RAPID PHENOTYPIC ASSESSMENT OF BIRD CHERRY-OAT APHID TOLERANCE IN WINTER WHEAT

By

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WHEAT

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

NOMENCLATURE

BCO . . . . . .bird cherry-oat

ELISA . . . . .enzyme linked immunosorbent assay

CHAPTER I

INTRODUCTION

The bird cherry-oat (BCO) aphid (*Rhopalosiphum padi*) reduces root and/or shoot growth in wheat (Riedell et al., 2003) and causes significant loss of grain yield (Pike and Schaffner, 1985; McPherson et al., 1986; Riedell et al., 1999) without obvious aboveground visual symptoms of injury. Exposure to the aphid, and thus detrimental affects associated with it, may be exasperated when winter wheat is planted early as a dual-purpose (graze-plus-grain) crop (Royer et al., 2004). As it is often the dominant cereal aphid in winter wheat during the fall, BCO aphid populations may persist until the soil surface freezes (Kieckhefer and Gustin, 1967). Direct injury from BCO aphid feeding can be attributed to depletion of phloem nutrients or to toxin injection (Hsu, 1963). Bird cherry-oat aphid feeding also may affect winter wheat cold hardiness by depleting fructans in crown tissue (Wellso et al., 1985). Feeding can occur throughout plant development from seedling to tillering stages. Indirect injury can be even more devastating if BCO aphids vector barley yellow dwarf virus (BYDV) (Riedell et al., 1999).

Mullins (1993) found that using imidacloprid, which exhibits both a systemic and contact insecticidal function, could be used in BCO aphid management strategies. Others have found antibiosis and tolerance to *R. padi* in some wheat accessions (Kazemi and van Emden, 1992; Papp and Mesterhazy, 1993, 1996; Lamb and MacKay, 1995). Given that genetic variation for BCO aphid tolerance exists within common wheat, a protocol should

be developed to allow discrimination among experimental lines typically evaluated in breeding program. Critical to this protocol is the capability to observe chronic effects of BCO aphid feeding on root and shoot growth, in contrast to bioassays for other aphids with acute effects and qualitative segregation of genotypic effects (Starks and Burton, 1977).

Baker et al. (2002) suggested that transparent seedling growth pouches could be used to observe shoot and root growth differences between BCO aphid-infested and non-infested treatments. Our primary objective was to optimize their procedure to develop a rapid, juvenile-plant bioassay for BCO aphid tolerance and a quantitative barometer of genotypic response. A secondary objective was to use this bioassay to identify sources of tolerance among divergent collections of winter wheat germplasm.

CHAPTER I I

LITERATURE REVIEW

Oklahoma, one of the largest wheat growing states in the nation, usually ranks in the top three for hard red winter wheat production. Farmers in Oklahoma and surrounding states including Kansas, New Mexico, Colorado, and Texas have management choices when it comes to wheat production. These include strictly grain only, forage-only, and grazing plus grain (Epplin et al., 2000). Of the more than six million acres planted in winter wheat in Oklahoma during 2003 (National Agricultural Statistics Service, 2003), of which approximately 31% was used for grain only, 20% for forage-only, and 49% for dual purpose (Hossain et al., 2004). Planting winter wheat for forage-only and grazing plus grain typically occurs four to eight weeks before planting winter wheat for grain only. The earlier planting provides ample fall and winter forage for grazing, but also increases the chances of crop damage due to pests such as *Rhopalosiphum padi* L., commonly known as the bird cherry-oat (BCO) aphid.

The BCO aphid has a soft, olive-green, pear-shaped body and can be winged or wingless. It varies from 1.3 to 2.6 millimeters in length with long antennae, dark colored legs, a reddish-orange area near the abdomen, and long dark tube-shaped cornicles, all of which distinguish this aphid from other aphid species. The BCO aphid reproduces both asexually (where adults over-winter on volunteer wheat or native grasses) and sexually with eggs typically laid on the bird-cherry tree, *Prunus padus*, in October before frost

(Delmotte et al., 2001). The eggs, which are highly resistant to freezing temperatures, overwinter until late April when nymphs emerge. Adult females are able to reproduce approximately 13 days after hatching, produce an average of 50 to 60 living young. This pattern continues throughout the summer.

Upon emergence the BCO aphid feeds on all parts of the wheat plant, including leaves, stems, and in some cases just below the soil surface near the roots. Growing shoots and leaves are a rich source of food for aphids, which use their stylet to feed from the phloem sap containing sugar, amino acids, plant hormones, mineral ions, and organic acids (Garsed and Galley, 1987). The aphid excretes a sticky waste called honey dew. During the feeding process, BCO aphids, along with 20 other aphids, are capable of vectoring barley yellow dwarf virus (BYDV) (Voegtlin and Halbert, 1995), a luteovirus which is transferred through regurgitation of the virus and nutrients between BCO aphid salivary glands and phloem tissue. A BCO aphid can acquire BYDV-rpv and BYDV-pav strains of BYDV in less than 1 hours of feeding. This virus has been considered the most widespread and economically important virus of cereal crops in the world; it may also be the most widely studied (Irwin and Thresh 1990).

