Chlorophyll degradation in wheat lines elicited by cereal aphid infestation
by Tao Wang

A thesis submitted in partial fulfillment. of the requirements for the degree of Master of Science in Entomology
Montana State University
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Abstract:

The Russian wheat aphid, Diuraphis noxia (Mordvilko) (Hemiptera: Aphididae), is a serious pest of cereal crops and causes chlorosis along with a multiple of other symptoms. Temporal changes of plant and aphid [i.e., D. noxia, Rhopalosiphum padi (L.), the bird cherry-oat aphid] biomass from 'Tugela' wheat and three near-isogenic lines (isolines) (i.e., Tugela-Dnl, Tugela-Dn2 and Tugela-Dn5) were tested to assess aphid resistance of the Tugela wheat lines. When infested by D. noxia, all D. u emu-resistant isolines sustained growth. Biomass of D. noxia collected from non-resistant Tugela was significantly higher than those plants with resistant Dn genes. Biomass of D. noxia collected from Tugela-Dn1 and Dn2 plants was not different from each other, but was lower than that from Tugela-Dn5 In contrast, there was no difference in R. padi biomass from the four wheat lines. Photosynthetic pigment (chlorophylls a and b, and carotenoids) concentrations, chlorophyll a/b ratio, and chlorophyll/carotenoid ratio among the four wheat lines were assayed. Concentrations of chlorophylls a and b, and carotenoids were significantly lower in D. noxia-infested plants when compared with R. padi-infested and the uninfested plants. However, no difference was detected in chlorophyll a/b or chlorophylls/carotenoids ratio among Tugela wheat lines with different aphid treatments. When infested by D. noxia, chlorophylls a and b contents were not different among the four wheat lines at day 3, but were lower in Tugela and Tugela-Dnl plants than in Tugela-Dn2 and Dn5 plants at days 6 and 12. To elucidate the biochemical mechanisms of chlorosis formation, activities of three chlorophyll degradation enzymes (i.e., chlorophyllase, Mg-dechelatase and chlorophyll oxidase) extracted from the wheat lines were measured. Chlorophyllase activities were higher on R. padi-infested wheat lines and lower on D. noxia-infested plants. Mg-dechelatase activities were higher on D. noxia-infested plants and lower on R. padi-infested plants. Experimental results confirmed the previous reports that Tugela is a D. noxia susceptible variety while Tugela Du plants are resistant to D. noxia. Chlorosis on Tugela wheat lines induced by D. noxia infestation was most likely driven by unbalanced chlorophyll biosynthesis and catabolism processes.

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ABSTRACT

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), is a serious pest of cereal crops and causes chlorosis along with a multiple of other symptoms. Temporal changes of plant and aphid [i.e., *D. noxia*, *Rhopalosiphum padi* (L.), the bird cherry-oat aphid] biomass from 'Tugela' wheat and three near-isogenic lines (isolines) (i.e., Tugela-*Dn1*, Tugela-*Dn2* and Tugela-*Dn5*) were tested to assess aphid resistance of the Tugela wheat lines. When infested by *D. noxia*, all *D. noxia*-resistant isolines sustained growth. Biomass of *D. noxia* collected from non-resistant Tugela was significantly higher than those plants with resistant *Dn* genes. Biomass of *D. noxia* collected from Tugela-*Dn1* and *Dn2* plants was not different from each other, but was lower than that from Tugela-*Dn5*. In contrast, there was no difference in *R. padi* biomass from the four wheat lines. Photosynthetic pigment (chlorophylls *a* and *b*, and carotenoids) concentrations, chlorophyll *a/b* ratio, and chlorophyll/carotenoid ratio among the four wheat lines were assayed. Concentrations of chlorophylls *a* and *b*, and carotenoids were significantly lower in *D. noxia*-infested plants when compared with *R. padi*-infested and the uninfested plants. However, no difference was detected in chlorophyll *a/b* or chlorophylls/carotenoids ratio among Tugela wheat lines with different aphid treatments. When infested by *D. noxia*, chlorophylls *a* and *b* contents were not different among the four wheat lines at day 3, but were lower in Tugela and Tugela-*Dn1* plants than in Tugela-*Dn2* and *Dn5* plants at days 6 and 12. To elucidate the biochemical mechanisms of chlorosis formation, activities of three chlorophyll degradation enzymes (i.e., chlorophyllase, Mg-dechelatase and chlorophyll oxidase) extracted from the wheat lines were measured. Chlorophyllase activities were higher on *R. padi*-infested wheat lines and lower on *D. noxia*-infested plants. Mg-dechelatase activities were higher on *D. noxia*-infested plants and lower on *R. padi*-infested plants. Experimental results confirmed the previous reports that Tugela is a *D. noxia* susceptible variety while Tugela *Dn* plants are resistant to *D. noxia*. Chlorosis on Tugela wheat lines induced by *D. noxia* infestation was most likely driven by unbalanced chlorophyll biosynthesis and catabolism processes.

