STUDIES OF PHYSIOLOGICAL ALTERATIONS IN CEREALS INDUCED BY GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI) AND RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* (MORDVILKO)

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STUDIES OF PHYSIOLOGICAL ALTERATIONS IN CEREALS INDUCED BY GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI) AND RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* (MORDVILKO)

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PART I

CHARACTERIZATION OF PLANT DAMAGE CAUSED BY RUSSIAN WHEAT APHID (HOMOPTERA: APHIDIDAE)

ABSTRACT

The effect of Russian wheat aphid, Diuraphis noxia (Mordvilko), on the growth patterns of 'TAM W-101' winter wheat seedlings was investigated. Results showed that a primary response of the wheat plant to aphid feeding involves the development of water imbalances expressed as a loss of turgor maintenance and reduced growth of the infested plants. A detailed growth analysis was performed to characterize specific plant damage components associated with aphid feeding and to evaluate host recovery. The analysis revealed that Russian wheat aphids caused substantial reductions in biomass to all plant components measured. When aphids were removed, absolute growth rates quickly recovered as relative growth rates increased. Net assimilation rates were found to be significantly higher in recovering plants despite reductions in leaf area ratios. When compared with undamaged controls, recovering plants were more efficient in terms of carbon accumulation, which resulted in higher relative growth rates and compensated for lowered leaf area ratios. Reduction in leaf area ratios result from decreased specific leaf areas caused by a combination of leaf stunting and the inhibition of new leaf unfolding. As plants recovered, stem weight ratios were

substantially reduced, indicating that carbon partitioning to the stem was decreased to compensate for leaf and root growth.

INTRODUCTION

Since its discovery in the United States in the spring of 1986, the Russian wheat aphid, Diuraphis noxia (Mordvilko), has spread from northwestern Texas and is now established in much of the western wheat and barley producing areas of the United States (Stoetzel 1987, Kindler and Springer 1989). It has caused cumulative economic losses in excess of 250 million dollars (Hein 1990).

Characteristic plant damage symptoms caused by Russian wheat aphid infestations include leaf rolling, longitudinal white leaf streaking, purple discoloration, and prostrate growth (Walters et al. 1980, Hewitt et al. 1984). These symptoms indicate physiological dysfunctions, yet few studies have focused on the physiological aspects of Russian wheat aphid damage.

Fouche et al. (1984) evaluated Russian wheat aphid damage at the ultrastructural level and found that chloroplasts and cellular membranes were destroyed during the feeding process and ascribed the destruction as being caused by a toxin that is injected into the leaf tissue during feeding. Other studies have shown that Russian wheat aphid damage can be expressed as plant stunting (Bush et al. 1989), interference with cold hardening and predisposition to winterkill (Thomas and Butts 1990), and, disruption of osmoregulatory processes and the occurrence of drought-stress symptoms in leaves of infested plants (Riedell 1989).

The objectives of this study were to quantify the effects of Russian wheat aphids feeding on the growth of wheat seedlings and to evaluate plant recovery.

MATERIALS AND METHODS

Plant Growth Analysis

Pre-germinated 'TAM W-101' winter wheat was planted in a greenhouse, one seed per container, with a fritted clay medium (Absorb-N-Dry, Balcones Minerals, Flatonia, Tex.) in cone-tainers (Supercell Cone-Tainer, Ray Leach Cone-Tainer Nursery, Canby, Oreg.) (Burton 1986). Plants were watered daily and starting 7 d after emergence were fertilized biweekly with Peters' Peat-Lite Special (analysis 15-16-17) (Peters Fertilizer Products, Fogelsville, Pa.), a water-soluble fertilizer. Plants were grown under natural light conditions (May - June) and greenhouse temperatures were maintained at 21 ± 5°C. Fourteen days after planting, at growth stage 13 (Zadoks et al. 1974), test plants were selected based upon developmental uniformity, and each aphid-treated entry was infested with 30 mature apterous Russian wheat aphids from greenhouse colonies that were established from a 1986 field collection from Bailey county, Tex. Both infested and noninfested control plants were covered with ventilated clear plastic cages (Starks and Burton 1977). The aphids were allowed to feed and reproduce freely for 7 d, after which they were removed and counted.

At harvest, plants were removed from the containers, and the roots were washed free of the fritted clay. Total number of leaves and tillers per plant were recorded. Leaves of each plant were clipped at the collar and measured for total leaf length. Total leaf area was then determined using a Li-Cor Model 3100 Area Meter (Li-Cor, Inc., Lincoln, Nebr.). Plant roots and shoots were separated at the hypocotyl and total root lengths were measured using a Comair root length scanner (Commonwealth Aircraft Corporation Limited, Melbourne, Australia). Root, leaf, and stem components were oven-dried at 60°C for 24 h and then weighed. Harvest data were used to calculate mean absolute growth rate (G), mean relative growth rate (R), mean net assimilation rate (E), mean leaf area ratio (F), mean leaf weight ratio (LWR), mean stem weight ratio (SWR), mean root weight ratio (RWR), and mean specific leaf area (SLA) using the procedures described by Hunt (1978).

The experimental protocol followed a paired-plot design (n=60) with sampling splits (controls, n = 10, infested, n = 10) at the time of aphid removal, followed by two samples at 7-d intervals.

Plant Water Status

Concomitant with experiment one, a second greenhouse study was conducted to determine the effect of Russian wheat aphid feeding on leaf turgor. Plant and Russian wheat aphid cultural techniques were similar to those for experiment one, but differed in both infestation level and duration. Aphid-treated plants were infested 14 d after planting, at growth stage 13 (Zadoks 1974), with 10 mature apterous aphids per plant. The duration of the aphid infestation was 14 d, after which the aphids were removed and counted. The experimental protocol followed a paired-plot design where n = 30; controls, n = 15, infested, n = 15.

To evaluate tissue water status, two 0.24 cm^2 leaf discs were excised from both control and infested plants using leaf-cutter psychrometers (J.R.D. Merrill Specialty Equipment, Logan, Utah). At the time of sampling, all plants had developed at least one tiller (growth stage 21, Zadoks 1974). The leaf-disc samples were taken 3 cm from the base of the fourth leaf of the main stem and 2 cm from the base of the first leaf of the coleoptilar tiller of each plant. Water potential, osmotic potential, and turgor pressure were determined using the procedures described by Johnson et al. (1984).

Statistical Analysis

Data analysis and computations were done with Statistical Analysis Systems (SAS 1988). The TTEST Procedure was used for all statistical tests except for the analysis of aphid counts in experiment one, which were analyzed using one-way analysis of variance (ANOVA).

RESULTS

Plant Growth Analysis

During the 7-d infestation period, the Russian wheat aphid populations increased to a mean well above 300 aphids per plant (Table 1). There were no significant differences in final aphid counts between sampling splits, indicating that the feeding stress imposed by the aphids was relatively equal.

At the time of aphid removal, all plant growth parameters measured were substantially reduced (Table 2). Tiller initiation on infested plants was virtually arrested during the 7-d infestation. All control plants had produced at least one tiller, whereas only one infested plant had tillered. However, during the 7 d after aphid removal, there was a substantial increase in the number of tillers on the aphid-treated plants; and by 14 d, subsequent tiller initiation rates on the infested plants had leveled off and were similar to those of the control plants. Leaf production was reduced ca. 23% during the 7-d infestation period. However, the level of reduction in the total number of leaves decreased after the aphids were removed, and by 14 d leaf initiation rates were equal to those of the controls. When compared with the reductions in leaf production (range, 17-25%), there were disproportionate decreases in the total leaf areas of infested plants (range, 56-62%). The sharp decline in leaf area is attributable to a combination of leaf stunting and an inhibition of unfolding of the damaged leaves. Total leaf and root lengths sustained proportionately equal amounts of growth reduction during the 7-d infestation and likewise, showed similar recovery patterns. At the time of aphid removal, shoot and root dry weights were reduced ca. 54 and 38%, respectively. As expected, biomass recovery trends were similar to those of total leaf and root lengths. Initially, root:shoot ratios were substantially higher for the infested plants, but did not differ significantly at 7 d and 14 d after aphid removal.

Aphid feeding significantly reduced mean absolute growth rate (G) during the week following aphid removal (Table 3). However, during the subsequent 1-wk growth period, G recovered to a level equal to the control plants. The root and shoot components of G followed the same recovery pattern. In contrast, mean relative growth rate (R) did not differ between plant treatments during the first

7-d recovery period, although the R value for roots was significantly lower for the infested plants. During the second recovery period, values for all R components were substantially higher for the infested plants. The subdivisions of R, mean net assimilation rate (E) and mean leaf area ratio (F), showed opposite recovery trends. E values were much higher for previously infested plants during both recovery periods while F values were significantly lower. F can be further subdivided into mean specific leaf area (SLA) and mean leaf weight ratio (LWR) components. Significant reductions in SLA of infested plants occurred in both recovery periods. Both LWR and mean root weight ratio (RWR) did not differ between treatments in either recovery period. Mean shoot weight ratio (SWR) was significantly lower for the infested plants.

Plant Water Status

The relationships between tissue water components, location on the plant, and aphid feeding are shown in Table 4. Water potentials were significantly lower (more negative) for infested plants in the leaves of both main stem and coleoptilar tillers. However, the accompanying osmotic potentials did not differ between aphid treatments at either plant location. As a result, turgor pressure was greatly reduced in the infested leaves.

