants.

Chlorophyll Concentration. Aphid-infested TAM 107 plants (first leaf blade) had similar chlorophyll concentrations to control plants on all harvest dates (Table 1). There were no significant differences in chlorophyll concentrations between aphid-infested and control resistant plants (Halt and Prairie Red) on day 3 or day 9. However, on day 6, there was a significant increase in chlorophyll in aphid-infested resistant plants compared with their respective control plants (Halt: $t = -2.1$, df = 120, P = 0.04; Prairie Red: $t = -2.0$, df = 120, P = 0.05). For all cultivars and harvest dates evaluated, there were no significant differences in the chlorophyll concentrations of the second leaf blade between infested and control plants (data not shown). Significant differences in the chlorophyll concentration for leaf blade 1 (F = 23.3; df = 2, 120; P = 0.0001) and leaf blade 2 (F = 23.6; df = 2, 121; P = 0.0001) were detected among the cultivars. TAM 107 and Prairie Red control plants consistently had higher chlorophyll concentrations compared with Halt control plants.

Previous studies have shown that susceptible cultivars incur chlorophyll loss before resistant plants (Rafi et al. 1997, Burd and Elliott 1996). After an extended period of infestation, resistant plants may

Table 1. Mean ± SE of chlorophyll loss of leaf blade 1 for resistant Halt and Prairie Red and susceptible TAM 107 wheat at 3, 6, and 9 d after exposure to Russian wheat aphid (RWA)

Cultivar	Mean ± SE chlorophyll (μmol m^{-2})								
	Day 3			Day 6			Day 9		
	Control	RWA	P value[a]	Control	RWA	P value[a]	Control	RWA	P value[a]
Halt	183.9 ± 30.9	165.9 ± 25.3	0.65	204.9 ± 27.7	282.7 ± 25.3	0.04	85.3 ± 30.9	112.7 ± 25.3	0.49
Prairie Red	275.3 ± 20.6	250.8 ± 20.6	0.40	329.1 ± 20.6	386.3 ± 20.6	0.05	167.5 ± 20.6	182.2 ± 20.6	0.62
TAM 107	271.6 ± 20.6	234.8 ± 20.6	0.21	333.2 ± 21.9	362.1 ± 20.6	0.33	143.8 ± 20.6	169.4 ± 20.6	0.38

[a] Significantly different at $P \leq 0.05$ by least significant difference.

exhibit losses in chlorophyll (Miller et al. 1994), but chlorophyll loss is not as great as in susceptible cultivars (Heng-Moss et al. 2003, Wang et al. 2004). Interestingly, in this study there were no significant differences in chlorophyll concentration between infested and control plants for the susceptible cultivar. Ni et al. (2002) showed that nonchlorotic areas of *D. noxia*-infested susceptible wheat compensated for damaged regions by having increased chlorophyll (a and b) and carotenoid concentrations. It is possible, that in our study, nondamaged areas with increased chlorophyll may have masked the reductions in chlorophyll concentration of damaged regions in susceptible plants. This could explain why no significant differences were observed between treatments of the susceptible cultivar.

A_{max}. Over the course of the study, infested TAM 107 and Prairie Red plants had A_{max} values that were not significantly different from their respective control plants (Figs. 2 and 4). Resistant Halt plants infested with *D. noxia* had significantly higher A_{max} values on day 3 and 9 compared with control plants (day 3: $t = -2.5$, df = 19, $P = 0.02$; day 9: $t = -3.5$, df = 19, $P = 0.001$) (Fig. 3), suggesting that *D. noxia* infestation in the resistant Halt was not associated with inhibiting the plants' ability to reach maximum photosynthetic capacity.

Stomatal Limitation. There were no significant differences in stomatal limitation across cultivars and treatments throughout the experiment. Similarly, stomatal conductance did not substantially differ among treatments, and stomatal conductance seemed to track with photosynthetic demand (data not shown).

Carboxylation Efficiency. Aphid infestation resulted in significantly lower CE for Prairie Red plants on day 6 ($t = 2.4$, df = 23, $P = 0.02$) and for TAM 107 plants on day 9 ($t = 2.1$, df = 23, $P = 0.05$). Throughout the experiment, infested Halt plants showed similar or increased CE values, although not statistically significant, compared with control plants (Figs. 2–4; Table 2), suggesting that the CE for this cultivar is not significantly impacted by *D. noxia.*

CO_2 Compensation Point. There was a significant day by variety by aphid interaction ($F = 3.8$; df = 4, 18; $P = 0.02$); however, compensation points between aphid treatments of interest were either not significantly different or did not seem to follow an apparent trend (data not shown).