Effects from nutrient loss can be observed in kernel weight and grain yield reductions if aphid numbers are sufficiently large (Papp and Mesterhazy, 1993, 1996; Riedell et al., 1999). Degree of aphid pressure depends greatly on environmental conditions (rain fall, wind, and temperature) and upon natural enemies (ladybird beetle adult, ladybird beetle larvae, syrphid fly larvae, aphid lion, and various parasitic wasps),

any of which can greatly reduce the possibility of an economically important outbreak. As air temperature increases, aphid populations migrate to numerous species of Gramineae including cereals (e.g. wheat, barely, oats, and maize) and pasture grasses, where numbers can reach over 3,000 aphids per row-foot. Parasitoids and other insects can suppress early-season populations of aphids, which would otherwise feed on wheat during the critical seedling stage (Kieckhefer and Kantack, 1980). Economic thresholds of 12 to 15 aphids per plant from seedling to boot stage have been established (Kieckhefer and Gellner, 1992). Many researchers in the USA use aphid days (number of aphids x number of infestation day) to establish these thresholds, and in Oklahoma, as few as 200 aphid-days can reduce yield by 5% (Royer, 2003). In European countries, thresholds are expressed both as percent tillers with aphids and aphids per tiller at a given growth stage, but these thresholds have less predictive value in later growth stages due to greater tolerance (Voss, et. al., 1997).

The BCO aphid and BYDV are becoming more widely recognized by farmers, prompting them to address the problem with chemical or biological treatments. Currently, insecticides and natural predators are the only plausible means for control. Some natural enemies of the BCO aphid are *Coccinella septempunctata* L. and *Aphidoletes aphidimyza* (predators), and *Aphidius rhopalosiphi* de Stefani Perez and *Lysiphlebus testaceipes* (parasitoids). Parasitoid activity can be chacterized by the presence of "mummies" (swollen on leaves), which are copper or tan colored aphid skeletons. Chemical treatments of carbofuran and disulfoton have been used as

insecticides to reduce aphid numbers (Araya and Foster 1987). Imidacloprid may be an economically feasible means to control aphid populations either as a foliar treatment or a seed treatment (Mullins, 1993; Royer, 2004). Winter wheat may recover from BCO aphid damage if aphids are absent for a period of time before tillering stage (Riedell, 2003).

Due to chemical and application costs, the best possibility for long-term control may come from resistant varieties of wheat. The lack of resistant varieties to the BCO aphid can be attributed to the complexity of establishing resistance. Detection of BYDV can be achieved by the use of an enzyme-linked immunosorbant assay (ELISA) and through observations due to yellowing of plant tissue. However, determining resistance to BCO aphid feeding cannot be accomplished with observations alone, because symptoms are not as easily observed as they are with other aphid species, such as the Russian wheat aphid (*Diuraphis noxia*) that causes yellow, purple, or reddish-purple longitudinal streaks on leaves and stems (Walters et al., 1980). These seemingly transparent symptoms seen from BCO aphid feeding may be attributed to the use of different oxido-reductases as salivary enzymes (catalyses verses peroxidase) in the Russian wheat aphid and BCO aphid, respectively (Xinzhi et. al., 2000).

A reliable screening tool for use in breeding programs is lacking even though early attempts were made to actively screen for BCO aphid resistance in cereals 40 years ago (Hsu & Robinson 1962, 1963). A reliable screening tool must discriminate for the presence or absence of defense mechanisms that plants use to discourage feeding. These

mechanisms include: (1) nonpreference (characters that make a plant undesirable), which was observed in wheat with reduced infestation level by invoking alterations in BCO probing or feeding behavior (Ullman et al., 1988); (2) antibiosis (adverse effects on development and reproduction), which has also been observed in wheat to cause low BCO aphid birth rates and/or high nymphal mortality (Wiktelius and Pettersson, 1986); and (3) tolerance or resistance (plant vigor unchanged), which has been observed in the hexaploid wheat accession MV4 (Hesler et al., 1999) and in the cultivar 'Halt' both of which have shown tolerance in shoot tissue.

Riedell (1995) indicated that BCO aphid feeding damage can have significant effects on root growth, suggesting both roots and shoots may play a key role as a possible means of characterizing plant tolerance. Others have looked at reactions to the aphid and disease together, finding a parallel between resistance to BYDV and to the BCO aphid in the form of antibiosis in perennial Gramineae (*Agropyron repens* and *Elymus angustus*) (Tremblay 1989). Molecular studies in barley (*Hordeum vulgare* subsp. spontaneum) indicated that resistance could occur through additive effects from several independent genes (Weibull, 1994). A bioassay capable of allowing visual assessment of damage from both viruliferous and aviruliferous BCO aphids may provide the best assessment of tolerance in wheat. Therefore more research in the area of BCO physiology, overall feeding effects, and resistance in wheat should occur in this insect pest.

CHAPTER I I I

MATERIALS AND METHODS

Two series of experiments were conducted in controlled-environment chambers to optimize the bioassay using a pair of genotypes with putative differences in BCO aphid tolerance and to verify the utility of the bioassay using four collections of germplasms with unknown responses. Certain procedural components were common to all experiments. Aphid colonies were maintained on 'Jagger' wheat and were confirmed by ELISA to be nonviruliferous for BYDV. Seeds were germinated and seedlings were grown in seed germination pouches (Mega International, Minneapolis, MN). Five uniform-size kernels were placed crease down in each pouch to allow pouch hydration. Two 7-mm diam holes were punched in the bottom of each pouch. Ten pouches were placed in a rack that was immersed in a 5.4-L tub containing 2 L tap water and 0.4 mL azoxystrobin (fungicide), plus 0.2 mL imidacloprid if the designated rack of pouches was assigned a non-infested treatment (control). The water level was allowed to rise approximately 4 cm above the bottom of the pouches. Preliminary tests showed that imidacloprid did not affect plant growth per se. To minimize border effect, each set of ten pouches was surrounded on each end with a border pouch treated similarly but not used in subsequent measurements. The tubs were placed in growth chambers (interior area of 1.4 m^2 , 185 PPFD) at 19 or 21 C, depending on the experiment. One week after initiation of germination, infestated Jagger leaves containing 40 to 60 aphids per leaf

were placed over the pouches for a target infestation level of 10 to 20 aphids per seedling. Aphids migrated from the dying Jagger leaf onto the test plants and began feeding. Root and shoot dry wt (48 h at 65 C in a dryer) were determined after removing all aphids.