DISCUSSION

During the growth analysis and water status experiments, Russian wheat aphid populations attained numbers considerably higher than the economic threshold levels previously reported for wheat seedlings in the field (Du Toit and Walters 1984, Du Toit 1986, Kriel et al. 1986). Under field conditions, visible damage symptoms caused by Russian wheat aphid feeding include chlorotic lesions, longitudinal white streaking, purple discoloration, and tightly rolled leaves. In the present studies, the most conspicuous response of 'TAM W-101' wheat to Russian wheat aphid feeding was convolutely rolled leaves. Russian wheat aphids typically aggregate on the new growth of the host to feed. Leaf rolling was confined to new growth where the aphids were feeding and was caused by preventing newly formed leaves from unrolling. Rolling of expanded leaves was not observed. Longitudinal streaks in the infested leaves did occur, but cell bleaching was invariably subsequent to the rolled-leaf condition. Chlorotic lesions were present on a few expanded leaves, but were limited to the most recently unfolded leaf at the time of infestation. Leaf purpling, caused by anthocyanin accumulation (Fouche et al. 1984), was not observed. Consequently, the observed primary event in the host damage sequence was leaf rolling.

In grasses, leaf unfolding and expansion are a function of growth resulting from the enlargement of bulliform and mesophyll cells arising from the intercalary meristem located at the base of the developing leaf (Esau 1977, Langer 1979). Eastham et al. (1984) reported a positive linear relationship between leaf turgor and leaf elongation rates in wheat. A minimum threshold turgor for growth has been observed in tissues of several plant species (Bradford and Hsiao 1982). The prevention of new-leaf unfolding and reduction in leaf size caused by Russian wheat aphid feeding apparently results from the reduction of leaf turgor below the threshold for elongation and cell wall extensibility. Osmotic adjustment is a major regulatory mechanism for minimizing the effect of lower water potential on leaf extension rates by maintaining osmotic potential through the active accumulation of intracellular solutes (Levitt 1980). The capacity of TAM W-101 to osmotically adjust to drought stress has been reported by Johnson et al. (1984). However, Russian wheat aphid infested plants showed no decrease in osmotic potential despite significant decreases in water potential. Similar observations were reported by Riedell (1989), where barley plants (Hordeum vulgare L. cv Hazen), subjected to drought stress following Russian wheat aphid infestation, failed to accumulate osmoregulatory solutes and were unable to adjust osmotically.

Characteristic interveinal white streaking, typically associated with Russian wheat aphid damage (Walters et al. 1980), developed longitudinally along the leaf blade of infested leaves. Ultrastructural analysis of Russian wheat aphid damage by Fouche et al. (1984), that described sequential events occurring at the cellular level, demonstrated the involvement of the chloroplast as a primary site of action. In their study, the initial response to Russian wheat aphid feeding was the retraction and convolution of the plasmalemma, implying a loss of cellular turgidity, followed by the distension of the chloroplast granal and stromal lamellae. As the damage progressed, a rapid disassembly of the chloroplast lamellae occurred which resulted in a substantial increase in the volume of plastoglobuli. Subsequent degeneration of the chloroplast envelope was followed by the disintegration of other cell organelle membranes, which culminated in cell bleaching.

In light-grown plants, cell bleaching can result from the process of photooxidation, where light induced overexcitation of chlorophyll molecules causes the formation of triplet chlorophyll which in turn reacts with molecular oxygen to form highly reactive singlet oxygen or superoxide (Hipkins 1985). These two forms of oxygen are highly destructive to chlorophyll pigments and membrane lipids (Cogdell 1988, Foyer 1984).

Protection against the photooxidative process is facilitated by carotenoids dispersed among the chlorophyll pigments embedded in the chloroplast lamellar membranes, which serve to directly quench the triplet chlorophyll and dissipate the excess energy as heat, and thereby aid in the prevention of photobleaching (Kok 1976). In addition to chlorophyll destruction, singlet oxygen can cause thylakoid degradation by lipid peroxidation of membrane polyunsaturated fatty acid side chains (Foyer 1984), which would account for the observed development of plastiglobuli in chloroplasts of Russian wheat aphid damaged tissue (Fouche et al. 1984), and could further promote pigment loss (Sandmann and Boger 1982). Carotenoid accumulation in etiolated wheat leaves is significantly reduced by water deficit, and under stimulatory light conditions, synthesis is greatly impaired (Duysen and Freeman 1974). Consequently, cell bleaching, associated with Russian wheat aphid damage may be caused by induced water imbalances that reduce constituent carotenoid levels by destruction or inhibition of biosynthesis, accompanied by the peroxidative destruction of chloroplast membranes.

The data have shown that, Russian wheat aphid feeding substantially reduces the growth and development of the wheat plants. Total biomass proved a good indicator of overall damage, and was correlated to the other response variables evaluated. The total number of leaves produced on

Russian wheat aphid infested plants was decreased. However, after aphid removal, leaf production resumed at a rate similar to that of the noninfested control plants. The resumption of leaf initiation appeared to be related to new tiller production, and not the result of damaged-tissue recovery. The most sensitive response measured was leaf area. Reduction of leaf area was directly correlated with leaf rolling and stunting which are attributable to a decrease in cell turgor (Levitt 1980). Despite the resumption of tiller and leaf development after the aphids were removed, the mean total leaf area of aphid-damaged plants remained substantially reduced after 2 wk. Following aphid removal, convolutely rolled leaves did not recover (unroll) and new growth remained trapped.

The ability of the plant to recover after aphid removal was clearly demonstrated by increased absolute and relative growth rates. Net assimilation rates of the aphid-damaged plants were significantly greater after the aphids were removed and accounted for the elevated relative growth rates. In contrast, leaf area ratios did not recover. Thus, recovering plants were more efficient in terms of carbon assimilation that resulted in higher relative growth rates and compensated for the lowered leaf area ratios. The reduction in the leaf area ratios resulted from diminished specific leaf areas, a direct consequence of leaf rolling. Stem weight ratios were significantly reduced indicating that carbon partitioning to the stem was diminished to compensate for leaf and root growth. The growth analysis data suggest that plant recovery from Russian wheat aphid attack may be influenced more by the timing and duration of the infestation than by the population level.

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TABLE 1

Mean, Standard Error (SEM), and Coefficient of Variation (CV) for Russian Wheat Aphid Populations on TAM W-101 Wheat

Sample	Mean	SEM	CV
Split I	387.0	20.1	17.2
Split II	385.5	15.9	13.0
Split III	345.0	27.8	25.4

TABLE 2

Summary of Mean Values (± SEM) for Measured Plant Parameters for Infested and Noninfested TAM W-101 Wheat at 0, 7, and 14 Days After Russian Wheat Aphid Removal

	0 Days After Removal	
Measurement	Infested	Control
Number of Tillers	0.1 (0.09)*	1.5 (0.15)
Number of Leaves	4.4 (0.18)*	6.1 (0.19)
Leaf Area (cm^2)	8.7 (0.47)*	22.9 (1.58)
Leaf Length (cm)	38.3 (1.29)*	65.0 (3.22)
Root Length (m)	3.19 (0.18)*	5.37 (0.34)
Stem Dry Weight (g)	0.008 (0.002)*	0.025 (0.005)
Leaf Dry Weight (g)	0.032 (0.004)*	0.062 (0.009)
Shoot Dry Weight (g)	0.040 (0.002)*	0.087 (0.003)
Root Dry Weight (g)	0.029 (0.003)*	0.047 (0.003)
Root:Shoot Ratio	0.725 (0.036)*	0.540 (0.027)

TABLE 2 (Continued)

	7 Days After Removal	
Measurement	Infested	Control
Number of Tillers	1.6 (0.05)*	2.1 (0.09)
Number of Leaves	8.5 (0.50)*	11.3 (0.41)
Leaf Area (cm^2)	25.4 (0.47)*	67.9 (3.32)
Leaf Length (cm)	94.6 (6.60)*	158.4 (6.80)
Root Length (m)	6.78 (0.60)*	10.43 (0.71)
Stem Dry Weight (g)	0.026 (0.004)*	0.072 (0.006)
Leaf Dry Weight (g)	0.091 (0.007)*	0.163 (0.011)
Shoot Dry Weight (g)	0.117 (0.010)*	0.234 (0.009)
Root Dry Weight (g)	0.040 (0.002)*	0.074 (0.003)
Root:Shoot Ratio	0.342 (0.026)	0.316 (0.019)

TABLE 2 (Continued)

	14 Days After Removal	
Measurement	Infested	Control
Number of Tillers	2.2 (0.32)*	3.2 (0.13)
Number of Leaves	11.8 (0.76)	14.2 (0.82)
Leaf Area (cm^2)	60.7 (8.10)*	138.7 (8.80)
Leaf Length (cm)	179.5 (14.9)*	248.0 (12.8)
Root Length (m)	11.36 (1.10)	14.93 (1.92)
Stem Dry Weight (g)	0.078 (0.009)*	0.139 (0.011)
Leaf Dry Weight (g)	0.222 (0.015)*	0.307 (0.023)
Shoot Dry Weight (g)	0.300 (0.028)*	0.446 (0.032)
Root Dry Weight (g)	0.064 (0.006)	0.084 (0.009)
Root:Shoot Ratio	0.213 (0.020)	0.188 (0.018)

*, Significant at the 0.05 probability level.