V_{cmax}. Susceptible and resistant plants exhibited trends for V_{cmax} values that were similar to CE (Table 2). At day 6, susceptible plants infested with *D. noxia* had reduced V_{cmax} values, and by day 9 infested plants showed significant reductions in V_{cmax} values compared with control plants ($t = 2.7$, df = 18, $P = 0.02$). These results indicate that the aphids are affecting the plant's ability to reach its maximum rate of rubisco-mediated carboxylation. Throughout the experiment, *D. noxia*-infested Halt plants had V_{cmax} values that were not significantly different from control plants. Prairie Red plants infested with *D. noxia* had similar values to control plants throughout the experiment, with the exception of day 6 ($t = 2.8$, df = 18, $P = 0.01$) when values for the infested plants were significantly lower than the control plants.

J_{max}. Susceptible plants infested with *D. noxia* had declining J_{max} values over the course of the experiment, with infested plants having significantly lower values compared with control plants on day 6 ($t = 2.1$, df = 18, $P = 0.05$) and day 9 ($t = 3.1$, df = 18, $P = 0.006$) (Table 2). In contrast, resistant plants infested with *D. noxia* only displayed significant reductions in J_{max} values on day 6 compared with control plants (Halt: $t = 2.3$, df = 18, $P = 0.03$; Prairie Red: $t = 3.7$, df = 18, $P = 0.002$). On days 3 and 9, infested Halt plants exhibited significantly higher J_{max} values compared with control plants (Table 2). There were no significant differences between control and infested Prairie Red plants on day 3 or day 9. Susceptible plants showed accelerated declines in RuBP regeneration in response to aphid feeding, whereas resistant cultivars generally showed comparable or increased RuBP regeneration rates.

Chlorophyll Fluorescence Response. There were no significant differences in the nonvariable F_o irrespective of aphid treatment for all of the experimental plants (data not shown). Similarities in the F_o values among aphid treatments strongly suggest that aphid feeding was not associated with photoinhibitory damage in the PSII reaction centers. Aphid infestation was associated with significant differences in F_m and the total amount of F_v, but there were no apparent trends between control and infested experimental plants (data not shown).

The photochemical efficiency of PSII (F_v/F_m) ratios provide information on the efficiency of the photochemical system, specifically how much light energy captured is being used by the reaction center and propagated through the photoelectron transport chain. *D. noxia* did not have a significant impact on F_v/F_m, indicating that the antennal chlorophyll complexes and electron transfer to the reaction center of PSII was not impacted negatively by aphids. Macedo et al. (2003a) also found that the soybean aphid, *Aphis glycines* Matsumura, regardless of aphid density, did not alter the photoelectron transport in soybean, *Glycine max* (L.) Merr. With sufficient time (i.e., experiments of longer duration), it is likely that photosystem injury can be associated with *D. noxia* infestation, but in this study we observed decreased photosynthetic activity without apparent changes in electron transport.

The yield (Y) of photosynthesis, which gives an indication of the total quantum yield produced by photosynthesis, was significantly impacted by *D. noxia* infestation for resistant plants, but not for susceptible plants (Table 3). Infested Prairie Red plants had significantly higher Y values compared with control plants on day 6 ($t = -4.9$, df = 462, $P = 0.0001$) and day 9 ($t = -3.3$, df = 462, $P = 0.001$). Infested Halt plants had similar Y values to control plants throughout the experiment, with the exception of day 6 when Y values of infested plants were significantly lower (day 6: $t = 5.2$, df = 462, $P = 0.0001$). Y is a good indication of the efficiency in light utilization, i.e., how