The following four experiments were conducted to determine optimal conditions relative to duration of aphid exposure (Exp. 1), growth temperature (Exp. 2), positioning of germination pouches in the racks (Exp. 3), and light exposure during germination (Exp. 4). Response variables for all experiments were root and shoot dry weight of viable plants, reported as weight per plant. Preliminary research (Baker et al., 2002) showed that 'Illinois Rustproof' (tolerant) and 'Patrick' (susceptible) responded differently to BCO aphid feeding, and thus these genotypes were used as reference genotypes in all experiments. Each genotype was assigned to separate pouches, arranged as five pairs per rack. For Exp. 1, aphid infestation periods of 10, 12, 14, 16, and 18 d were compared at 19 C. For Exp. 2, root and shoot dry weight were determined at 19 and at 21 C using a 14-d aphid exposure period. In Exp. 3, the pouches were paired and arranged in facing (plants oriented toward each other) or reverse position during a 14-d aphid exposure period at 21 C. In Exp. 4, Exp. 3 was repeated except that all pairs of pouches were arranged in facing position, and the seeds of one pouch were covered with sterilized sand, while those of the other pair member remained exposed. The two genotypes were arranged as five pairs of pouches per rack or tub. Multiple tubs were used to accommodate the various treatments tested in Exp. 1, 3, and 4. Optimal conditions were

predicated on maximum separation of these genotypes under aphid infestation. Each experiment was repeated over time to provide two replications.

Another set of experiments was conducted sequentially using the optimized bioassay, each containing a different set of genotypes with the following derivation: 30 hard winter wheat elite breeding lines and cultivars from the Oklahoma State University (OSU) wheat breeding program (provided by B. F. Carver); 23 hard winter wheat elite breeding lines and cultivars from the Colorado State University (CSU) wheat breeding program (provided by S.D. Haley); 50 hard winter wheat genotypes tested in the 2004 Southern Regional Performance Nursery (SRPN) (provided by R.A. Graybosch); and 48 genotypes produced at CIMMYT and provided by A. R. Klatt containing 44 primary synthetics (*T. durum* x *T. tauschii*) and synthetic derivatives, and four non-synthetic spring wheat lines. Two treatments (aphid-infested and non-infested control) comprised each experiment and were applied to a complete set of genotypes. Each treatment was represented by two tubs (replicates), and genotypes were assigned to pouches (one genotype per pouch) within tubs. Each pouch contained up to 5 uniform-size plants of one genotype. After a 14-d aphid exposure at 21 C, root and shoot dry weights were determined as described above. Analysis of variance and means comparisons were generated with the MIXED procedure of SAS (SAS Institute, 2001), assuming effects associated only with treatments were fixed.

CHAPTER I V

RESULTS AND DISCUSSION

Bioassay development

A bioassay that establishes a response relationship to a range of infestation periods might give a more complete assessment of tolerance to BCO feeding, but such an assay would not be practical for screening germplasm collections or breeding populations. We examined five infestation periods in Exp. 1 to determine the duration of aphid exposure that would allow greatest differentiation between the two reference genotypes, Illinois Rustproof (tolerant) and Patrick (susceptible). Root weight of Patrick did not increase beyond the initial exposure period of 10 d, whereas root weight of Illinois Rustproof peaked at 14 d (Table 1), producing a significant infestation period x genotype interaction ($P<0.05$). Genotypic differences for shoot weight were also maximum after 14-d aphid exposure but without a significant interaction.

Genotypic differences were not observed in Exp. 2 between growth chamber temperatures maintained at 19 or 21 C at 14-d aphid exposure (Appendix A), and all subsequent experiments were conducted at 21 C. Orientation of the pouches in the racks did not influence genotypic responses to 14-d aphid exposure in Exp. 3, though we hypothesized that the greater light penetration allowed by reverse positioning would negatively impede root growth (Appendix B). In all subsequent experiments, pouches were arranged in the same direction for convenience. Consistency of seed germination in the pouches was improved with the addition of sand in Exp. 4. Pouches lacking sand

TABLE I

ROOT AND SHOOT DRY WT. FOR TWO WHEAT GENOTYPES EXPOSED TO BCO APHIDS FOR FIVE INFESTATION PERIODS (TOLERANT MEMBER OF GENOTYPE PAIR LISTED FIRST)

Infestation	Roots		Shoots	
period	Illinois Rustproof	Patrick	Illinois Rustproof	Patrick
d	---------- mg plant^{-1} ----------			
10	10.0	9.8	39.9	31.5
12	7.9	11.6	36.7	37.4
14	14.8	9.8	48.0	38.6
16	14.3	9.3	44.4	37.7
18	10.1	7.1	33.1	32.5
LSD† (0.05)	3.6		5.0	

† LSD value used to compare infestation periods.

produced one to five viable seedlings for biomass determination, whereas pouches with sand produced three to five viable seedlings. For subsequent experiments, seeds were covered with sterilized sand prior to germination inside the pouches.