TABLE 3

Summary of Mean Values (± SEM) for Measured Plant Parameters for Infested and Noninfested TAM W-101 Wheat for Growth Intervals of 0 to 7 and 7 to 14 Days After Russian Wheat Aphid Removal

	0 to 7 Day Growth Interval	
Measurement	Infested	Control
G_{root} (mg/day)	1.5 (0.02)*	3.9 (0.06)
G_{shoot} (mg/day)	11.0 (0.91)*	20.9 (1.30)
G_{total} (mg/day)	12.6 (1.01)*	24.9 (1.62)
R_{root} (mg/mg/day)	0.046 (0.008)*	0.065 (0.002)
R_{shoot} (mg/mg/day)	0.153 (0.015)	0.141 (0.010)
R_{total} (mg/mg/day)	0.117 (0.011)	0.119 (0.008)
E (mg/cm^2/day)	0.807 (0.051)*	0.600 (0.029)
F (cm^2/mg)	0.144 (0.008)*	0.196 (0.011)
SLA (cm^2/mg)	0.275 (0.021)*	0.395 (0.018)
LWR	0.522 (0.035)	0.496 (0.029)
SWR	0.140 (0.012)*	0.210 (0.008)
RWR	0.337 (0.009)*	0.296 (0.006)

TABLE 3 (Continued)

	7 to 14 Day Growth Interval	
Measurement	Infested	Control
G_{root} (mg/day)	3.9 (0.71)	2.5 (0.83)
G_{shoot} (mg/day)	26.0 (1.93)	30.2 (1.37)
G_{total} (mg/day)	30.0 (1.05)	32.7 (1.44)
R_{root} (mg/mg/day)	0.067 (0.006)*	0.018 (0.002)
R_{shoot} (mg/mg/day)	0.135 (0.009)*	0.092 (0.005)
R_{total} (mg/mg/day)	0.120 (0.008)*	0.078 (0.002)
E (mg/cm^2/day)	0.729 (0.093)*	0.320 (0.056)
F (cm^2/mg)	0.164 (0.014)*	0.241 (0.017)
SLA (cm^2/mg)	0.276 (0.020)*	0.435 (0.031)
LWR	0.594 (0.026)	0.554 (0.025)
SWR	0.190 (0.011)*	0.248 (0.013)
RWR	0.215 (0.009)	0.200 (0.010)

*, Significant at the 0.05 probability level.

TABLE 4

Mean Values (MPa ± SEM) for Leaf Water Potential, Osmotic Potential, and Turgor Pressure for Infested and Noninfested TAM W-101 Wheat

Leaf Location	Water Potential	Osmotic Potential	Turgor Pressure
Main Stem			
Infested	-0.77 (0.02)*	-0.94 (0.03)	0.17 (0.02)*
Control	-0.36 (0.03)	-0.97 (0.04)	0.61 (0.03)
First Tiller			
Infested	-1.05 (0.02)*	-1.11 (0.05)	0.06 (0.03)*
Control	-0.60 (0.03)	-1.02 (0.04)	0.42 (0.04)

*, Significant at the 0.05 probability level.

PART II

EVALUATION OF RUSSIAN WHEAT APHID DAMAGE ON DIFFERENT HOST SPECIES WITH COMPARISONS OF DAMAGE RATINGS TO QUANTITATIVE PLANT MEASUREMENTS

ABSTRACT

Plant entries that had previously identified to be resistant or susceptible to Russian wheat aphid, Diuraphis noxia (Mordvilko), were used to evaluate the relationships between damage rating indices and quantitative measurements of plant damage. I measured the degree of association between percentage leaf chlorosis, leaf rolling, and plant stunting, and found that they occurred as independent damage symptoms. Damage ratings based on percentage chlorosis, accurately measured highly resistant and highly susceptible entries but failed to adequately describe intermediate plant responses. The most reliable indicator of plant damage was plant stunting expressed as percentage height of a noninfested control. No significant relationships between leaf rolling and quantitative plant measurements were found. Aphid-caused reductions in leaf turgor and the number of aphids per unit shoot mass were closely related to the amount of chlorosis.

INTRODUCTION

The Russian wheat aphid, Diuraphis noxia (Mordvilko), has become a major pest of cereals in much of the western wheat and barley producing areas of the United States (Stoetzel 1987, Kindler and Springer 1989, Hein 1990). At low population densities, it is capable of disrupting osmoregulatory processes (Riedell 1989), cold hardening (Thomas and Butts 1990), and causing interveinal chlorosis, rolled leaves, head trapping (Kiriac et al. 1990), and substantial yield losses (Kriel et al. 1986, Fouche et al. 1984, Du Toit and Walters 1984).

Generally, the relationship between plant injury and yield varies with the plant growth stage at the time of infestation (Bardner and Fletcher 1974) and therefore, plant resistance screening evaluations should be conducted during the growth stage(s) that are prone to attack in the field (Ortman and Peters 1980). However, Russian wheat aphids have the potential to infest plants throughout the growing season (Girma et al. 1990) and the most appropriate growth stage(s) to evaluate resistance remains to be determined. Methods currently used to evaluate Russian wheat aphid resistance have been largely based upon protocols developed for greenbugs (Starks and Burton 1977a), but also can include separate leaf rolling and plant stunting indices (Bush et al. 1989, Du Toit 1989, Frank et al. 1989, Nkongolo et al. 1990a, Quick et al. 1991, Smith et al. 1991, Webster 1990, Webster et al. 1987, 1991, Zemetra et al. 1990).

The objectives of this study were to determine the relationships among the different Russian wheat aphid resistance evaluation indices and their reliability in predicting plant damage in relation to quantitative plant measurements.

MATERIALS AND METHODS

Plant Material and Insects

For this study, plant entries which had been previously identified as resistant or susceptible to Russian wheat aphids in plant resistance screening evaluations were used. Plant materials were as follows: oat (Avena sativa L.), 'Okay', resistant (Webster et al. 1987); triticales (xTriticosecale Wittmack), PI 386148, resistant (Webster 1990) and 'Beagle 82', susceptible (Webster et al. 1987); and wheats (Triticum aestivum L.), PI 372129, resistant (Quick et al. 1991) and 'TAM W-101', susceptible (Webster 1990).

Pre-germinated seed of each entry was planted in a greenhouse, one seed per container, 3 cm deep, in a fritted clay medium (Absorb-N-Dry, Balcones Minerals, Flatonia, Tex.) in cone-tainers (Supercell Cone-Tainer, Ray Leach

Cone-Tainer Nursery, Canby, Ore.) (Burton 1986). Plants were watered daily and starting 7 d after emergence were fertilized biweekly with Peters' Peat-Lite Special (analysis 15-16-17) (Peters Fertilizer Products, Fogelsville, PA). Plants were grown under natural light conditions (February - March) and greenhouse temperatures were maintained at 21 ± 5°C. Fourteen days after planting, at growth stage 12 (Zadocks et al. 1974), test plants were selected based upon developmental uniformity, and each aphid-treated entry was infested with 25 mature apterous Russian wheat aphids from greenhouse colonies that were established from a 1986 field collection from Bailey county, Tex. Both infested and noninfested control plants were covered with ventilated clear plastic cages (Starks and Burton 1977a). The Russian wheat aphids were allowed to feed and reproduce freely for 14 d, after which they were removed and counted.

The experimental protocol followed a paired-plot, randomized complete block design, with 12 blocks ($\underline{n}$ = 120; infested = 12, control = 12, per plant entry).

Plant Evaluation

Plant damage was qualitatively evaluated by rating the relative amount of chlorosis, leaf rolling, and plant stunting. The amount of foliar chlorosis was measured using a 1 to 9 scale (Webster et al. 1991), where; 1 = plants appear healthy, may have small isolated chlorotic spots; 2 =

isolated chlorotic spots prominent; 3 = chlorosis ≤ 15% of total leaf area, chlorotic lesions coalesced; 4 = chlorosis > 15% but ≤ 25% of total leaf area, streaky appearance; 5 = chlorosis > 25% but ≤ 40% of total leaf area, well-defined streaks; 6 = chlorosis > 40% but ≤ 55% of total leaf area; 7 = chlorosis > 55% but ≤ 70% of total leaf area; 8 = chlorosis > 70% but ≤ 85% of total leaf area; 9 = plant death, or beyond recovery. Leaf rolling was rated using a 1 to 3 scale, where: 1 = no leaf rolling; 2 = one or more leaves conduplicately folded; 3 = one or more leaves convolutely rolled. Plant stunting was measured by comparing the height of aphid-treated plants with paired noninfested controls, where: 1 = plant height equal to control; 2 = plant height < 100% but ≥ 75% of control; 3 = plant height < 75% but ≥ 50% of control; 4 = plant height < 50% but ≥ 25% of control; 5 = plant height < 25% of control.

Leaf water status was measured by excising 0.24 cm^2 leaf discs from both control and aphid-treated plants using leaf-cutter psychrometers (J.R.D. Merrill Specialty Equipment, Logan, Utah). The leaf-disc samples were taken 3 cm above the base of the most recent fully expanded leaf on the main stem of each plant. Water potential, osmotic potential, and turgor pressure were determined using the procedures described by Johnson et al. (1984).