CHAPTER 1

INTRODUCTION

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), is a serious pest of cereal crops worldwide, except in Australia. This species completes its entire life cycle on a wide range of cereal grains and grasses. Estimated economic losses caused by *D. noxia* feeding to wheat, *Triticum aestivum* L., and barley, *Hordeum vulgare* L., in the western U. S. between 1987 and 1993 averaged over $127 million per year (Webster et al. 1994). In recent years, various management perspectives (e.g., biological control, chemical control, cultural control, and plant resistance) have been studied to reduce *D. noxia* populations and the economic impact of this pest on cereals.

Diuraphis noxia typically aggregates on the new growth of the host plants. The most obvious plant injury symptom is chlorosis, which can subsequently develop into longitudinal white streaks on leaves (Hewitt et al. 1984). Other injury symptoms include folding or convolute leaf rolling, blasted heads, and purple discoloration. These injury symptoms can lead to plant stunting, prostrate growth or even sterility, and finally loss of yield.

Von Wechmar and Rybicki (1981) showed that the typical injury symptoms of plants associated with *D. noxia* feeding were induced by a salivary toxin injected during feeding. Although the nature of the toxin was unknown, Kruger and Hewitt (1984) reported that *D. noxia* extracts adversely affect photosynthetic rates *in vitro* and proposed this could be secondary to the degradation of the chloroplast membrane or *vice versa*. Haile et al. (1999) found that *D. noxia* feeding caused less reduction of photosynthetic rates in PI 262660 (*D. noxia* tolerant) when compared with PI 137739 (antibiotic) and 'Arapahoe' (susceptible) wheat. They suggested this phenomenon might be caused by varying resistance mechanisms that provided different photosynthetic compensation abilities to aphid feeding injury. Ni et al. (2000) studied the hydrolases and oxido-reductases in the homogenate of aphids and plant tissues. Pectinesterase, polygalacturonase, cellulose, and amylase activities were examined in the hydrolase group, while catalase, peroxidase, catechol oxidase, superoxide dismutase, and ascorbate oxidase activities were examined in the oxido-reductases group. They detected the same hydrolase activities from *D. noxia* and bird cherry-oat aphid, *Rhopalosiphum padi* (L.), but different oxido-reductase activities. Catalase activity was detected from *D. noxia* salivary tissue but not from *R. padi*, whereas peroxidase was detected from *R. padi* salivary tissue but not *D. noxia*. They suggested that this salivary enzyme difference between the two aphid species is important in the development of injury symptom formation on susceptible wheat plants. Ni et al. (2001a) detected a peroxidase increase in resistant 'Halt' wheat and suggested the increase may have contributed to *D. noxia* resistance. Ni et al. (2001b) demonstrated that herbivore-elicited chlorophyll loss differed from chlorophyll degradation in naturally senescing plants because neither chlorophyllase nor oxidative bleaching activities described by Janave (1997) and Matile et al. (1999) in the senescing plants was detected in aphid-infested wheat. *Diuraphis noxia*-infested susceptible wheat (Arapahoe) showed a significantly higher Mg-dechelatase activity than the uninfested wheat. The finding suggested chlorosis is the result of increased Mg-dechelatase activity and possibly other plant physiological changes.

The research reported in this thesis was a continuation of previous research by Ni et al. (2001b, 2002) to understand the biochemical mechanisms of aphid-elicited chlorosis using 'Tugela' and three wheat near-isogenic lines (isolines) (i.e., Tugela-*Dn1*, Tugela-*Dn2* and Tugela-*Dn5*) and two species of cereal aphids (i.e., chlorosis-eliciting *D. noxia* and non-chlorosis-eliciting *R. padi*). The objectives were to assess the resistance mechanisms of Tugela and Tugela isolines through analysis of aphid and plant biomass; to quantify photosynthetic pigments (i.e., chlorophylls *a* and *b*, and carotenoids) in wheat plants with different aphid treatments (i.e., *D. noxia*, *R. padi*, and control); and to assay changes in chlorophyll degradation enzyme (chlorophyllase, Mg-dechelatase and chlorophyll oxidase) activities elicited by aphid infestation in wheat plants and their correlation with chlorosis formation. Chapter three focused on resistance assessment and pigment quantifications of aphid-infested wheat plants, while Chapter four reports measurement of chlorophyll degradation enzyme activities in wheat plants elicited by aphid feeding. The experimental results of chlorosis rating and quantifications of photosynthetic pigments of aphid-infested wheat plants in Chapter three were basal to the further discussion of enzymatic chlorosis formation in *D. noxia*-infested wheat plants in Chapter four.