Using the established protocol, Illinois Rustproof and Patrick were re-evaluated in infested and non-infested treatments (Table 2). Unlike the results produced from a series of aphid infestation periods in Exp. 1 (Table 1), these genotypes could not be differentiated for a single infestation period without the addition of a non-infested control treatment. Illinois Rustproof and Patrick did not differ in root biomass following a 14-d exposure, but relative to their biomass in the control treatment, Patrick suffered a greater reduction in root biomass (48%) than Illinois Rustproof (27%) as evidenced by a significant genotype x treatment interaction ($P<0.01$). A similar pattern was observed for shoot biomass, in which the respective reductions equaled 31% and 17%.

Using the same experimental design but a different pair of reference genotypes, the more susceptible genotype, 'Scout 66', suffered a greater reduction in root biomass (48%, averaged across four experiments) and shoot biomass (37%, averaged across four experiments) than did the more tolerant genotype, 'Skala' (29% reduction for root weight and 24% for shoot wt.) (Appendix C). Differential responses of Scout 66 and Skala to aphid damage was validated by significant genotype x treatment interactions detected in all but one of the eight F-tests for shoot and root biomass among the four experiments. As with Patrick and Illinois Rustproof, however, Skala did not necessarily produce greater root or shoot biomass than Scout 66 within the infested treatment alone. Conclusions drawn from this series of experiments were highly repeatable, as indicated by the lack of experiment x treatment and experiment x genotype x treatment interactions ($P>0.05$).

TABLE I I

ROOT AND SHOOT BIOMASS RESPONSES OF BCO-TOLERANT AND BCO-SUSCEPTIBLE GENOTYPES IN THE ABSENCE (CONTROL) AND PRESENCE OF APHIDS (14-d EXPOSURE)

Genotype	Treatment	Root	Shoots
		---------- mg plant^{-1} ----------	
Illinois Rustproof	Control	18.6	51.8
	Infested	13.5	42.8
	t-test[†]	*	NS
Patrick	Control	28.6	54.9
	Infested	15.0	37.9
	t-test[†]	**	**

[*, **] Treatments significantly different at the 0.05 and 0.01 probability level respectively; NS, not significant.

[†] Based on two replicates of 3-5 plants per replicate.

This bioasssay can be used to effectively and rapidly detect genotypic differences in tolerance to BCO feeding, but detection depends upon a non-infested treatment to establish a baseline for expected biomass produced by a given genotype.

Utility of bioassay in germplasm screening

Extending the bioassay to breeding lines of Great Plains and CIMMYT origin, aphid feeding produced variation in root and shoot biomass detectable at the 0.10 probability level in three of four sets (Table 3). However, indigenous genetic variation also existed in the absence of aphid feeding, making direct comparisons in the infested treatment alone tenuous and possibly confounded with differences in biomass per se. Furthermore, simple correlations between treatments were generally low (r = 0.03 - 0.24, P>0.05) and non-significant except within the CIMMYT genotypes (r= 0.35 and 0.48, P<0.01). Information gained from both treatments would not be considered repetitive and should be considered simultaneously, such as a ratio of infested-to-control biomass, to more accurately assess BCO tolerance. Individual genotype ratios are given in Appendices D, E, F, and G.

Based on the ratio calculated within replicates, phenotypic variation was detected at the 0.10 probability level for either tissue source in three of the four germplasm sets. The minimum and maximum ratio values were consistent within each set, varying from about 0.5 to values >1.0. For a few genotypes, aphid feeding produced an unexpected positive effect on biomass, either shoot or root tissue, but never both (data not shown). The mean biomass ratio consistently hovered around 0.7 for both tissue sources. Thus, the severity of BCO injury was not tissue-dependent even though feeding was restricted entirely to leaf tissue. This series of experiments revealed some consistency between

TABLE I I I

SUMMARY OF VARIABILITY IN ROOT AND SHOOT DRY WEIGHT, AND THEIR RATIO OF INFESTED/CONTROL TREATMENTS, WITHIN FOUR SETS OF WHEAT GERMPLASMS

Source	Tissue	Infested treatment	Control treatment	Infested/ control ratio	Ratio				Correlation coefficient
					Min	Max	Mean	LSD (0.05)	
		----------*P* value † --------							
Oklahoma	Root	0.88	0.03	0.89	0.47	0.94	0.61	0.08	0.15
	Shoot	0.07	0.01	0.01	0.53	1.02	0.73	0.02	
Colorado	Root	0.04	0.13	0.10	0.45	0.99	0.68	0.09	0.80 **
	Shoot	0.02	0.01	0.21	0.59	0.98	0.78	0.08	
CIMMYT	Root	0.07	0.01	0.28	0.33	1.35	0.66	0.11	0.68 **
	Shoot	<0.01	<0.01	0.43	0.44	1.03	0.68	0.07	

TABLE I I I (Cont.)

Source	Tissue	Infested treatment	Control treatment	Infested/ control ratio	Ratio				Correlation coefficient
					Min	Max	Mean	LSD (0.05)	
		----------*P* value † --------							
SRPN	Root	0.03	0.17	0.02	0.48	1.68	0.69	0.09	0.59 **
	Shoot	0.02	<0.01	0.19	0.47	1.45	0.74	0.08	

** Correlation coefficient for ratio vs. shoot ratio significant at the 0.01 probability level.
† From the *F*-test for phenotypic variation, with two replicates per genotype and 3-5 plants per replicate.

shoot and root responses to aphid damage in all sets except the Oklahoma materials (r = 0.59-0.80, P<0.01, for ratios derived from roots vs. shoots). However, we recommend evaluation of BCO tolerance based on damage assessment of both shoot and root growth when the objective is to identify the highest level of tolerance.