At harvest, the number of tillers and leaves of each plant were recorded. Next, the leaves were excised at the collar and measured to determine total leaf length per plant. Total leaf area for each plant was determined using a Li-Cor Model 3100 Area Meter (Li-Cor, Inc., Lincoln, Nebr.). The plant shoots were oven-dried for 24 h at 65°C, after which they were weighed.

Statistical Analysis

Data analyses and computations were done with Statistical Analysis Systems (SAS Institute 1988a, 1988b). Means for the number of tillers and leaves, leaf area, leaf length, and shoot weight were analyzed using the TTEST procedure for paired samples. Regression and correlation analyses were done to describe the relationships between measured plant parameters and plant damage evaluation schemes.

RESULTS AND DISCUSSION

Means for aphid populations and plant rating scores for the different plant entries are shown in Table 1. PI 372129 supported the highest aphid population followed by 'TAM W-101', 'Beagle 82', 'Okay', and PI 386148. Significant differences ($\underline{P} \leq 0.05$) in aphid populations were not observed between plant entries except for the resistant triticale, PI 386148. Because aphids were confined on individual test plants, the significantly smaller population level observed on PI 386148 indicates a substantial antibiotic response.

Russian wheat aphid resistance in triticale has generally been attributed to an antibiotic effect (Frank et al. 1989, Webster et al. 1987, Webster et al. 1991) which is thought to come from the rye (Secale cereale L.) background (Nkongolo et al. 1990b). This is in agreement with Kindler and Springer (1989) who reported that Russian wheat aphid populations were reduced by 85 and 95% on cereal rye when compared to a susceptible wheat and barley, respectively.

As a group, the resistant entries, 'Okay', PI 386148, and PI 372129, did not develop convolutely rolled leaves. In contrast, on both susceptible entries, 'Beagle 82' and 'TAM W-101', new leaves did not unfold and leaf trapping was evident. It is important to note that screening tests conducted within greenhouses may not accurately characterize the leaf-rolling response. Quick et al. (1991) observed that leaf rolling was much less pronounced in greenhouse screening when compared to field evaluations. An accurate assessment of leaf rolling is important because the biological fitness of Russian wheat aphids may be closely linked with its capacity to induce the rolled-leaf condition.

There is a strong correlation between the presence of well-developed cornicles and the development of chemical alarm signals in aphids. Generally, aphids, such as the Russian wheat aphid, that lack or have poorly developed cornicles, typically live within protective plant galls or the soil to escape predators (Nault and Phelan 1984). Thus, from a biological control standpoint, selection for plants that inhibit or diminish the Russian wheat aphid's ability to create a protective gall-like niche within tightly rolled leaves would appear highly desirable.

Despite the lack of leaf-rolling in 'Okay' and PI 372129, aphid dispersion patterns were not appreciably altered from those observed on 'Beagle 82' and 'TAM W-101'. Within 24 h after infestation, the Russian wheat aphids had formed compact aggregations located exclusively on the new growth. Because aphid populations did not differ significantly between these rolled and nonrolled entries, it appears that leaf-rolling is not a requisite for reproductive or nutritional success. In contrast, colonization behavior differed greatly on PI 386148; the aphids failed to aggregate on the new growth and tended to be widely dispersed on the plant. Daily observations of aphids on PI 386148, indicated a heightened 'restlessness' that was demonstrated by a varied random-distribution of the aphids on the plants. A similar behavioral response has been reported for greenbugs, where an increased restlessness was observed on resistant hosts (Starks and Burton 1977b). Moreover, it was evident that the greatly reduced mean population level on PI 386148 was primarily due to decreased reproductive rates rather than poor nymphal survival (data not shown).

Aphid aggregations may have an adaptive role in aphid performance through a conditioned improvement of the quality of host tissues as food (Hayamizu 1984, Dorshner et al. 1987, Dorshner 1990). A positive relationship between aggregate development and aphid fecundity has been shown for aphid species which, under natural conditions, form compact aggregations (Way and Cammell 1970, Hayamizu 1984). When compared to the other plant entries, the reluctance of Russian wheat aphids to form aggregations on PI 386148 suggests poor host suitability and an inability of the aphid to alter that condition.

The percent of reduction in plant height differed significantly among the plant entries, and was greatest for 'TAM W-101' and 'Beagle 82' (Table 1). 'Okay' exhibited an intermediate response but did not differ statistically ($\underline{P} \geq 0.05$) from the susceptible entry, 'Beagle 82'. Although PI 372129 scored better than the susceptible entries, however, when compared with a noninfested check, it was substantially stunted. PI 386148 was the only plant entry that did not exhibit at least a 25% reduction in plant height, and when compared to the other plant entries, the amount of stunting was significantly less ($\underline{P} \leq 0.05$).

As expected, both susceptible plant entries, 'TAM W-101' and 'Beagle 82', had significantly higher ($\underline{P} \leq 0.05$) damage-rating scores than 'Okay' and PI 386148 (Table 1). However, the mean damage score for PI 372129 was intermediate and considerably higher than those reported from comparable tests (damage rating = 5.08 vs 2.0 to 2.8, Nkongolo et al. 1990a, Quick et al. 1991). Likewise, the mean damage rating for 'Okay' was higher than previously reported (Webster et al. 1987). Oats and PI 372129 are generally considered resistant to Russian wheat aphids and have been widely used in plant screening evaluations as resistant checks (Walters et al. 1980, Harvey and Martin 1990, Nkongolo et al. 1990a, Quick et al. 1991, Smith et al. 1991, Zemetra et al. 1990). Nonetheless, in this test, damage ratings were higher and, although leaf rolling did not occur, the plants were substantially stunted. The superior performance of these entries in resistance screening tests may reflect an antixenotic response that occurs with unrestricted infestations coincident with the presence of more preferred host plants. In this study, Russian wheat aphid performance based on population levels, clearly indicated that 'Okay' and PI 372129 were suitable hosts, having intermediate levels of resistance that were expressed primarily as tolerance. The mean damage score for PI 386148 was significantly less ($\underline{P} \leq 0.05$) than the scores of all other entries and was consistent with ratings reported from comparable studies (Nkongolo et al. 1990b, Webster et al. 1991).

Linear correlation analysis ($\underline{P} = 0.05$, Pearson product-moment correlations, SAS Institute 1986b) was used to measure the intensity of the association between damage ratings, leaf rolling scores, plant stunting scores, and aphid counts for all plant entries. The only significant relationship found among the plant rating indices was a moderate correlation between mean damage rating and plant stunting for the entry 'Okay' ($r^2 = 0.72$). Thus, the damage parameters measured by the plant rating indices appeared to occur independently and in varying sequences. Similar observations have been reported for wheat (Smith et al. 1991), triticale (Frank et al. 1989), and barley (Hordeum vulgare L.) (Webster et al. 1987). No significant correlations ($\underline{P} \geq 0.05$) between the mean number of aphids per plant and the damage indices were detected within entries, implying a damage-response threshold, beyond which, increases in the aphid population do not proportionately increase the amount of chlorotic damage.

Except for PI 386148, all Russian wheat aphid infested plants, when compared to noninfested control plants, sustained substantial reductions in all quantitative plant parameters measured (Table 2). However, because of 'Beagle 82's' limited-tillering growth pattern, there was no statistical difference ($\underline{P} \geq 0.05$) in the number of tillers between infested and control plants, despite a 52% reduction in tiller development.

Damage rating scores (Table 1) were consistent with the relative performance of the infested plants (Table 2); those entries having the lowest damage scores performed best, while entries with the highest damage scores exhibited the lowest values for the parameters measured. However, plant performance, based on mean differences between infested and noninfested plants, was much different. Mean scores for plant damage, leaf rolling, and plant stunting for 'Okay' and PI 372129 differed significantly ($\underline{P} \leq 0.05$) from those of 'TAM W-101', suggesting a resistant response to the Russian wheat aphid. In contrast to the plant rating indices, the quantitative evaluation of plant performance, based on comparisons with noninfested control plants, showed no significant differences in tiller initiation, leaf area, leaf length, or shoot dry weight between 'Okay', PI 372129, and 'TAM W-101'. Moreover, 'Beagle 82', which rated susceptible, generally outperformed 'TAM W-101' and was equal to or better than 'Okay' and PI 372129 based on the quantitative measurements, except for leaf area, where only PI 386148 differed.

Regression analysis was used to evaluate the relationships between the plant rating schemes and the measured plant parameters (Table 3). No significant relationships ($\underline{P} \geq 0.05$) between leaf rolling scores and the quantitative plant measurements were revealed by regression analysis. Damage ratings accounted for ca. 80% of the variation in shoot weight and leaf area reductions (significant, $\underline{P} \leq 0.05$), but showed no significant relationship with reductions in total leaf length, tiller development, or number of leaves. Overall, Russian wheat aphid damage, in terms of the quantitative responses measured, was best described by the plant stunting scores. Ratings for plant stunting accounted for over 90% of the variation for reductions in shoot weight, leaf area, and tiller development, and for ca. 80% of the reductions in total leaf length and numbers of leaves. These results are in agreement with Bush et al. (1989) who concluded that plant height, expressed as a percentage of a noninfested control, was a nonsubjective measure of the plant's response to Russian wheat aphids. The failure of damage ratings based on percentage chlorosis to adequately describe intermediate levels of resistance was clearly evident. Similar discrepancies have been observed by Du Toit (1989) and Webster et al. (1987), where reductions in plant height and plant biomass were not consistent with corresponding damage ratings.