CHAPTER 2

LITERATURE REVIEW

History and Economic Impact

Diuraphis noxia is native to the area between the Caucasus Mountains and the Tian Shan Mountain of Central Asia. It was first described in the early 1900s when outbreaks occurred in Moldova and Ukraine, followed by a period of relative obscurity (Halbert and Stoetzel 1998). It was introduced into the Republic of South Africa where it became a serious pest in 1978 (Halbert and Stoetzel 1998). *Diuraphis noxia* was detected in Mexico in 1980 and in the U. S. at Muleshoe, TX in March 1986 (Stoetzel 1987). The aphid soon spread to the seven Great Plains states (Colorado, Kansas, Nebraska, New Mexico, Oklahoma, Texas and Wyoming) and the following year spread north and west, increasing its range to include seven more western and northwestern states (Arizona, Idaho, Montana, South Dakota, Oregon, Utah and Washington). In North America, the estimated cumulative total economic losses to the U.S. cereal industry from *D. noxia* from 1987 through 1993 have been over $900 million (Webster et al. 1994). Morrison and Peairs (1998) estimated the associated cost (i.e., crop loss and use of pesticides) in the U. S. between 1986 and 1994 from *D. noxia* damage was 893.1 million dollars.

Diuraphis noxia has established itself as an important pest of small grains in the arid and semi-arid areas of the western U. S. The Canadian provinces of Alberta, British Columbia, and Saskatchewan have also reported the aphid's presence and damage on cereals (Jones et al. 1989). To date, *D. noxia* occurs in all major cereal production areas of the world, except Australia (Halbert and Stoetzel 1998).

Host Range

Hosts of *D. noxia* are restricted to the Graminae, including barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), rye (*Secale cereale* L.), and triticale (*X Triticosecale* Wittmack). It also can survive and reproduce on several other cool-season and warm-season grasses (Kindler and Springer 1989). Wheat, barley, and some cool-season grasses are particularly favorable host plants.

It is clear that volunteer wheat is the most important oversummering host for this aphid (Hewitt et al. 1984). Suitability of some cool-season grasses appears to vary geographically. The most important grass hosts of *D. noxia* in the Great Plains are crested wheatgrass, *Agropyron cristatum* (L.) Gaertner; Canada wildrye, *Elymus Canadensis* (H.); jointed goatgrass, *Triticum cylindricum* (Host) Ces.; and downy bromegrass, *Bromus tectorum* (L.) (Hammon et al. 1989, Armstrong et al. 1991, Montandon et al. 1993). Crested wheatgrass and Canada wildrye are reported to be the grass species most capable of supporting oversummering populations of *D. noxia* (Armstrong et al. 1991).

Life Cycle

Throughout the summer season, all populations of *D. noxia* reproduce parthenogenetically. The live forms (viviparae) can be winged or wingless. Winged morphs (alatae) appear in response to a decline of the host plant, an increase of population density, and possibly other environmental stimuli (Kawada 1987, Baugh and

Phillips 1991). Some populations that are holocyclic produce males and oviparae. They mate to produce overwintering eggs in the autumn. Other populations are anholocyclic and do not produce males or oviparae and overwinter as viviparae.

Diuraphis noxia is holocyclic in its native range of Central Asia (Kiriac et al. 1990), Hungary (Basky 1993), and Spain (Fernandez et al. 1992). There are no reports of sexual forms occurring in the Middle East or in South America. Similarly, the South African population of *D. noxia* is anholocyclic (Hewitt et al. 1984). In the U. S., oviparae of *D. noxia* were found in Idaho in 1989, both in the southwest Treasure Valley and in the northern Idaho Palouse region (Kiriac et al. 1990). However, no males have been found to date in North America, and eggs are not viable without fertilization (Halbert and Stoetzel 1998).

Survival of *D. noxia* during winter depends on the duration of cold temperatures, cold temperature extremes, and precipitation patterns (Butts 1989, 1992; Armstrong 1994). *Diuraphis noxia* does not seem to suffer adverse effects from cold until temperatures are below 0°C. However, numbers decrease slowly and almost linearly throughout winter when soil surface temperatures remain at or below 0°C. As soil surface temperatures decrease below -5°C, mortality increases at a faster rate and, when temperature is below -25°C, mortality is rapid and extensive (Armstrong 1994).