CHAPTER V

SUMMARY AND CONCLUSIONS

In summary, this protocol provides a relatively rapid (3 wk) and repeatable bioassay for BCO tolerance in wheat that has greatest utility in breeding and germplasm evaluation programs. Essential to effective discrimination among genotypes is a non-infested control treatment as a baseline comparison for each genotype. Given the sizes of the growth chamber, seedling pouches, and plant containers used in this study, as many as 200 non-replicated genotypes could be feasiblely managed by one operator, including four replicates of three reference genotypes as a source of experimental error for statistical tests if required. A more rapid assessment might be achieved by visual assessment of root and shoot biomass, if the primary objective is to identify genotypes either highly susceptible or highly resistant to BCO feeding.

CHAPTER V I

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Appendix A: Analysis of variance for root and shoot biomass for two wheat genotypes exposed to 19 and 21ºC temperatures in both the presence and absence of aphids.

		Root		Shoot	
Source	df	Mean square	F-value	Mean square	F-value
Temperature (Temp)	1	107.6	1.1 NS	7.2	0.5 NS
Aphid treatment (Treat)	1	1404.2	14.6 **	14.2	0.9 NS
Temp X Treat	1	53.8	0.6 NS	51.1	3.3 NS

*, ** Significant at the 0.01 probability level, respectively; NS, not significant.

Appendix B: Analysis of variance for root and shoot biomass for two wheat genotypes whose pouches were arranged in one of three different orientations in both the presence and absence of aphids.

		Root		Shoot	
Source	df	Mean square	F-value	Mean square	F-value
Pouch treatment (P)†	2	3.5	0.1 NS	12.5	1.5 NS
Aphid treatment (T)	1	<0.1	<0.1 NS	2.0	0.2 NS
P X T	2	27.5	0.9 NS	7.4	0.9 NS

*, ** Significant at the 0.01 probability level, respectively; NS, not significant.

† Pouches having foil, or without foil and facing each other, or facing same direction.

Appendix C: Root and shoot biomass responses for BCO-tolerant and BCO-susceptible genotypes in the absence and presence of aphids over four experiments.

		Trial 1		Trial 2		Trial 3		Trial 4	
Genotype	Treatment	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
		----------mg----------							
Scout 66	Control	18	43	18	39	21	39	20	40
	Infested	11	29	9	25	9	24	11	24
	t-test	**	**	**	**	**	**	**	**
Skala	Control	16	33	16	32	16	27	18	29
	Infested	13	28	12	23	11	21	11	20
	t-test	NS	*	**	*	**	**	**	**
F-test, Genotype x Treatment		*	**	*	*	**	**	NS	**

*, ** Treatments significantly different at the 0.05 and 0.01 probability level, respectively; NS, not significant.

Appendix D: Biomass estimates and percent losses for root and shoot weights of advanced lines from the Oklahoma State University wheat breeding program.

		Root			Shoot		
Selection	Pedigree	Control	Infested	Loss†	Control	Infested	Loss†
		mg^{-1}		%	mg^{-1}		%
OK98690	OK91724/Karl	18.0	11.1	38	27.4	19.0	31
OK94P549-21	HBY756A/Siouxland//2180	20.1	9.6	51	36.5	19.7	46
OK94P549-11	HBY756A/Siouxland//2180	16.9	11.9	30	31.4	18.9	40
OK96705-38	2180/OK88803//Abilene	16.4	9.6	41	26.7	21.7	19
OK95548-54	OK86216/Cimarron sib//2180	18.1	11.9	34	32.9	25.4	23
OK95616-56	TXGH13622/2180	15.6	10.9	30	28.5	17.8	38
OK98699	TAM 200/HBB313E//2158 Seln	16.7	9.5	43	29.1	20.6	29
OK98697	TAM 200/HBB313E//2158 Seln	16.2	10.6	35	29.8	21.1	29
OK99212	Tomahawk/2174//Tonkawa	15.4	10.2	34	24.4	21.4	12
OK99219	OK91P609/Cimarron//2174	19.4	12.1	38	34.6	24.5	29
OK99215	AgSeco 7853/2*2174	16.0	9.9	38	32.4	20.9	35
OK00514	KS93U206//KS82W418/Stephens F3:9	16.0	14.1	12	26.8	25.9	3
OK00515	KS93U206//KS82W418/Stephens F3:9	21.0	13.7	35	31.5	23.0	27
OK00614	OK90604/Rio Blanco	19.0	11.5	39	33.3	24.6	26
OK00520	OK91724/2180//Pecos	16.0	9.7	39	28.8	21.5	25
OK00227	Tonkawa/2137	22.4	10.4	54	28.1	22.0	22
OK00223	OK88767-15/Arlin//Tonkawa	13.8	8.5	38	28.6	20.3	29
OK00125	Tonkawa/Cimarron	17.5	11.4	35	29.2	19.0	35
OK00229	Tonkawa/Arlin//Tonkawa	16.6	10.1	39	29.6	25.0	16
OK00411	ER6789-86/Karl 92//Tonkawa	19.5	9.2	53	32.9	26.1	21
OK00316	TAM 202/2163//Tonkawa	14.9	10.0	33	26.4	26.9	-2
Ok00413	Lut 10488/Chisholm//Karl92	16.0	10.3	36	27.0	26.0	4
OK00608W	Karl 92/OK90604	16.8	9.5	43	32.0	18.9	41
Ok00618W	Intrada/WI89-163W F2:8	15.3	9.8	36	29.0	18.6	36
Intrada	Intrada	20.0	9.2	54	27.5	18.5	33
OK101	Ok101	14.2	9.8	31	29.9	22.5	25
OK102	Ok102	17.4	9.9	43	28.9	21.7	25

Appendix D (cont.)