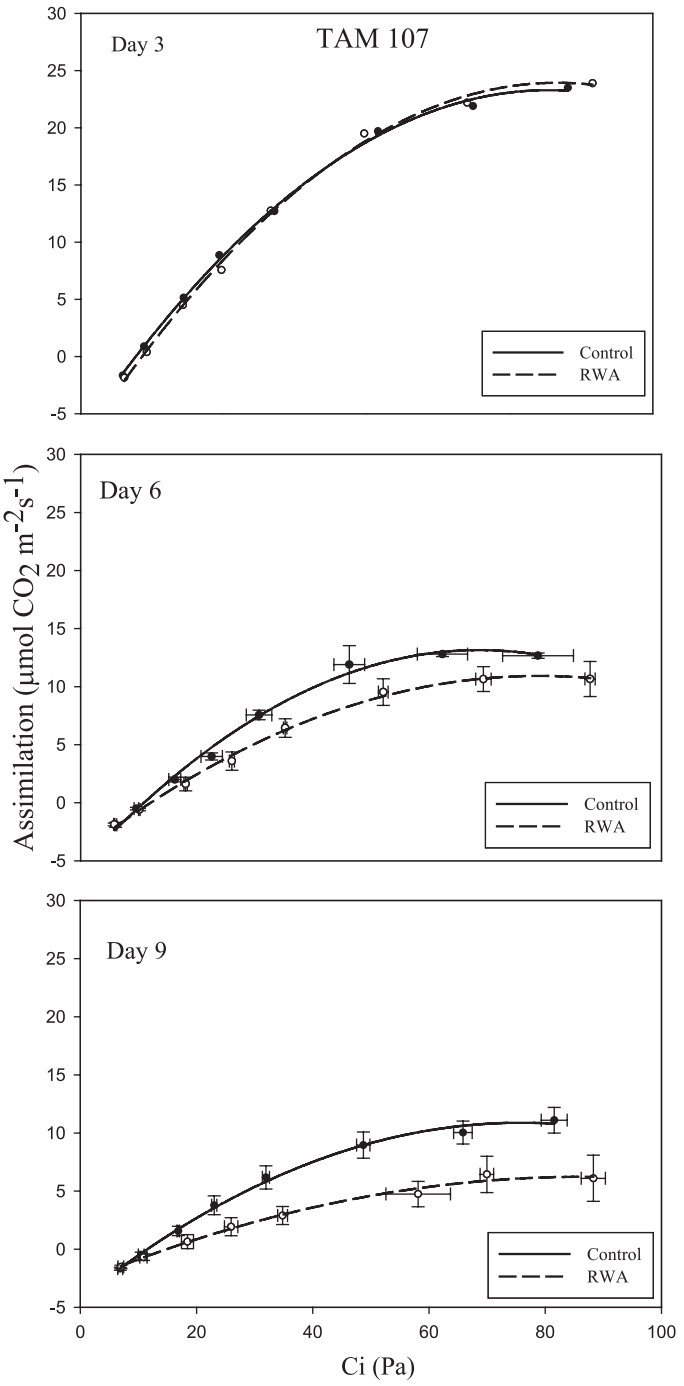

Fig. 2. Assimilation (μmol CO_2 m^{-2} s^{-1}) versus intercellular CO_2 concentration (Ci) in pascals (Pa) for susceptible TAM 107 at 3, 6, and 9 d after Russian wheat aphid (RWA) exposure.

efficiently photons absorbed were converted into chemical products (Malkin and Niyogi 2000). Our results suggest that aphid infestation of resistant plants may play a role in the efficiency of charge separation.

Photochemical fluorescence quenching (qP) was significantly impacted by aphid infestation (Table 3). Throughout the experiment, infested susceptible plants had significantly higher qP values compared with control plants (day 3: $t = -2.4$, df = 471, $P = 0.02$; day 6: $t = -2.0$, df = 471, $P = 0.05$; day 9: $t = -5.3$, df = 471, $P = 0.0001$). Photochemical quenching for infested Halt plants was significantly higher at day 3 ($t =$