Economic Thresholds

The concept of plant tolerance to pest injury was discussed by scientists as early as 1934; however, the original concepts of the Economic Injury Level (EIL) and Economic Threshold (ET) were first proposed in a landmark paper by Stern et al. (1959). Economic damage is defined as the amount of injury that will justify the cost of control. The EIL is proposed as the lowest population density of pests that will cause economic damage, while the ET is defined as the density of pests at which control measures should be taken to prevent the pest population from reaching the EIL (Peterson and Higley 2002). The ET is a basic component of integrated pest management (IPM) and determinations of the EIL and ET for an insect species are important to an IPM program (Legg and Archer 1998). Poston et al. (1983) listed four categories of thresholds: (1) nonthresholds, where a pest is so damaging and the threshold is too low to be employed; (2) nominal threshold, which is determined by field observation and/or experience rather than data; (3) simple threshold, a threshold developed by research using an average or nominal system for a single pest and is based on correlations of damage potential of the pest, crop market value, costs of control, and potential crop yield; and (4) comprehensive threshold, which incorporates multiple pest and stress factors and is regarded as the ideal model.

Determination of the ET for *D. noxia* was complicated because small grains are grown in several climate zones throughout the U. S. with various cultural practices. *Diuraphis noxia* also infests both winter and spring grains at any plant growth stage. Consequently, EILs and ETs must be applicable to a wide range of climatic zones, production practices, and plant stages. This requires knowledge of aphid injury and yield loss interactions, insect and plant developmental rates, and the effects of stress on the interaction between the aphid and plant (Legg and Archer 1998).

The first economic threshold for *D. noxia* was determined in South Africa by du Toit (1986). He calculated an EIL level of 14% infested plants at growth stage 59 (seed spike clear of the leaf sheath, Tottman and Makepeace 1979) using the formula proposed by Stone and Pedigo (1972) and Ogunlana and Pedigo (1974); $EIL = (ED)/b$, where ED is the economic damage and b is slope of the crop yield on pest infestation level regression. The ET was calculated at 4-7% infested plants at growth stage 31 (first node) by using the formula $ET = EIL\ (C^{-x})$, in which C is the amount of increase in infestation between growth stages 31 and 59 and x is the time in weeks between growth stages 31 and 59 (du Toit 1986). Archer and Bynum (1992) reported a threshold of *D. noxia* on dryland winter wheat which predominates in the western United States and Canada. They concluded an estimate of 0.46% and 0.48% yield loss for each 1% increase in damaged and infested tillers respectively. Girma et al. (1993) calculated an economic injury level of 2.2 and 4.0 aphids per 7 plants for fall infestations in northeastern Kansas in 1988 and 1989, respectively. The spring infestation caused more damage than the fall infestation with economic injury levels of 0.9 and 2.4 aphids per tiller, respectively.

Recently, U. S. scientists realized that the thresholds reported previously were not dynamic because they did not include other factors such as chemical control costs and crop value. Therefore, in the U.S., a dynamic economic threshold was developed that factored in the changing cost of chemical control and crop value into the decision process. This enabled a comprehensive threshold that was sensitive to regional differences. The comprehensive threshold was equally useful in the northern states where emphasis is placed on fall infestations in winter grain, and in southern states where spring infestations are of the most concern. Pesticide use then evolved from being conservative policy against any *D. noxia* population to an as-needed policy based on output from varying economic environment (Legg and Archer 1998). Based on this concept, Archer et al. (1998) concluded a comprehensive economic threshold by different infestation seasons and regions. For spring infestation through all regions, they suggested the formula proposed by Peairs et al. (1991): EIL = (CC*loss per unit) / (EY*MV), where CC = cost of control, EY = estimated yield, and MV = market value of wheat.. However, economic thresholds for fall infestation vary by state: in southwestern states, the loss in bulk seed weight will be 4% per 1% of infested or damage tillers and 0.3% per infestation or damage day. In the central Great Plains or Pacific Northwest states, the value of loss will be 0.7% per percentage of infested or damaged tiller and 0.6% per infestation or damage day. In the coldest states or Canada, the loss value will be 1% per percentage infested or damaged tillers and 1.1% for infestation or damage days.

Management Strategies

In recent years, various management tactics have been studied and applied to manage *D. noxia*. These include: biological control, chemical control, cultural control practices, and host plant resistance.