Selection	Pedigree	Root			Shoot		
		Control	Infested	Loss†	Control	Infested	Loss†
		mg^{-1}		%	mg^{-1}		%
Chisholm	Chisholm	14.9	10.5	30	30.3	19.3	36
Skala	Skala	19.0	10.2	46	26.6	19.6	26
Scout 66	Scout 66	15.5	10.5	32	43.9	23.0	48

† $\left(\frac{\text{Control} - \text{Infested}}{\text{Control}}\right)$ x 100, where negative values equal a percentage increase in biomass caused by aphid feeding.

Appendix E: Biomass estimates and percent losses for root and shoot weights of advanced lines from the Colorado State University wheat breeding program.

		Root			Shoot		
Selection	Pedigree	Control	Infested	Loss†	Control	Infested	Loss†
		mg^{-1}		%	mg^{-1}		%
CO970547	Ike/Halt	17.9	11.7	35	31.7	22.7	28
CO970547-2	Ike/Halt	21.9	10.0	54	27.7	19.9	28
CO970547-7	Ike/Halt	16.8	10.8	36	30.3	25.1	17
CO980376	CO850034 T-57//5*TAM 107/3	17.9	11.6	35	32.3	25.6	21
CO980607	Yuma/T-57//TAM 200/3/4*Yuma/4	25.3	11.4	55	37.4	25.8	31
CO980630	Yuma/T-57//TAM 200/3/4*Yuma/4	17.7	9.5	46	32.3	22.4	31
CO99141	Ike/Halt	16.4	11.4	30	29.0	23.4	19
CO99177	Longhorn/Halt	20.7	13.7	34	34.7	26.9	22
CO99W314	TX91V4931/Halt	16.2	9.2	43	22.9	21.7	5
CO99W183	KS92WGRC25/Halt	19.6	11.9	39	26.4	26.5	-1
CO99W188	KS92WGRC25/Halt	15.8	15.7	1	25.7	23.0	11
CO99W192	KS92WGRC25/Halt	15.4	11.3	27	27.6	21.2	23
CO99W254	CO931037/Halt	16.4	14.3	13	23.4	24.5	-5
CO99W277	CO931037/Halt	15.2	10.7	30	29.2	28.0	4
CO99W329	CO931037/Halt	15.1	11.4	25	25.0	23.8	5
CO00D007	Yumar//TXGH12588-120*4/FS2	16.3	13.4	18	26.4	23.0	13
CO00D011	Yumar//TXGH12588-120*4/FS2	20.5	14.9	27	33.0	28.6	13
CO991057	Akron//TXGH12588-26*4/FS2	18.9	18.2	4	27.9	25.3	9
CO991132	Jagger//TXGH12588-120*4/FS2	25.8	16.1	38	38.3	24.6	36
CO991350	Yumar//TXGH12588-26*4/FS2	21.0	12.5	40	30.7	24.5	20
CO991407	Yumar//TAM 110*4/FS2	19.0	13.6	28	31.0	29.3	5
Skala	Skala	17.0	9.9	42	23.0	23.4	-2
Scout 66	Scout 66	17.6	9.2	48	34.3	21.3	13

† $\left(\frac{\text{Control} - \text{Infested}}{\text{Control}}\right)$ x 100, where negative values equal a percentage increase in biomass caused by aphid feeding.

Appendix F: Biomass estimates and percent losses for root and shoot weights of breeding lines tested in the 2004 SRPN.

Selection	Pedigree	Root			Shoot		
		Control	Infested	Loss†	Control	Infested	Loss†
		mg^{-1}		%	mg^{-1}		%
Kharkof	Kharkof	20.1	11.1	45	39.0	21.0	46
Scout 66	Scout 66	17.3	13.9	20	30.6	22.6	26
TAM-107	TAM-107	18.5	11.0	41	26.4	17.1	35
Trego	Trego	16.0	11.0	31	25.5	17.7	31
G990191	OK90604/KS6397//SNOWWHITE	18.6	12.6	32	31.9	23.5	26
G982238-2	N87V107/BETTY	18.9	11.5	39	31.8	21.6	32
G991324	97 8/64 MASA	19.2	10.0	48	26.4	16.7	37
G980143	OK88767-11/JAGGER	18.7	11.4	39	31.8	17.8	44
AP01T1112	TAM 105/3/NE70654/BBY//BOW"S"/4/Century*3/TA2450	19.0	11.5	39	29.5	24.5	17
AP01T1114	TAM 105/3/NE70654/BBY//BOW"S"/4/Century*3/TA2450	18.8	9.0	52	27.3	19.4	29
AP01T3131	W94-320/3/KS85W663-2-4/2W81-133/Thunderbird	21.6	12.6	42	25.0	20.6	18
NW99L7068	KS84HW196*8/RioBlanco/HBY762A//Halt	17.5	12.0	31	33.0	15.0	55
T135	T81/97T2688	19.9	11.9	40	26.1	18.2	30
T136	Jagger/T811	19.4	15.3	21	26.6	18.8	29
T140	93WGRC27/T811	16.6	9.1	45	22.8	17.2	25
T141	T441/T13	15.6	8.2	47	24.5	14.9	39
OK00611W	KS96WGRC39/Jagger	16.9	11.4	33	22.9	15.9	31
OK00618W	Intrada/W189-163W	12.6	8.9	29	18.9	16.2	14
OK00514	KS96WGRC39/Jagger	12.5	11.1	11	22.2	19.7	11
OK99212	Tomahawk/2174//Tonkawa	18.0	10.0	44	27.6	19.7	29
OK00614	OK90604/Rio Blanco	16.4	9.6	41	23.3	19.8	15
KS950811-5-1	Ogallala/KS95WGRC33//Jagger	19.3	13.1	32	26.6	16.5	38
KS00F5-14-7	Bulk Selection	18.9	11.4	40	25.1	16.0	36
KS00F5-20-3	Bulk Selection	21.3	12.3	42	26.9	18.9	30
KS00F5-57-8	Bulk Selection	15.6	10.1	35	25.3	17.1	32
CO970547-7	Ike/Halt	11.6	11.8	-2	17.5	16.1	8
CO980607	Yuma/T-57//TAM 200/3/4*Yuma/4/NEWS08	20.9	15.4	26	28.5	23.7	17