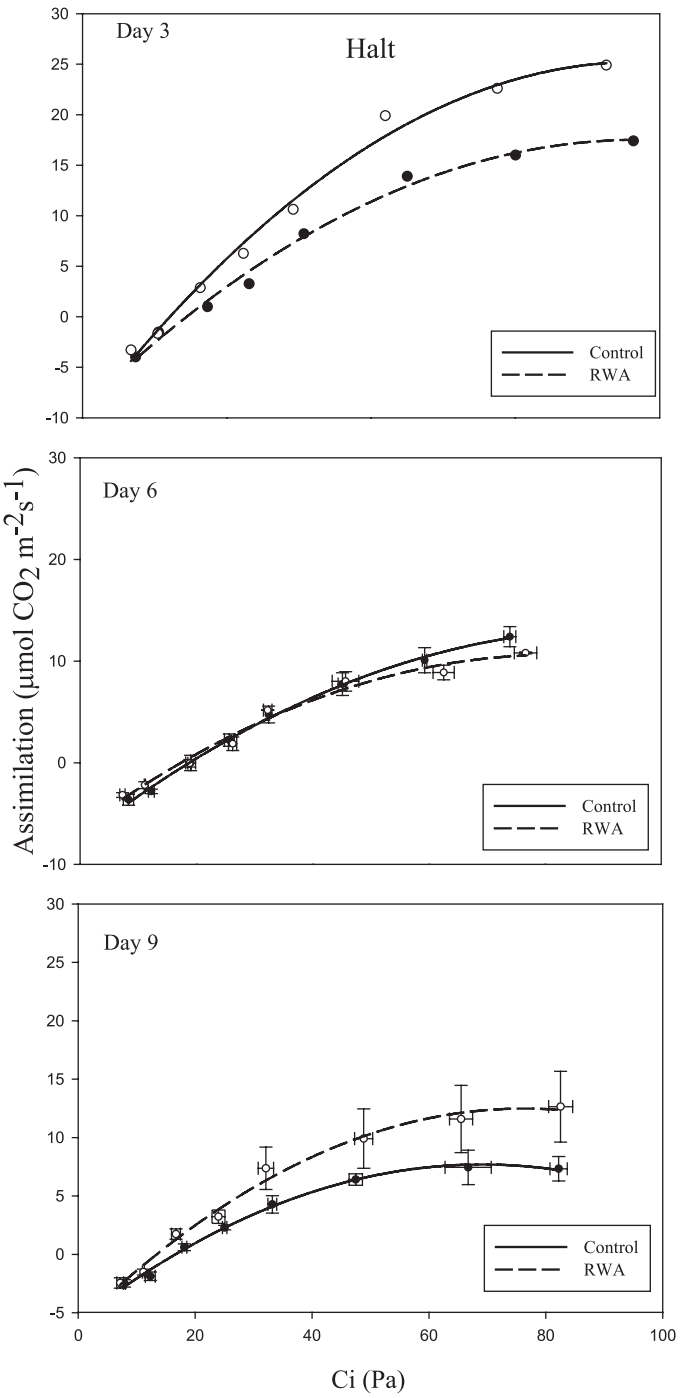

Fig. 3. Assimilation (μmol CO_2 m^{-2} s^{-1}) versus intercellular CO_2 concentration (Ci) in pascals (Pa) for resistant Halt at 3, 6, and 9 d after Russian wheat aphid (RWA) exposure.

-5.2, df = 471, $P = 0.0001$) and then remained similar to control plants on days 6 and 9. Infested Prairie Red plants exhibited qP values that were not significantly different from control and infested plants throughout the course of the experiment.

These data suggest that the resistant plants are able to adjust for changes accompanying aphid feeding that result in minimal disruption of mechanisms that impact qP (Roháček 2002). In contrast, the susceptible genotype seems to be unable to compensate for pho-