Biological Control

Even though endemic natural enemies do not seem to play a significant role in regulating *D. noxia* populations in the U. S., biological control is still recognized as a potential tactic for managing *D. noxia*. According to Prokrym et al. (1998), four exotic parasitoid species *Aphidius uzbekistanicus* (Luzhetzki) (Hymenoptera: Aphidiidae), *Aphidius colemani* (Viereck) (Hymenoptera: Aphidiidae), *Aphelinus albipodus* Hayat and Fatima (Hymenoptera: Aphelinidae), and *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) have been established in the U. S. to control *D. noxia*. *Aphelinus* spp. can be found in most regions where *D. noxia* infestation occurs. They are solitary endoparasitoids of nymphs and adults of a variety of aphid species. They are regarded as promising candidates for *D. noxia* biological control because they are active during the season when *D. noxia* is active; they occur throughout most of *D. noxia*'s geographic distribution range, live longer, and can parasitize a large number species of aphids (Hopper et al. 1994). Syrphid flies (Diptera: Syrphidae) are another group of important natural enemies of *D. noxia.* According to Hopper et al. (1994), the most common species found in *D. noxia* colonies are *Episyrphus balteatus* (De Geer), *Eupeodes corollae* (F.), and *Sphaerophoria scripta* L. The adults feed on nectar, honeydew, and pollen, but their larvae prey on aphids. These three species of syrphids are promising predators for *D. noxia* biological control because of their frequent occurrence, large consumption rate, and vigor of their larvae. Gonzalez et al. (1990) summarized in their survey of natural enemies of *D. noxia* that aphelinids and chamaemyiids provided the best potential for rearing and colonizing for *D. noxia* management. Syrphids are next in order of importance, while *Aphidius colemani* (Viereck) and *Aphidius matricariae* (Haliday) are the third.

Other promising species reported by previous researchers include, predators [e.g., *Chrysopa* spp. (Neuroptera: Chrysopidae); *Aphidoletes aphidimyza* (Rondani); *Leucopis* spp. (Diptera: Chamaemyiidae); *Coccinella septempunctata* L.; *C. transversoguttata* Brown; *Propylea quatuordecimpunctata* (L.); *Scymnus frontalis* Fabricius; and *Adonia variegata* (Goeze), (Coleoptera: Coccinellidae)] (Bosque-Pérez et al. 2002), parasitoids [e.g., *Lysiphlebus* spp.; *Aphidius picipes* (Nees); *Aphidius rhopalosiphi* DeStefani-Perez; *Ephedrus plagiator* (Nee); *Diaeretiella rapae* (McIntosh); and *Praon gallicum* Stary

(Hymenoptera: Braconidae) (Brewer et al. 2001)], parasites (e.g., mites), and fungal pathogens [e.g., *Pandora neoaphidis* (Remaudière and Hennebert)] (Feng et al. 1990, 1991).

Chemical Control

Diuraphis noxia colonization of the flag leaves of wheat can be inhibited and significant yield losses prevented by applying insecticides to plants when the first node is visible (du Toit and Walters 1984). Systematic insecticides have been recommended for *D. noxia* control. Contact insecticides are ineffective because *D. noxia* secludes itself within rolled leaves typically associated with the injury symptoms (Webster 1990). Mixing contact and systemic insecticides has reportedly resulted in effective control (Botha 1984, Valiulis 1986).

Numerous registered and unregistered insecticides in the U.S. have been tested against *D. noxia*. Materials most commonly used commercially include dimethoate, disulfoton, phorate, methyl parathion, and parathion. Other promising insecticides such as endosulfan, chlorpyrifos, esfenvalerate, and ethyl parathion also are effective (Pike and Suomi 1988).

Meyer et al. (1990) compared imidacloprid with other granular formulations of disulfoton, phorate terbufos, and carbofuran as seed treatments to control *D. noxia* and obtained effective control with imidacloprid. An evaluation of systemic insecticides applied in furrow at planting time for *D. noxia* control on winter wheat by Armstrong et al. (1989) showed that carbofuran plus liquid fertilizer were effective for aphid control. However, carbofuran was ineffective when applied with dry fertilizer. Hill and Butts

(1990) compared chlorpyrifos with dimethoate and malathion commonly used for aphid control in Canada. They found that chlorpyrifos was effective for *D. noxia* control because of its toxic vapor, efficient penetration through the plant epi-cuticular wax layer, and rapid diffusion to aphid feeding sites.