A primary plant response to Russian wheat aphid feeding involves the development of drought-like symptoms caused by tissue water imbalances (Riedell 1989) which are directly related to the loss of turgor (Burd 1991). In this study, leaf water potentials for all plant entries were significantly reduced (more negative) by aphid feeding except for PI 386148 (Table 4). However, accompanying osmotic potentials were higher (less negative) or remained unchanged, indicating an inability of the infested plants to adjust osmotically to the reduced leaf water potential, and consequently, leaf turgor was reduced. Compared to noninfested control plants, turgor pressure was significantly lower for PI 372129, 'Beagle 82', and 'TAM W-101', but did not differ ($\underline{P} \geq 0.05$) for 'Okay' or PI 386148.

Plant pigment loss may be directly related to the tissue water deficits caused by Russian wheat aphids and indeed, in this study, there was a significant relationship ($\underline{P} \leq 0.05$) between mean turgor reduction and the amount of chlorosis among all plant entries tested (Fig. 1). Likewise, there also was a close relationship between the number of aphids per unit shoot mass and the amount of chlorosis (Fig. 2). Nonetheless, plant damage, in terms of reduced biomass, was more severe on some plant entries than was accounted for by damage ratings based on the percentage chlorosis.

Turgor plays an important role in leaf unfolding and leaf expansion (Eastham et al. 1984), however, the minimum turgor threshold required for leaf growth varies among different plant species (Bradford and Hsiao 1982). This was evident in this study, where 'Beagle 82' and PI 372129 sustained similar reductions in leaf turgor, yet leaf rolling occurred only on 'Beagle 82'. Consequently, the leaf rolling scores may not accurately describe the relative amount of turgor reduction.

Methods used to evaluate Russian wheat aphid resistance in mass screening programs have been largely based upon protocols developed for the greenbug (Schizaphis graminum) (Webster et al. 1987, Du Toit 1988). However, discrepancies in the expression of Russian wheat aphid damage, both in the occurrence and sequence of damage events, has lead to the augmentation of rating schemes so that distinct plant responses, principally, chlorosis, leaf rolling, and plant stunting, could be independently addressed (Webster et al. 1987, 1991, Webster 1990, Frank et al. 1989, Bush et al. 1989, Nkongolo et al. 1990a, Smith et al. 1991, Zemetra et al. 1990). I measured the degree of association between these plant responses, and the data indicate that they occur as independent damage symptoms. Analysis of the relationships among the different evaluation indices with plant growth measurements showed that the most consistent indicator of plant damage was plant stunting expressed as percentage height of a noninfested control. Although leaf rolling was not directly related to plant growth, it is an important damage criterion because it appears to be closely linked with the biological fitness of the Russian wheat aphid and may be a primary factor in limiting the aphid's host-plant range. Consequently, a more accurate account of the leaf rolling response seems necessary, especially when plants respond differently between greenhouse and field environments (Quick et al. 1991).

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TABLE 1

Summary of Mean Values (SEM) for Aphid Populations and Damage Ratings for Infested Plant Entries

Plant entry	Aphids/plant	Damage Rating	Leaf rolling	Stunting
Okay oat	353.3a (29.9)	3.25c (0.35)	1.00b (0.00)	3.41ab (0.31)
PI 386148 triticale	54.1b (21.0)	1.16d (0.16)	1.08b (0.08)	1.50c (0.19)
Beagle 82 triticale	356.6a (40.2)	5.75a (0.25)	2.91a (0.09)	4.16ab (0.27)
PI 372129 wheat	478.3a (43.5)	5.08b (0.31)	1.25b (0.13)	3.08b (0.28)
TAM W-101 wheat	394.1a (27.5)	6.41a (0.39)	2.75a (0.16)	4.33a (0.18)

Means in a column followed by different letters are significantly different ($P < 0.05$; Tukey's HSD test).

TABLE 2

Summary of Mean Values (SEM) for Measured Plant Parameters for Infested and Noninfested Plant Entries

Measurement	Okay oat	PI 386148 triticale	Beagle 82 triticale	PI 372129 wheat	TAM W-101 wheat
Number of tillers					
control	2.16* (0.16)	1.58 (0.14)	0.33 (0.18)	2.00* (0.00)	2.08* (0.08)
infested	1.50 (0.15)	1.41 (0.22)	0.16 (0.11)	0.91 (0.25)	0.75 (0.13)
mean difference	0.66a (0.22)	0.17b (0.24)	0.17b (0.16)	1.09a (0.25)	1.33a (0.14)
Number of leaves					
control	9.16* (0.38)	8.08 (0.52)	4.41* (0.19)	8.08* (0.08)	9.41* (0.31)
infested	6.58 (0.37)	7.66 (0.43)	3.08 (0.08)	5.41 (0.51)	4.66 (0.30)
mean difference	2.58b (0.49)	0.42d (0.66)	1.33c (0.14)	2.67b (0.52)	4.75a (0.39)
Total leaf area (cm^2)					
control	58.61* (1.42)	43.26 (2.88)	47.54* (2.73)	53.21* (4.58)	46.07* (1.50)
infested	25.68 (2.10)	35.97 (2.75)	20.15 (3.32)	24.28 (2.99)	14.34 (0.89)
mean difference	32.93a (2.25)	7.29b (3.41)	27.39a (4.03)	28.93a (5.71)	31.73a (1.68)
Total leaf length (cm)					
control	119.45* (4.63)	96.28 (6.66)	91.38* (4.01)	117.23* (9.98)	114.07* (3.29)
infested	59.75 (4.80)	92.07 (7.23)	54.60 (7.83)	66.60 (8.87)	42.57 (3.61)
mean difference	59.70ab (5.55)	4.21c (4.72)	36.78b (7.67)	50.63ab (13.18)	71.50a (4.19)
Shoot dry weight (g)					
control	0.186* (0.011)	0.101 (0.006)	0.104* (0.009)	0.165* (0.013)	0.150* (0.007)
infested	0.076 (0.008)	0.089 (0.015)	0.041 (0.004)	0.074 (0.006)	0.042 (0.007)
mean difference	0.110a (0.014)	0.012c (0.017)	0.063b (0.010)	0.091ab (0.018)	0.108a (0.014)

Means in a row followed by different letters are significantly different ($P < 0.05$, Tukey's HSD test).

*, Significant at the 0.05 probability level (Student's *t* test)

TABLE 3

Coefficients of Determination (r^2) and Change in Mean Percent Increase in Plant Component Reduction (Slope) for Incremental Increases in Plant Evaluation Method

Evaluation method	Shoot weight reduction		Leaf area reduction		Leaf length reduction		Tiller reduction		Leaf reduction	
	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
Damage rating	9.71	0.798*	8.39	0.816*	8.46	0.672	9.92	0.737	6.91	0.703
Plant stunting	19.69	0.925*	16.79	0.920*	17.43	0.802*	16.27	0.903*	12.68	0.837*
Leaf rolling	14.05	0.332	12.15	0.339	10.60	0.209	15.56	0.475	10.27	0.366

*, r^2 value significant at the 0.05 probability level.

TABLE 4

Mean Values (MPa + SEM) for Water Potential, Osmotic Potential, and Turgor Pressure for Infested and Noninfested Plant Entries

	Water potential		Osmotic potential		Turgor pressure		
Plant entry	Control	Infested	Control	Infested	Control	Infested	mean difference
Okay oat	-0.258* (0.028)	-0.407 (0.052)	-1.032 (0.039)	-1.066 (0.057)	0.773 (0.033)	0.659 (0.63)	0.114c (0.046)
PI 386148 triticale	-0.215 (0.038)	-0.320 (0.046)	-1.120 (0.037)	-1.124 (0.041)	0.905 (0.051)	0.803 (0.55)	0.102c (0.034)
Beagle 82 triticale	-0.308* (0.043)	-0.604 (1.032)	-0.996 (0.048)	-0.897 (0.128)	0.688* (0.058)	0.293 (0.56)	0.395b (0.075)
PI 372129 wheat	-0.227* (0.048)	-0.491 (1.000)	-1.134 (0.061)	-1.052 (0.096)	0.907* (0.056)	0.561 (0.45)	0.346b (0.058)
TAM W-101 wheat	-0.184* (0.035)	-0.509 (0.058)	-1.044* (0.035)	-0.741 (0.062)	0.859* (0.028)	0.231 (0.55)	0.628a (0.065)

Means followed by a different letter are significantly different ($P < 0.05$, Tukey's HSD test).
*, significant at the 0.05 probability level (Student's t test).