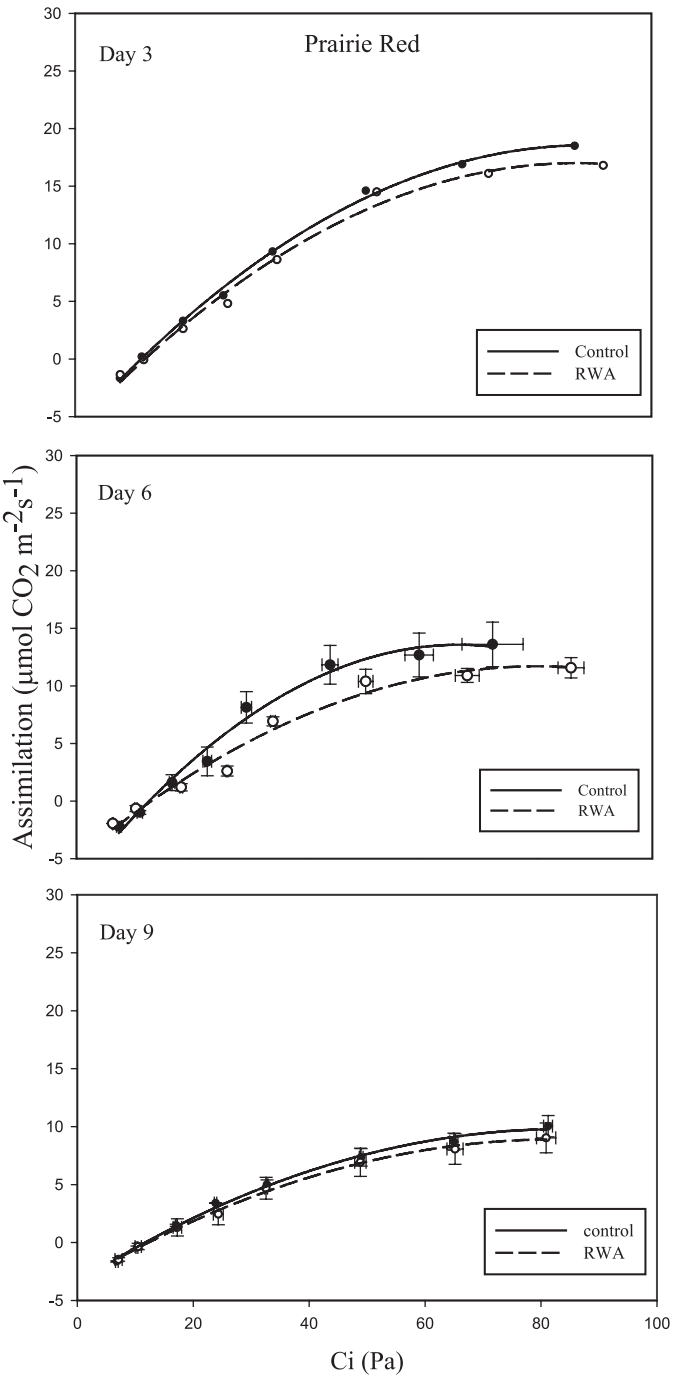

Fig. 4. Assimilation (μmol CO_2 m^{-2} s^{-1}) versus intercellular CO_2 concentration (Ci) in pascals (Pa) for resistant Prairie Red at 3, 6, and 9 d after Russian wheat aphid (RWA) exposure.

tochemical fluorescence quenching under aphid infestation, potentially due to loss of PS-2 centers and/or loss of associated electron-transfer process.

Nonphotochemical fluorescence quenching (qN), a value that relies more on emissions from thylakoid membrane energization (Müller et al. 2001), also was affected by *D. noxia* infestation (Table 3). Infested Halt and TAM 107 plants had significantly higher qN values compared with control plants on day 3 (Halt: $t = -5.9$, df = 472, $P = 0.0001$; TAM 107: $t = -2.3$, df = 472, $P = 0.02$), but subsequently, there were no significant differences between treatments for these two

Table 2. Mean ± SE for gas-exchange responses of resistant Halt and Prairie Red and susceptible TAM 107 wheat at 3, 6, and 9 d after exposure to Russian wheat aphid (RWA)

	Mean ± SE gas-exchange responses								
	CE			V_{cmax}			J_{max}		
	Control	RWA	*P* value[a]	Control	RWA	*P* value[a]	Control	RWA	*P* value[a]
Day 3									
Halt	0.44 ± 0.07	0.63 ± 0.07	0.09	71.6 ± 8.1	91.0 ± 8.1	0.11	119 ± 9.6	167 ± 9.6	0.002
Prairie Red	0.50 ± 0.07	0.42 ± 0.07	0.46	64.8 ± 8.1	61.7 ± 8.1	0.80	109 ± 9.6	103 ± 9.6	0.67
TAM 107	0.77 ± 0.07	0.71 ± 0.07	0.56	75.4 ± 8.1	87.3 ± 8.1	0.31	134 ± 9.6	144 ± 9.6	0.47
Day 6									
Halt	0.35 ± 0.04	0.30 ± 0.05	0.50	57.4 ± 5.7	41.5 ± 5.7	0.07	105± 6.8	83.0 ± 6.8	0.03
Prairie Red	0.38 ± 0.04	0.23 ± 0.04	0.02	62.7 ± 4.7	43.9 ± 4.7	0.01	103 ± 6.8	67.5 ± 6.8	0.002
TAM 107	0.36 ± 0.04	0.27 ± 0.04	0.16	54.7 ± 4.7	42.8 ± 5.7	0.13	88 ± 5.5	71.7 ± 5.5	0.05
Day 9									
Halt	0.29 ± 0.04	0.36 ± 0.04	0.22	32.1 ± 5.7	44.3 ± 5.7	0.15	65.7 ± 6.8	97.1 ± 6.8	0.04
Prairie Red	0.29 ± 0.04	0.23 ± 0.04	0.30	23.2 ± 4.7	21.1 ± 4.7	0.76	59.6 ± 5.5	54.1 ± 5.5	0.49
TAM 107	0.31 ± 0.04	0.18 ± 0.04	0.05	35.2 ± 5.7	15.3 ± 4.7	0.02	66.1 ± 5.5	41.5 ± 5.5	0.006