Chemical applications have limited success because temperature may affect the rate of insecticide absorption into insects and, in the case of a systemic insecticide, the rate of uptake by plants. Rainfall, occurring immediately after application, may dissolve and move active ingredients or mechanically dislodge residues. Volatility, which is directly related to temperature, can result in significant loss of insecticide from leaf surfaces after application (Johnson and Kammerzell 1990). The expense of insecticide application for managing *D. noxia* might be prohibitive and the cost to the environment is of concern (Burd et al. 1994). For instance, Flickinger et al. (1991) reported the poisoning of more than 200 Canada geese, *Branta canadensis* Lamark, in Texas as a direct result of parathion applications to winter wheat for control of *D. noxia*. Moreover, the use of insecticides for controlling *D. noxia* also has been reported to contribute significantly to the recent occurrence of insecticide-resistant greenbugs, *Schizaphis graminum* (Rondani) (Shotkoski et al. 1990, Shufran et al. 1993). Research programs directed toward the control and management of *D. noxia* populations in agroecosystems have emphasized the development of environmentally friendly alternative means of control such as plant resistance and biological and cultural controls (Burd et al. 1994).

Cultural Control

Cultural control can be defined as the alteration of crop environment through modification of normal farm practices for the purpose of either discouraging target pests or of encouraging biological control agents (Peairs 1998). Cultural controls are important management tactics especially for pests of extensively grown, low-return crops where profit margins leave little room for insecticide use (Holtzer et al. 1996). The production of dryland winter wheat typifies such a system. For instance, in 1992 in northeastern Colorado, the average return on winter wheat without federal price support payments on 1 ha of winter wheat was ≈$125 (Nitchie and Schaubert 1993). Cultural controls also help in postponing the development of insecticide resistance.

Various cultural control techniques have been studied for the management of *D. noxia*: modifications in crop diversity, sanitation, grazing, fertilization, irrigation, row spacing, and planting dates (Peairs 1998). Increasing crop diversity is one of the practices that may possibly increase the abundance of the natural enemies of *D. noxia*, however, there are no studies of this technique.

Sanitation is the process of removing volunteer crop plants, crop residues, weeds, alternate hosts, and other potential pest refuges that can be sources of reinfestation (Peairs 1998). A number of alternate hosts of *D. noxia*, mostly cool-season grasses, as indicated above, have been identified (Kindler and Springer 1989, Armstrong et al. 1991). Volunteer wheat is generally considered to be the most important infestation source for the newly emerging crops. Management of volunteer wheat is very important because these plants compete with the crop for moisture, and act as alternative host for many other pests including cereal aphids, the wheat curl mite (*Aceria tosichella* Keifer), and the Hessian fly [*Mayetiola destructor* (Say)] (Stuckey et al. 1989).

Grazing is a very common cultural practice in the southern Great Plains especially in southeastern Colorado where ≈ 50% of the wheat is grazed (Peairs 1998). The frequency of grazing decreases in north regions because of the severe winter conditions. Grazing can affect insects by trampling, ingestion, or competition for host plant tissues. Therefore, under proper utilization, grazing can be an effective cultural control.

Fertilization of wheat is another popular practice in the management of *D. noxia*. Appropriate fertilization will improve the plant's vigor and enable the plant to better tolerate the stresses from *D. noxia* and other unfavorable conditions (Peairs 1998). Proper plant fertilization can also alleviate aphid injury and result in less yield loss. For instance, Riedell (1990) reported a ≈50% more grain produced in the presence of *D. noxia* in 'Marshall' wheat plants provided with 100% of normal nitrogen requirements in comparison with wheat plants given only 10% of the same nutrients. Changes in plant nutrition may also affect the reproductive potential of *D. noxia* (Peairs 1998). Soil moisture can be a limiting factor affecting fertilizer utilization. According to the research by Walker et al. (1990b), the application of varying levels of nitrogen has been beneficial under irrigated conditions when compared with dryland sites. *Diuraphis noxia* is more likely to cause economic losses in dryland wheat than in irrigated wheat, which is most likely attributed to the drought stress in plants (Peairs 1998). Therefore, modification of irrigation practices may provide an opportunity to improve *D. noxia* management. The direction in which a field is irrigated also correlates the severity of *D. noxia* infestation (Hammon and Peairs 1992). In western Colorado, the percentage of infested plants or tillers and aphids per plant or tiller in early spring were ≈ 10 times greater in fields irrigated from east to west than irrigated from north to south on furrow-irrigated 'Schuyler' barley. This was due to the higher temperature accumulations during the winter on the south-facing slopes of fields between the irrigation furrows.