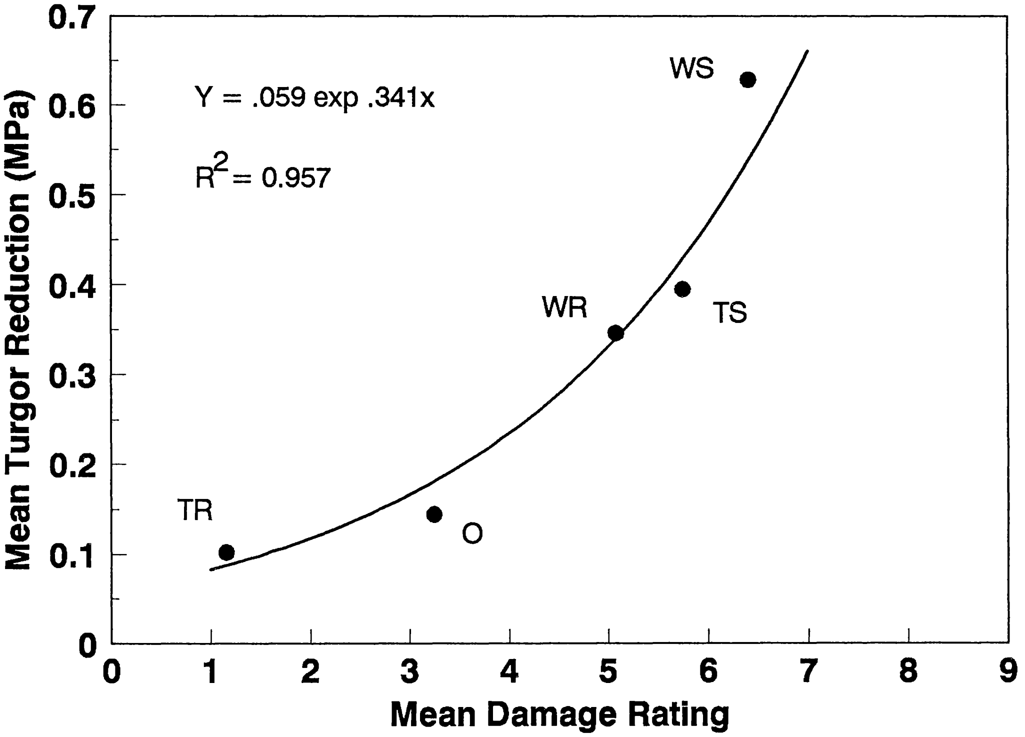

Figure 1. Relationship between mean turgor reduction and mean damage rating for Russian wheat aphid infested plant entries (O, 'Okay', oat; TR, PI 386148, triticale; TS, 'Beagle 82', triticale; WR, PI 327129, wheat; WS, 'TAM W-101', wheat).

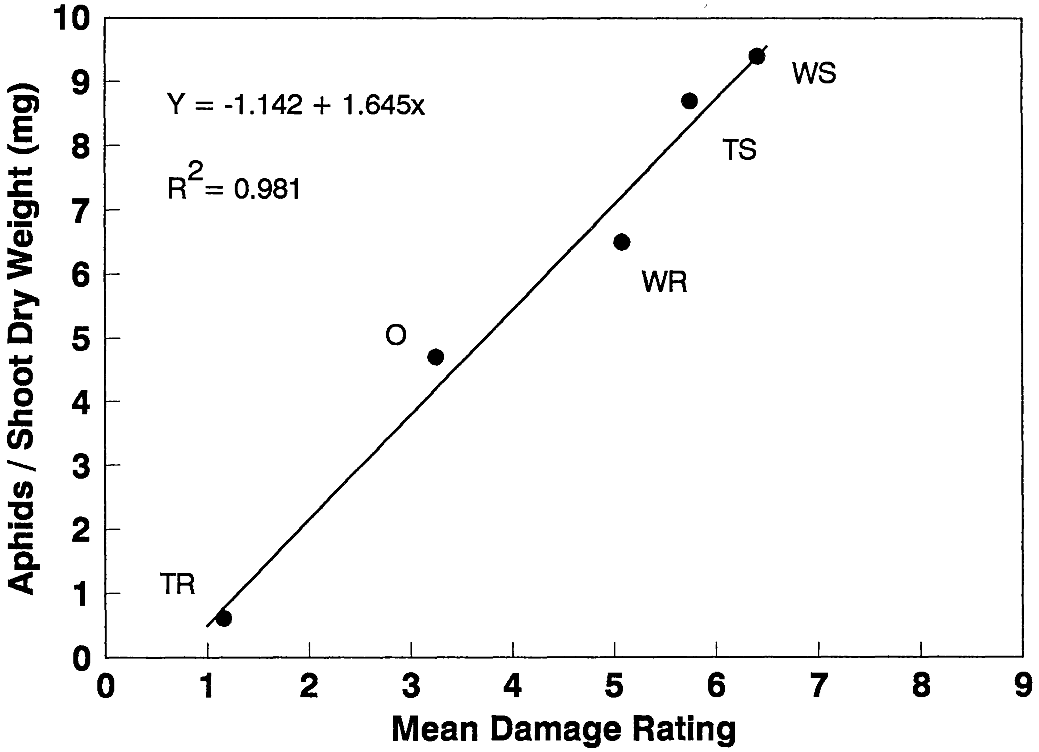

Figure 2. Relationship between mean number of aphids per unit shoot mass and mean damage rating for Russian wheat aphid infested plant entries (O, 'Okay', oat; TR, PI 386148, triticale; TS, 'Beagle 82', triticale; WR, PI 327129, wheat; WS, 'TAM W-101', wheat).

PART III

INHIBITION OF ^{14}C MOVEMENT CAUSED BY GREENBUG (HOMOPTERA: APHIDIDAE)

ABSTRACT

IAA-1-^{14}C and ^{14}C-sucrose labels were used to study the effects of greenbug, Schizaphis graminum (Rondani), feeding on phloem transport in wheat seedlings. Greenbug feeding significantly reduced ^{14}C translocation from the immediate feeding site, although, phloem integrity was not impeded. Similar results were obtained when resistant and susceptible wheats were infested with three different greenbug biotypes. Greenbugs fed artificial diets containing ^{14}C-sucrose injected detectable levels of salivary material that was translocated to both root and shoot systems.

INTRODUCTION

Greenbug, Schizaphis graminum (Rondani), is an important pest of wheat in the United States. Economic infestations occur annually and are primarily controlled by insecticides. Reliance on chemicals to control insect pests has lead to environmental concerns which have stimulated research focusing on alternate methods of insect control. One alternative approach to greenbug management has been the development and use of resistant crops. However, the occurrence of new greenbug biotypes has been a major obstacle to the deployment of resistant wheat cultivars. Therefore, it is important that a fundamental understanding of the mechanisms of greenbug damage be established to facilitate new approaches for evaluating resistant plant sources.

Early cytological work by Chatters and Schlehuber (1951), focusing on greenbug damage at the feeding site, maintained that it is the injection of toxic saliva and not the uptake of food that is the primary cause of damage, and therefore, that greenbug resistance was physiological. This hypothesis agrees with ultrastructural studies of Saxena and Chada (1971) and Al-Mousawi et al. (1983) who attributed greenbug resistance to biochemical and physiological factors. Al-Mousawi et al. (1983) indicated that the visible damage (chlorosis/necrosis) at the feeding site is biochemically associated with the feeding track of the aphid. A biochemical basis of the feeding-site damage was suggested in a review article by Dreyer and Campbell (1987), where a model was presented in which salivary pectinases played the key role in the damage response.

Other greenbug-induced physiological changes in wheat (Triticum aestivum L.) have been reported. Ryan et al. (1987) found significant reductions in total chlorophyll, carbon assimilation rates, transpiration rates, and stomatal conductance in a susceptible wheat cultivar. Gerloff and Ortman (1971) reported similar results for greenbug susceptible barley (Hordeum vulgare L.). Greenbug-caused physiological damage to wheat was described by Dorschner et al. (1986), where greenbug feeding disrupted the drought stress acclimation process. Dorschner et al. (1987) showed that significantly increased levels of free amino acids in greenbug-damaged susceptible wheat were closely correlated with the greenbug's ability to cause senescence-like damage at the feeding site.

Greenbugs feed from the phloem (Campbell et al. 1982), yet little is known of how the greenbug seemingly exploits this tissue. The objectives of this study were to determine the effects of greenbug feeding on phloem function and to evaluate the movement and accumulation within the plant of greenbug-injected salivary compounds.

MATERIALS AND METHODS

Experiment I

Pre-germinated 'TAM W-101' winter wheat was planted in cone-tainers (Supercell Cone-Tainer, Ray Leach Cone-Tainer Nursery, Canby, Oreg.), with one seed per container in a fritted clay medium (Absorb-N-Dry, Balcones Minerals, Flatonia, Tex.) (Burton 1986). Plants were grown in environmental chambers (Sherer Model CE 38-15HLE, Rheem Manufacturing Company, Asheville, N. C.) at 21°C, 70 ± 10% relative humidity, and a 16 h photophase. Plants were watered daily and beginning 7 d after emergence were fertilized biweekly with Peters' Peat-Lite Special (analysis 15-16-17) (Peters Fertilizer Products, Fogelsville, Pa.), a water-soluble fertilizer. Fourteen days after planting, at growth stage 13 (Zadoks et al. 1974), 10 mature apterous biotype E greenbugs from greenhouse colonies, reared on barley (Hordeum vulgare L. cv Wintermalt), were confined in a ventilated transparent plastic cage (3 cm X 3 cm X 1 cm) on aphid-treated plants (cf. Pathak et al. 1958), 10 cm below the apical tip of the second leaf. Noninfested control plants were also caged at the same location and all cages were supported by wood blocks to maintain the leaves at their natural position. The greenbugs were allowed to feed and reproduce for 7 d, after which the cages and aphids were removed.