[a] Significantly different at $P \leq 0.05$ by least significant difference.

cultivars on days 6 and 9. Infested Prairie Red plants exhibited qN values that were not significantly different from control and infested plants throughout the course of the experiment. These data suggest that qN may not be as important a factor in these genotypes and that all three plants possess adequate mechanisms to correct for emissions arising from thylakoid membrane energization.

Significant changes in the quenching coefficients, especially in the susceptible cultivar, suggest that aphid feeding may influence the photoprotective xanthophyll cycle by altering the pH gradient across the thylakoid membrane (Macedo et al. 2003a). Changes in *trans*-thylakoid pH might compromise synthesis of zeaxathin by the xanthophylls deepoxidase enzyme, which could lead to increased formation of triplet state chlorophyll and singlet state oxygen, thereby decreasing the efficiency of photosynthesis (Malkin and Niyogi 2000). Our findings, which are similar to Macedo et al. (2003a), indicate that chlorophyll may not be directly impacted by aphid injury but rather by biochemical mechanisms involved with quenching may be more immediately impacted by aphid feeding.

Protein Content. Total protein content was not significantly different between aphid-infested and control plants for the three cultivars evaluated (Table 4). Over the course of the experiment, there was a significant decline in total protein for all three cultivars ($F = 6.3$; df = 2, 54; $P = 0.004$). Changes in protein followed a normal senescence pattern among all wheat cultivars (Dangl et al. 2000).

Peroxidase Specific Activity. Peroxidase specific activity was not significantly different between aphid-infested and control plants for the three cultivars evaluated ($F = 0.5$; df = 4, 53; $P = 0.73$). In general, aphid feeding resulted in the up-regulation of peroxidase activity in resistant cultivars, but not in susceptible plants (Table 4). However, the timing of up-regulation differed between resistant cultivars with the greatest increase for Prairie Red at day 3 and day 6 for Halt.

Previous studies by van der Westhuizen et al. (1998) also have documented increased peroxidase activity in Russian wheat aphid-resistant wheat. They investigated the intercellular peroxidase and chitinase activities of three wheat cultivars ('Tugela DN', 'Molopo DN', and 'Betta DN') resistant to the Russian wheat

Table 3. Mean ± SE of chlorophyll fluorescence responses for resistant Halt and Prairie Red and susceptible TAM 107 wheat at 3, 6, and 9 d after Russian wheat aphid (RWA) exposure

	Mean ± SE chlorophyll fluorescence responses								
	Y			qP			qN		
	Control	RWA	*P* value[a]	Control	RWA	*P* value[a]	Control	RWA	*P* value[a]
Day 3									
Halt	0.09 ± 0.005	0.11 ± 0.005	0.07	0.58 ± 0.3	2.47 ± 0.3	0.0001	0.31 ± 0.06	0.62 ± 0.06	0.0001
Prairie Red	0.09 ± 0.005	0.11 ± 0.008	0.17	1.22 ± 0.3	1.53 ± 0.3	0.45	0.24 ± 0.06	0.35 ± 0.07	0.06
TAM 107	0.08 ± 0.005	0.08 ± 0.005	0.63	1.23 ± 0.3	2.08 ± 1.3	0.02	0.26 ± 0.06	0.37 ± 0.06	0.02
Day 6									
Halt	0.15 ± 0.009	0.09 ± 0.007	0.0001	0.59 ± 0.10	0.70 ± 0.08	0.79	0.81 ± 0.02	0.83 ± 0.02	0.73
Prairie Red	0.09 ± 0.007	0.13 ± 0.007	0.0001	0.35 ± 0.08	0.66 ± 0.08	0.38	0.81 ± 0.02	0.82 ± 0.02	0.91
TAM 107	0.13 ± 0.007	0.13 ± 0.007	0.22	0.42 ± 0.08	1.12 ± 0.08	0.05	0.78 ± 0.02	0.85 ± 0.02	0.18
Day 9									
Halt	0.11 ± 0.007	0.10 ± 0.008	0.16	0.79 ± 0.3	0.74 ± 0.4	0.89	0.84 ± 0.01	0.83 ± 0.02	0.83
Prairie Red	0.10 ± 0.007	0.14 ± 0.008	0.001	0.49 ± 0.3	0.54 ± 0.4	0.89	0.81 ± 0.01	0.74 ± 0.02	0.21
TAM 107	0.09 ± 0.007	0.09 ± 0.008	0.89	0.39 ± 0.3	2.5 ± 0.4	0.0001	0.81 ± 0.01	0.89 ± 0.02	0.20