Finally, modification of planting date is another common cultural tactic because crops can be planted within a range of dates that still have acceptable quality and yield. Planting date recommendations for *D. noxia* generally propose that spring wheat should be planted as early as possible to maximize crop maturity by the time *D. noxia* migrates from fall-sown, small-grain crops. This is based on the assumption that more mature plants will be less attractive to the aphids. Meanwhile, the plants will suffer less damage from *D. noxia* infestation when they are closer to maturity (Peairs 1998). On the other hand, decisions on fall seeding are more site-specific. In regions where spring infestation of *D. noxia* is of concern, early fall seeding is recommended because it enables the plants to mature early and be less attractive to aphids during spring *D. noxia* dispersal. Where fall infestations are severe, however, late fall seeding is suggested. During fall aphid dispersal, the crops are still small and presumably less attractive to aphids. Some crops may emerge after aphid dispersal (Peairs 1998). For instance, Butts (1992) in Alberta and Kammerzell and Johnson (1990) in Montana recommended late fall seeding in comparison with Walker et al. (1990a) in Colorado who support an early or intermediate fall seeding date.

Host Plant Resistance

Among the various pest management strategies, host plant resistance may be the most effective strategy, both from economical and environmentally sound perspectives, to manage *D. noxia* on cereal crops. Other management tactics, such as using biological control agents and chemical applications, have limited success because aphid feeding causes leaf rolling that protects aphids from natural enemies and direct contact of insecticides. Consequently, the major initial management tactics for this pest have been limited to systemic insecticides and cultural practices such as delayed plantings in late 1980's (Pike 1988, Smith et al. 1991).

The phenomena of plant resistance to insects are usually based on heritable traits (Panda and Khush 1995). Nevertheless, some characteristics are quite plastic and fluctuate widely under the influence of environmental conditions. The environment may favor the plant or the insect unequally and may prevent or aggravate damage; therefore, it is likely to affect the expression of insect resistant traits. Accordingly, plant resistance to insects may be classified as "genetic," implying characteristics are under the primary control of genetic factors, or "ecological," implying the characteristics are under the primary control of environmental factors.

In terms of plant genetic resistance, Painter (1951) defined insect-resistance as nonpreference, antibiosis, and tolerance. Kogan and Ortman (1978) then proposed to replace the term "nonpreference" with "antixenosis" because the word "nonpreference" is mostly related to the insect feeding behavior instead of host plant's intrinsic response to pest infestation. Plant resistance to insects can be categorized as antibiosis, antixenosis, tolerance or combination of the three categories (Painter 1951, Kogan and Ortman 1978).

Antixenosis is the resistance mechanism employed by the plant to deter or reduce colonization by the insects. Generally, insects orient themselves toward plants for food, oviposition sites, or for shelter. However, because of certain characteristics, the plant deters the insects. In certain situations, even though the insect may come in contact with the plants, the antixenotic characteristics of the plant do not allow the insect to colonize. Sometimes, the antixenosis mechanism is so effective that the insects starve and die (Painter 1968). Antibiosis is the resistance mechanism that functions after the insects have colonized and have started feeding. When an insect feeds on a plant exhibiting antibiotic resistance, its growth, development, reproduction, and survival will be affected. The antibiotic effects may result in a decline in insect size or weight, reduced metabolic processes, increased restlessness, and greater larval or preadult mortality. Indirectly, antibiosis may result in an increased exposure of the insect to its natural enemies. In certain cases antibiosis cannot be clearly separated from antixenosis because of the chemicals or physical factors in the plant may both negatively affect the insect's biological or physiological perspective and be deterrent to the pest. Tolerance is a genetic characteristic that enables the plant to support an insect population that will damage a susceptible host variety. There is no economic yield loss occurring when a plant is tolerant to insect damage. Tolerance does not affect the increase of the pest population but does raise the threshold level.

Not all host plant insect resistance phenomena can be distinguishingly assigned to a single category of resistance. These categories of resistance do not exclude each other, but are correlated along with other biotic and abiotic factors in the expression of resistance. Therefore, different cultivars may possess the same level of resistance but with different resistant mechanisms (Panda and Khush 1995). This phenomenon has also been found in the wheat accessions examined by Souza (1998) for *D. noxia* resistance.

Ecological resistance has been categorized as pseudoresistance and induced resistance (Panda and Khush 1995). Pseudoresistance in the host plant does not result from the inherent characters, but from some temporary favorable environmental conditions (Painter 1951). As a consequence, certain crop varieties may pass through a susceptible stage rapidly and avoid pest damage. Induced resistance is either qualitative or quantitative enhancement of the plant's defense capability against the pests (Panda and Khush 1995).