Appendix F (cont.)

Selection	Pedigree	Root			Shoot		
		Control	Infested	Loss†	Control	Infested	Loss†
		mg^{-1}		%	mg^{-1}		%
CO00D007	Yumar//TXGH12588-120*4/FS2	12.2	10.3	16	17.4	12.5	28
CO00016	CO940606/TAM107R-2	20.5	12.1	41	24.5	17.0	31
CO00698	CO931083/Oro Blanco//Halt	13.8	10.7	22	24.4	14.6	40
TX96D1073	TX86D1310/Kavkaz//TX86D1308 (=WX87D144-10-99-12-18)	18.8	13.7	27	28.6	22.0	23
TX00V1117	ARLIN/TX89V4213 (CO723594/YACO'S'//TX81V6582)	20.4	13.8	32	29.2	21.5	26
TX00V1131	TX87V1613/KS91WGRC11	14.8	10.2	31	27.4	16.1	41
TX01D3232	TX92U3060/TX91D6564 (=X95U104-P66)	17.2	10.3	40	24.6	20.4	17
TX00D1390	TX89D1253*2/TTCC404 (=WX93D208-9-1-17-13)	20.2	10.0	50	28.7	17.0	41
TX01A5936	JAGGER/3/PSN 'S'/BOW 'S'//T200	22.4	12.6	44	28.8	21.8	24
NE00403	PRONGHORN/ARLIN//ABILENE	15.3	12.7	17	21.2	16.3	23
NE00435	WI87-018/2*ARAPAHOE	18.7	10.1	46	27.4	16.2	41
NE01481	NE92458 (=OK83201/REDLAND)/Ike	13.4	11.1	17	15.1	16.2	-7
NE00564	T81/NE91635 (=NE82761/NE82599)	17.4	13.0	25	21.8	18.2	17
W99-194	059E//Jagger/Pecos	20.1	13.7	32	30.2	17.9	41
W96x1311-01	W91-376-20/W9-084	18.5	16.9	9	24.5	25.4	-4
W98-159-7	Ponderosa/Jagger	19.8	11.9	40	33.8	21.2	37
W03-20	Ogallala/KSU94U261//Jagger	15.2	10.8	29	29.0	17.4	40
KS01HW152-6	TREGO/BTY SIB	14.9	13.1	12	25.4	17.7	30
KS01HW163-4	TREGO/BTY SIB	22.3	12.2	45	25.9	15.5	40
KS02HW34	TREGO/JGR 8W	14.4	10.4	28	21.6	16.3	25
SD97W604	SD89333/Abilene	21.1	10.7	49	24.9	15.2	39
CO991132	Jagger//TXGH12588-120*4/FS2	20.2	11.9	41	26.9	21.0	22
NW98S097	WA691213-27/N86L177//AP-WI89-163	19.0	12.0	37	25.6	18.6	27
Skala	Skala	10.7	10.2	1	16.5	16.2	2

† $\left(\frac{\text{Control} - \text{Infested}}{\text{Control}}\right)$ x 100, where negative values equal a percentage increase in biomass caused by aphid feeding.

Appendix G: Biomass estimates and percent losses for root and shoot weights of breeding lines developed by the CIMMYT wheat breeding program.