In two separate tests, IAA-1-^{14}C (57 mCi/mmol) and ^{14}C-sucrose (560 mCi/mmol) (Amersham Corporation, Arlington Heights, Ill.) were used to assess the impact of greenbug feeding on translocation. Label preparations were as follows; for the IAA-1-^{14}C test, 140 ul of IAA-1-^{14}C was dried and made to 320 ul by adding 50% EtOH + 0.4% Triton X-100 (Sigma Chemical Company, St. Louis, Mo.), and for the ^{14}C-sucrose test, 20 ul of ^{14}C-sucrose was added to distilled water + 0.5% Triton X-100 to make 0.3 ml.

Labelling started 4 h after the onset of the photophase, and was done by placing four 2-ul droplets of the appropriate label on the adaxial surface of the previously caged 3-cm leaf section.

Plants were harvested 4- and 8 h following application of the IAA-1-^{14}C and ^{14}C-sucrose, respectively. Test plants were partitioned into four components, the apical tip of the treated leaf, the labeled 3-cm leaf section, the remainder of the shoot, and the roots, and each plant part was monitored for radioactivity.

The treated leaf sections were washed for 20 s in 50% EtOH. The partitioned samples were then lyophilized, ground in 5 ml of 100% EtOH, and 1 ml from each sample was counted in 15 ml of Complete Liquid Counting Cocktail (Research Products International, Mount Prospect, Ill.) using a

Beckman LS-100 liquid scintillation system (Beckman Instruments, Inc., Fullerton, Ca.).

The experimental protocol for the test followed a paired-plot design where n = 20; controls, n = 10, and infested, n = 10.

To assess the impact of greenbug feeding on phloem integrity, aphids were allowed to feed and reproduce for 7 d on the caged leaf sections. Four hours after the onset of the photophase, 8 ul of the ^{14}C-sucrose label was applied to the apical portion of the caged leaf. Plants were harvested 4 h after labelling and were partitioned into treated leaf, shoot, and root components, and measured for radioactivity as described above. In addition to the plant assays, the greenbugs were removed from the infested plants and measured for radioactivity.

The experimental protocol followed a split-plot design where n = 20; controls, n = 10, and infested, n = 10.

Experiment II

Three biotypes of the greenbug, biotype B (GBB), C (GBC), and E (GBE), and the Russian wheat aphid, Diuraphis noxia (Mordvilko), were evaluated in combination with resistant and susceptible wheat entries for their impact on phloem translocation. The plant entries tested were: 'TAM W-101', susceptible to GBB (Webster et al. 1986), GBC (Burton et al. 1985), GBE (Burton 1986) and the Russian wheat aphid (Webster 1990); 'Amigo', resistant to GBB and GBC but susceptible to GBE (Tyler et al. 1987) and Russian wheat aphids (Bush et al. 1989); and 'Largo', resistant to GBC and GBE but susceptible to GBB (Tyler et al. 1987) and Russian wheat aphids (Bush et al. 1989).

The plants were grown on greenhouse benches under natural light conditions (December - January) and greenhouse temperatures were maintained at 21 ± 5°C, otherwise, the materials and methods used were identical to those in experiment I. Ten plants of each entry (growth stage 13, Zadoks et al. 1974) were infested with 10 aphids of one of the four aphid treatments, and 10 noninfested control plants were included per entry. The aphids were caged 10 cm from the apical tip of the second fully expanded leaf for 7 d, after which they were removed and counted. Next, 8 ul of the ^{14}C-sucrose label was applied to the previously caged leaf section. Plants were harvested 4 h after labelling and were partitioned into treated leaf, shoot, and root components, and measured for radioactivity as described above.

The experimental protocol followed a randomized complete block design, where n = 50, treatments = 5, and blocks = 10, for each plant entry tested.

Experiment III

To evaluate the movement of salivary materials, greenbugs were fed for 72 h on an artificial diet labelled with ^{14}C-sucrose. The artificial diet consisted of a 35% sucrose solution, with pH adjustment to 7.6 (Cress and Chada 1971) by adding 0.001M KOH, combined with 600 ul of ^{14}C-sucrose to make 10 ml of diet. The artificial diet was presented to the aphids in sachets made by sandwiching the diet solution within a stretched parafilm envelope (see Mittler and Dadd 1964).

The greenbugs were then placed on a non-test plant ('TAM W-101') for 24 h to allow the aphids time to clear the artificial diet from their stylets and gut. Next, the aphids were transferred from the non-test plants to 'TAM W-101' wheat seedlings (Growth stage 13, Zadoks et al. 1974) for evaluation. Fifteen greenbugs were caged on each plant 10 cm from the apical tip of second fully expanded leaf. The test plants (n = 24) were grown under the same environmental conditions as described for experiment I. The greenbugs were allowed to feed and reproduce on the plants for 7 d, after which they were removed, and the plants were harvested and measured for radioactivity as described above. The plant parts measured were, the leaf above the feeding site, the feeding site, the leaf below the feeding site, the remainder of the shoot, and the roots.

Statistical Analysis

The amount of radioactivity within each plant part was expressed as a percentage of total radioactivity recovered (excluding the labelled leaf wash). Data analysis and computations were done with Statistical Analysis Systems (SAS Institute 1988). The TTEST procedure was used for all statistical tests in experiment I. Data from experiment II were analyzed using the ANOVA procedure, and when appropriate, means were separated using Tukey's studentized range test ($\underline{P} \leq 0.05$, SAS Institute 1988).

RESULTS AND DISCUSSION

Greenbug feeding significantly reduced the amount of ^{14}C exported to both the root and shoot from mature leaves (Table 1). The phloem translocation of exogenous IAA after application to mature tissue has been shown to occur (see Ziegler 1975), and in this study, exported ^{14}C recovered from IAA-1-^{14}C treated leaf sections accounted for ca. 27% of the total percentage of label recovered in noninfested plants compared to less than 3% for those infested with aphids. Translocation of sucrose, which is the principal sugar translocated in the phloem (Geiger 1975), was significantly reduced. Following ^{14}C-sucrose application, the ^{14}C exported from the leaf sections of noninfested plants accounted for ca. 70% of the total radioactivity recovered compared to ca. 36% for infested plants.

Phloem blockage caused by aphids has been reported by Wood et al. (1985) and potentially could account for the observed decrease in ^{14}C translocation. However, phloem translocation of ^{14}C was not impeded by greenbugs that were caged downstream from the labelling site, and the pattern of ^{14}C movement to the root and shoot was not altered (Table 2).

Both IAA and sucrose are actively loaded into the phloem of mature leaves (Bandurski and Nonhebel 1984, Giaquinta 1983). Electrogenic proton pumps, probably membrane-bound ATPase complexes, serve as the active vein loading system by creating a pH generated transmembrane electrochemical gradient that is coupled to a carrier-mediated cotransport system (Marschner 1986, Spanswick 1981). Because IAA and sucrose do not share the same protein carrier (Kursanov 1984), one possible explanation for the reduction of ^{14}C movement caused by greenbugs, may be a localized inactivation of the electrogenic pump system. Moreover, phloem loading of amino acids, which is also coupled to the 'proton-motive' force arising from these electrogenic pumps (Reinhold and Kaplan 1984), would be similarly affected, and the efflux of amino acids from the greenbug feeding site should be reduced. Evidence for a greenbug induced inhibition of amino acid efflux was reported by Dorschner et al. (1987) who observed that greenbugs caused the amount of free amino acids in wheat leaves to significantly increase at the infestation sites.

In a subsequent experiment, the effect of different greenbug biotypes and the Russian wheat aphid on ^{14}C movement in resistant and susceptible wheats was investigated. Aphid population growth on the different wheat entries is shown in Fig. 1A. Compared to the greenbug biotypes, the mean number of Russian wheat aphids was generally lower, and may be attributable to an inherently lower reproductive rate (Webster and Starks 1987). Population means for GBB were significantly lower than those of GBC and GBE on 'TAM W-101' (GBB susceptible) and 'Amigo' (GBB resistant), nonetheless, GBB caused a substantial amount of visible damage to 'TAM W-101'.

On the susceptible wheat entries, virulent greenbug biotypes induced a characteristic phytotoxic response, that initially appeared as small necrotic lesions (< 1 mm diameter) surrounded by chlorotic halos (Al-Mousawi et al. 1983, Puterka and Peters 1988). As the greenbug populations increased, the chlorotic halos coalesced, and on some plants, the entire caged section became chlorotic. However, the chlorosis was restricted to the feeding site and never extended beyond the boundary of the cages. Greenbug resistant wheats did not exhibit pronounced visible symptoms, necrotic lesions did not occur, and visible damage was limited to an occasional chlorotic spot (< 1 mm diameter). Damage caused by Russian wheat aphids visibly differs from that of greenbugs and is typified by the development of longitudinal white streaks on infested leaves (Walters et al. 1980). In this study, Russian wheat aphids caused considerable chlorosis on all wheat entries tested, however, no leaf streaking nor necrosis was observed.

Based on the percentage of translocated ^{14}C recovered, all greenbug biotypes significantly decreased ^{14}C movement in all wheat entries tested when compared to a noninfested control, and the level of reduction did not differ significantly among the different plant entries (Fig. 1B). The fact that ^{14}C movement was inhibited on both resistant and susceptible entries, irrespective of the greenbug biotype, suggests that this plant response occurs independent of the visible damage symptoms. In contrast, Russian wheat aphids had much less of an impact on ^{14}C movement, and though generally reduced, the percentage of ^{14}C translocated did not differ significantly from noninfested controls.