[a] Significantly different at $P \leq 0.05$ by least significant difference.

Table 4. Mean ± SE protein concentration and peroxidase specific activity of resistant Halt and Prairie Red and susceptible TAM 107 wheat at 3, 6, and 9 d after Russian wheat aphid (RWA) exposure

	Protein (mg/g)			Peroxidase activity (μ mol/min/mg)		
	Control	RWA	*P* value[a]	Control	RWA	*P* value[a]
Day 3						
Halt	14.6	12.5	0.48	0.60	0.59	0.97
Prairie Red	10.2	12.5	0.45	0.63	0.97	0.11
TAM 107	14.0	15.9	0.52	0.76	0.77	0.97
Day 6						
Halt	10.5	8.2	0.45	0.41	0.75	0.09
Prairie Red	8.2	8.8	0.82	0.41	0.49	0.70
TAM 107	9.6	8.9	0.83	0.43	0.56	0.54
Day 9						
Halt	11.8	10.2	0.61	0.50	0.63	0.49
Prairie Red	10.4	8.0	0.42	0.36	0.50	0.50
TAM 107	11.3	10.8	0.86	0.53	0.56	0.86

SE: protein = 2.1; peroxidase activity = 0.1.

[a] Significantly different at $P \leq 0.05$ by least significant difference.

Table 5. Mean ± SE NSC concentrations of resistant Halt and Prairie Red and susceptible TAM 107 wheat at 3, 6, and 9 d after Russian wheat aphid (RWA) exposure

	NSC carbohydrates (glucose equivalents/g dry wt)		
	Control	RWA	*P* value[a]
Day 3			
Halt	51.8 ± 14.0	45.5 ± 11.4	0.73
Prairie Red	50.8 ± 14.0	52.6 ± 14.0	0.93
TAM 107	43.0 ± 11.4	40.2 ± 11.4	0.86
Day 6			
Halt	151.8 ± 14.0	173.0 ± 14.0	0.29
Prairie Red	210.7 ± 11.4	237.5 ± 11.4	0.11
TAM 107	183.0 ± 14.0	173.8 ± 11.4	0.62
Day 9			
Halt	70.8 ± 14.0	131.2 ± 14.0	0.005
Prairie Red	143.0 ± 14.0	157.0 ± 14.0	0.49
TAM 107	187.8 ± 14.0	166.2 ± 11.4	0.24

[a] Significantly different at $P \leq 0.05$ by least significant difference.

aphid and three corresponding near-isogenic susceptible cultivars ('Tugela', 'Molopo', and 'Betta'). In all cultivars, the peroxidase activity in control susceptible and resistant plants was very low and remained constant throughout the experiment. Peroxidase activity was shown to increase in response to aphid feeding in all three resistant plants, whereas susceptible plants infested with aphids showed no significant increase (Tugela) in peroxidase activity or a delayed increase at a very late infestation stage (Molopo and Betta).

The accumulation of peroxides, a process that takes place during plant senescence patterns, is normally a highly regulated and subtle signaling mechanism in viable cells. When a plant is stressed by injury, such as aphid feeding, alternative senescence patterns or plant cell death pathways may be initiated (Leitner et al. 2005) that cause increased production of oxygen radicals in plants. Susceptible plants may not be able to combat the unregulated production of oxygen radicals. The accumulation of these unregulated oxygen radicals can lead to altered senescence patterns (Dangl et al. 2000).