Selection[†]	Pedigree	Root			Shoot		
		Control	Infested	Loss[‡]	Control	Infested	Loss[‡]
		mg^{-1}		%	mg^{-1}		%
3084	SHA3/SERI//2*PSN/BOW	20.3	12.2	40	40.2	30.1	25
3087	WUH1/VEE#5//CBRD	18.9	9.9	48	36.1	29.3	19
3095	VORONA//PRL/VEE#6	22.7	11.7	48	45.8	25.7	44
3098	TC-14 SPEAR 2	17.9	13.0	27	35.8	26.9	25
3113	CROC 1/AE.SQ. (205)//2*BCN	17.4	10.8	38	36.3	24.8	32
3114	DVERD 2/AE.SQ. (214)//2*BCN	11.4	10.2	11	31.0	19.2	38
3115	DVERD 2/AE.SQ. (214)//2*BCN	13.2	9.4	29	25.0	21.2	15
3116	DVERD 2/AE.SQ. (214)//2*BCN	13.8	11.5	17	34.5	23.5	32
3117	ALTAR 84/AE.SQ (219)//3*ESDA	21.5	11.0	49	39.6	31.0	22
3118	ALTAR 84/AE.SQ.(J BANGOR)//ESDA	17.1	14.4	16	39.5	28.5	28
3158	ALTAR 84/T.TAUSCHII (ACC. 198)	6.8	8.9	-30	27.8	24.4	12
3159	DUERGAND/T. TAUSCHII (ACC. 22)	14.2	8.5	40	32.9	19.5	41
3160	ALTAR 84/T. TAUSCHII (ACC. 223)	13.2	8.6	35	30.6	27.4	10
3161	CHEN 'S'/T. TAUSCHII (ACC. 224)	10.9	8.3	24	35.8	22.0	39
3162	ALTAR 84/AE.SQ.(J BANGOR)//ESDA	20.3	14.0	31	45.7	26.3	42
3163	GAN/AE.SQ.(236)//CETA/AE.SQ.(895)/3/MAIZ	13.6	9.6	29	41.9	26.1	38
3164	SCOOP1/AE.SQ.(434)//CETA/AE.SQ.(895)/3/MAIZ	15.3	9.5	38	38.6	24.2	37
3165	SCOOP1/AE.SQ.(434)//CETA/AE.SQ.(895)/3/MAIZ	16.5	9.2	44	41.8	24.5	41
3166	DOY1/AE.SQ.(447)//CETA/AE.SQ.(895)/3/MAIZ	19.7	13.1	34	37.3	24.4	35
3168	YUK/AE.SQ.(217)	14.9	9.8	34	39.2	24.3	38
3169	ALTAR 84/AE.SQ.(224)	18.0	12.1	33	35.0	28.8	18
3170	ALTAR 84/AE.SQ.(224)	15.4	8.7	44	31.4	22.8	27
3171	68112/WARD//AE.SQ.(369)	18.1	12.7	30	36.7	26.1	29
3172	YAV 3/SCO//JO69/CRA/3/YAV79/4/ AE.SQ.(498)	15.8	9.2	42	37.8	21.7	43
3173	DOY1/AE.SQ.(511)	16.2	8.4	48	43.5	31.2	28
3174	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQ.(629)	14.7	10.9	26	41.1	26.9	35
3175	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQ.(878)	16.7	6.8	59	40.7	29.0	29

Appendix G (cont.)

Selection[†]	Pedigree	Root			Shoot		
		Control	Infested	Loss[‡]	Control	Infested	Loss[‡]
		mg^{-1}		%	mg^{-1}		%
3176	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQ.(878)	16.6	10.2	39	45.0	24.5	46
3177	LCK59.61/AE.SQ.(324)	24.9	8.1	67	46.6	23.5	50
3178	SCA/AE.SQ.(518)	19.6	10.8	45	46.0	24.5	47
3179	BOTNO/AE.SQ.(620)	14.3	13.4	6	33.2	29.2	12
3180	SNIPE/YAV79//DACK/TEAL/3/AE.SQ.(700)	14.6	9.0	38	39.3	23.5	40
3181	GAN/AE.SQ.(897)	19.8	13.0	34	41.8	25.0	40
3182	SCA/AE.SQ.(409)	18.4	12.5	32	45.8	32.9	28
3183	CETA/AE.SQ.(1024)	14.5	8.9	39	36.9	24.5	34
3184	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQ.(498)	7.4	9.6	-30	26.6	20.9	21
3186	CETA/AE.SQ.(1024)	13.0	7.3	44	28.0	17.8	36
3187	ALTAR 84/AE.SQ.(205)	10.1	7.7	24	21.3	19.7	8
3188	D67.2/P66.270//AE.SQ.(220)	12.7	10.0	21	30.0	19.1	36
3190	YAV_2/TEZ//AE.SQ.(895)	16.8	13.8	18	36.9	19.5	47
3191	GAN/AE.SQ.(897)	11.8	9.9	16	28.2	19.0	33
3193	CROC_1/AE.SQ.(879)	12.5	4.5	64	31.2	18.7	40
3194	DVERD_2/AE.SQ.(221)	12.9	7.7	40	23.4	16.0	32
3195	ALTAR 84/AE.SQ.(192)	12.8	10.9	15	24.4	26.8	-10
3197	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQ.(498)	13.8	6.5	53	26.9	20.9	22
3198	YAR/AE.SQ.(783)	15.3	9.2	40	27.7	18.2	34
Skala	Skala	16.6	8.4	49	23.4	16.3	30
Scout 66	Scout 66	17.2	9.0	48	31.1	24.8	20

[†] 3084-3098 are spring wheats, 3113-3118 are synthetic derivatives, and 3158-3198 are primary synthetics.

[‡] $\left(\frac{\text{Control} - \text{Infested}}{\text{Control}}\right)$ x 100, where negative values equal a percentage increase in biomass caused by aphid feeding.

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Pages in Study: 33 Candidate for the Degree of Master of Science

Major Field: Plant Breeding and Genetics

Abstract: *Rhopalosiphum padi* L., or the bird cherry-oat aphid, causes significant damage to winter wheat (*Triticum aestivum* L.) in the Great Plains. Our objective was to develop a juvenile-plant bioassay for BCO tolerance that allows rapid phenotypic characterization of tolerance in a growth chamber study using root and shoot weight measurements of 3-wk old seedlings produced in seed germination pouches. Based on preliminary results, bioassay methods were used in verification experiments conducted on one hundred and forty-nine Oklahoma, Colorado, SRPN, and CIMMYT lines indicated levels of responses to feeding (roots being greater than shoots), but both averaging around a 30 % reduction. Findings of indigenous genetic variation in six of the eight control treatments ($P>0.05$) further exonerated the need for control plants to curve variation by a ratio of infested-to-control. Correlation relationships between three of the four sources showed that both roots and shoots are key to finding tolerance.

Advisor's approval: Brett Carver