Aphids can alter host tissues and therefore nutrient availability in the immediate vicinity of the feeding site (Way and Cammell 1970, Dixon and Wratten 1971). The ability of greenbugs to cause senescent-like damage at the feeding site has been associated with substantial increases of free amino acids which enhance the diet quality of the plant and in turn results in an increased aphid fitness (Dorschner et al. 1987). Results from these studies indicate that greenbugs may significantly reduce the rate of phloem loading, which could further benefit the aphid by the retention and accumulation of essential amino acids at the feeding site.

It is generally thought that plant damage caused by greenbugs results from a toxin-like substance that is injected into the plant during feeding (Chatters and Schlehuber 1951). Greenbugs that were radiolabelled with ^{14}C-sucrose were used to identify the presence of salivary materials in the host tissues. The results showed that greenbug saliva was injected into the plant and was translocated to both root and shoot tissues (Table 3). The majority of the injected material, ca. 61%, was found in the infested leaf, while ca. 30% was recovered from the roots.

Although greenbugs cause substantial damage to the infested leaves of the plant, significant damage, in terms of biomass reduction, also occurs in root systems (Ortman and Painter 1960, Daniels 1965, Burton 1986). Moreover, Holmes et al. (1991) reported that the damage to roots caused by greenbugs is not a direct result of depleted photosynthate pools. Potentially, the greenbug salivary materials that are translocated to the roots may induce a phytotoxic response, which results in root damage.

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TABLE 1

Mean (± SEM) Percentage of Total ^{14}C Exported from Labelled Leaf Sections for Infested and Noninfested TAM W-101 Wheat

	Percentage Recovery of Applied Label	
Plant Part	^{14}C-IAA	^{14}C-Sucrose
Apical Tip		
Infested	1.0 (0.7)	1.1 (0.2)
Control	0.9 (0.2)	1.2 (0.2)
Treated Section		
Infested	96.2* (12.4)	62.5* (9.6)
Control	71.9 (10.1)	28.4 (7.8)
Shoot		
Infested	1.3* (0.3)	3.8* (0.9)
Control	17.8 (3.2)	10.9 (1.4)
Root		
Infested	1.3* (0.4)	32.6* (5.3)
Control	9.4 (0.9)	59.5 (11.8)

*, Significant at the 0.05 probability level.

TABLE 2

Mean (± SEM) Percentage of Total ^{14}C Exported from Infested and Noninfested Leaves of TAM W-101 Wheat

	Percentage Recovery of Applied Label	
Plant Part	Infested	Control
Treated Leaf	76.6 (15.1)	81.7 (10.9)
Shoot	3.9 (0.6)	2.9 (0.8)
Root	13.3 (7.3)	15.3 (4.1)
GBE[a]	6.2 (1.8)	

[a], Greenbug biotype E.

TABLE 3

Mean (± SEM) Percentage of Total ^{14}C Recovered from TAM W-101 Wheat Following Infestation with Radiolabelled Aphids

Plant Part	Percentage Recovery of Label
Infested Leaf	
Feeding Site	42.9 (9.1)
Leaf Tip	4.8 (1.3)
Leaf Base	13.1 (2.4)
Shoot	9.4 (3.7)
Root	29.8 (7.2)

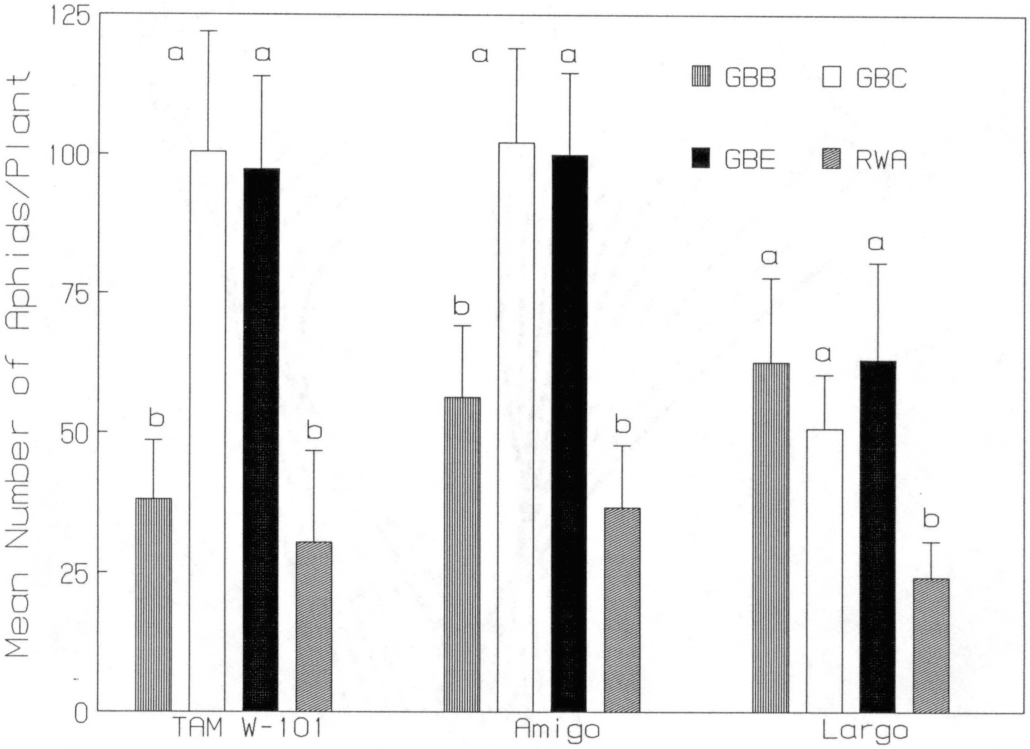

Figure 1. Mean number of aphids per plant (GBB, biotype B; GBC, biotype C; GBE, biotype E; RWA, Russian wheat aphid). Vertical lines at tops of bars represent the SEM. For each wheat entry, bars with different letters are significantly different ($\underline{P} \leq 0.05$; Tukey's studentized range test [SAS Institute 1988]).

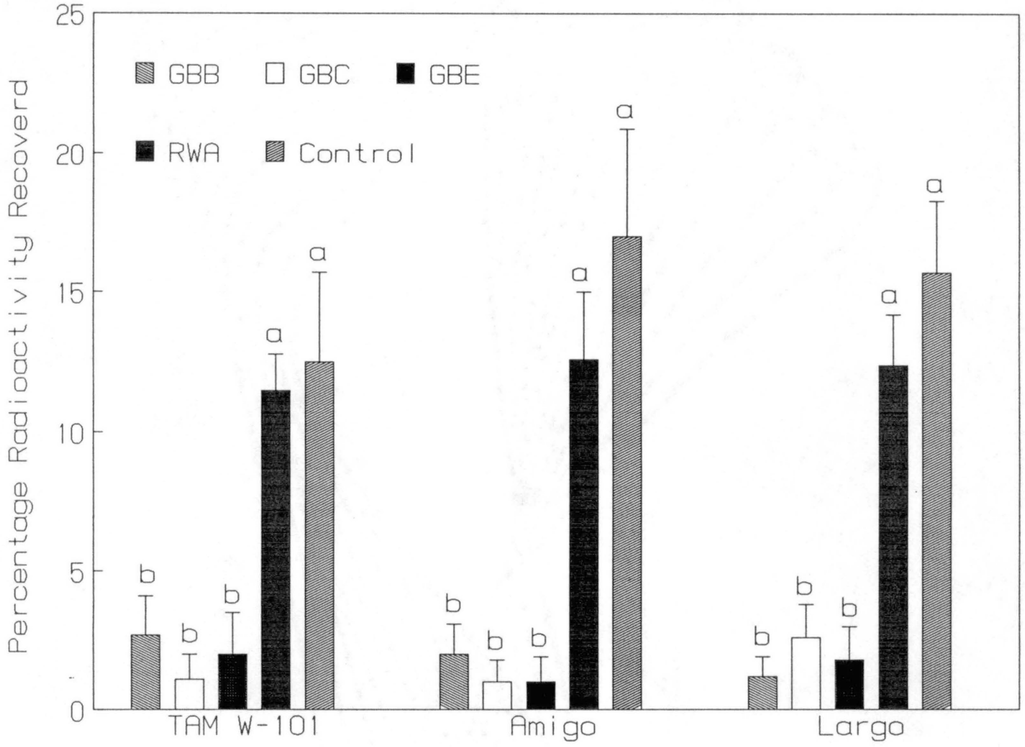

Figure 2. Mean percentage of translocated radioactivity recovered in resistant and susceptible wheats (GBB, biotype B; GBC, biotype C; GBE, biotype E; RWA, Russian wheat aphid). Vertical lines at tops of bars represent the SEM. For each wheat entry, bars with different letters are significantly different ($\underline{P} \leq 0.05$; Tukey's studentized range test [SAS Institute 1988]).

VITA

John Daniel Burd

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES OF PHYSIOLOGICAL ALTERATIONS IN CEREALS INDUCED BY GREENBUG, *SCHIZAPHIS GRAMINUM* (RONDANI) AND RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA* MORDVILKO

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