Nonstructural Carbohydrate Concentration. The effect of *D. noxia* feeding on NSC profiles also was investigated (Table 5). Although there were significant differences, no consistent trend was observed. The only significant interaction of interest between treatments for a wheat cultivar was observed for Halt plants on day 9 ($t = -3.1$, df = 25, $P = 0.005$), where infested plants had significantly higher NSC levels from control plants.

The effect of *D. noxia* on soluble carbohydrates (expressed as percentage dry weight) did not seem to deplete or cause altered partitioning patterns for Prairie Red and Halt. TAM 107 plants infested with *D. noxia* consistently had similar or reduced NSC levels compared with control plants, suggesting that alterations in photosynthetic rates may have been correlated with declining NSC levels. Nonstructural carbohydrate levels can be an informative plant parameter, but in this case, may not be precise enough to detect differences in partitioning patterns (alterations toward storage forms versus translocated forms of carbohydrate) resulting from aphid feeding. In the future, different forms of carbohydrates (starch, sucrose, hexose, and fructose) should be measured in relation to NSC.

Integrated Responses

Overall, there was a decline in photosynthetic capacity for all wheat plants over the course of the experiment, but aphid infestation seemed to have a different effect on resistant and susceptible cultivars. Generally, as plants senesce there are declines in photosynthetic capacity as well as alterations in plant metabolism. The observed decline in photosynthetic capacity may have involved the cytochrome b6f electron transport complex, PSII, the carbon-fixing reaction catalyzed by rubisco, or a combination (Lambers et al. 1998). Results from this study suggest that resistant plants subjected to *D. noxia* feeding maintained or compensated for aphid injury by altering their senescence pathways, whereas susceptible plants seemed to have an accelerated senescence pattern.

Infested Halt plants exhibited delayed senescence patterns by maintaining a steady photosynthetic activity over time despite aphid feeding, whereas the control plants exhibited normal declines in photosynthetic capacity as the plant aged. However, infested and control Prairie Red plants had similar photosynthetic rates throughout the experiment. Based upon these results, it is likely that infested Prairie Red plants exhibited photosynthetic compensation. Even though Halt and Prairie Red both possess the *Dn4* gene (for *D. noxia* resistance) (Randolph et al. 2005a), the mechanisms of resistance seem to be different with respect to gas-exchange responses.

Gas-exchange processes rapidly respond to external factors and provide an immediate indication of plant stress (Peterson and Higley 1993) as well as a common biological basis for comparison of herbivore impact on different plants (Welter 1989). In this study, we ob-

served asymptomatic plants to have reduced gas-exchange responses despite the lack of visual chlorosis or reductions in chlorophyll content. Based upon these observations, it is evident that gas-exchange responses are a more sensitive physiological parameter than chlorophyll measurements. Moreover, it seems likely that altered chlorophyll levels may be a secondary effect and not the primary trigger of declined host plant function (Macedo et al. 2003a).

Previous studies on how aphids alter plant gas-exchange processes have indicated that photosynthetic inhibitions occur before CO_2 assimilation with rubisco (Ryan et al. 1987, Haile et al. 1999). In general, we found that TAM 107 (susceptible cultivar) plants infested with *D. noxia* had decreased rubsico activity and RuBP regeneration rates compared with control plants. These results suggest that as the rate of incorporation and/or export of photosynthetic products declines, photosynthesis becomes restricted by "feedback inhibition" (Lambers et al. 1998) and results in alterations of source–sink feedback signals (Peterson and Higley 1993). Resistant plants infested with Russian wheat aphids had comparable or increased RuBP rates, which suggest faster regeneration of RuBP.

This study provides evidence to support our working hypothesis that end-product inhibition plays an important role in reduced photosynthetic rates of susceptible plants (Macedo 2003). Resistant plants seem to counteract deleterious effects of aphid herbivory on leaves through up-regulation of detoxification mechanisms and faster regeneration of photosynthetic active centers and RuBP. In contrast, our data suggest that leaves of susceptible plants are unable to sustain these processes and become senescent. Overall, it would seem that the initial response in all wheat plants to aphid feeding is similar, but the extent of recovery (level of resistance) is determined by several complimentary cellular pathways (i.e., elicitor pathways and cellular metabolism). These pathways apparently allow a source leaf on a resistant plant to overcome the negative effects of aphid feeding and maintain adequate rates of photosynthesis that supports continued plant growth.

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