In the U. S., the first source of significant level of resistance found in wheat to *D. noxia* was in PI 372129 (Turcikum 57 = T-57) in Colorado (Quick et al. 1991). PI 372129 is plant introduction line collected from Russia but generally unadapted to modern agriculture. In 1991, CORWA1, a hard, red winter wheat germplasm, was released for breeding and experimental purposes (Quick et al. 1991). The first North American Russian wheat aphid-resistant hard red winter wheat cultivar, known as 'Halt' (a sister line to CORWA1) was released by Colorado Agricultural Experiment Station in 1994 (Quick et al. 1996). The Western Regional Coordinating Committee No. 66 (WRCC-66) facilitated the identification of *D. noxia*-resistant genes and deployment of these genes in cultivated cereals. Systematic surveys were conducted of landraces and old cultivars from the former USSR, Central Asia, and the Near East. Research groups participating within WRCC-66 have evaluated >25,000 accessions, and identified at least 86 accessions to have reproducible resistance to *D. noxia*. Harvey and Martin (1990), Smith et al. (1991), Webster et al. (1991), and Porter et al. (1993) identified new sources of *D. noxia* resistance in wheat, barley, and triticale accessions originated from these same areas (Souza 1998). These researchers also were able to confirm *D. noxia* resistance in PI 137739, PI 262660, and PI 294994 wheat that were initially identified in South Africa by du Toit (1987).

In recent years, scientists have identified a number of plant introduction lines that possessing at least one main category of resistance. Budak et al. (1999) reported PI 137739 and PI 294994 as antibiotic lines. Of particular interest are accessions that have been identified with strong sources of tolerance and limited or no antibiosis. These wheat accessions include PI 262660 (du Toit 1989, Budak et al. 1999), CI 15465 (Formusoh et al. 1992), Sando collection accession SS36 and SS385 (Formusoh et al. 1994), and PI 366447 (Webster et al. 1991). The sources of *D. noxia* resistance in current cereal breeding programs represent ten unique wheat genes (Table 1) (Quisenberry and Clement 2002).

The biochemical and physiological mechanisms associated with aphid resistance genes are not clearly understood. The resistance factors in PI 386148 triticale and PI 372129 wheat seem to minimize the loss of plant cell turgor pressure that normally result from *D. noxia* feeding (Burd and Todd 1992). *Diuraphis noxia* infestation induces the suppression of large and small subunits of Ribulose 1, 5-bisphosphate carboxylase on wheat and barley (Porter 1992, Rafi et al. 1993, 1996, Miller et al. 1994). Different protein biosynthesis between resistant and susceptible genotypes has also been documented in wheat and barley following *D. noxia* feeding (Miller et al. 1994, Rafi et al. 1996). Ni et al. (2001a) detected peroxidase increase in resistant 'Halt' wheat and suggested the increase may have contributed to *D. noxia* resistance. Preliminary evidence indicates that some of the induced proteins (i.e., ≈32, 33, and 35k polypeptides) may be related to the resistance and early senescence in *D. noxia*-resistant wheat lines (e.g., PI 137739) (Rafi et al. 1996).

Table 1. Conserved cereal germplasm identified with *D. noxia* resistance (Quisenberry and Clement 2002)

Source	Origin	Wheat type or class	Gene	Chromosome location
PI 137739	Iran	Hard white	*Dn1*	7DS
PI 262660	Russia	Hard white	*Dn2*	7DS
SQ 24	Unknown	*Aegilops tauschii*	*dn3*	Unspecified
PI 372129	Russia	Hard white	*Dn4*	1DS
PI 294994	Hungary(Bulgaria)	Hard red	*Dn5*	7DS
			Dn8	7DS
			Dn9	1DL
PI 243781	Iran	White	*Dn6*	Unspecified
Turkey 77	Turkey	*Secale cereale*	*Dn7*	1RS
PI 220127	Afghanistan	Red	*Dnx*	7DS

Etiology of Injury Symptoms

Diuraphis noxia typically aggregates and feeds on the new growth of susceptible host plants. *Diuraphis noxia* is a leaf-gall adapted aphid that induces "pseudogalling" by preventing newly formed leaves from unrolling (Burd and Burton 1992). *Diuraphis noxia* is well adapted to this niche because of its tubular body shape, waxy excretory products, and lack of well-developed cornicles (Nault and Phelan 1984, Wool 1984). Nonetheless, different aphid colonization behavior and plant response have been observed by Burd et al. (1993). Some aphids fail to aggregate on new growth and tend to be widely dispersed on the resistant wheat such as PI 386148. This suggested poor host suitability and an inability of the aphids to alter the feeding site condition. Aphid aggregations may have an adaptive role in aphid performance through a conditioned improvement of the quality of host tissues as a food source (Hayamizu 1984, Dorschner et al. 1987, Dorschner 1990). A positive relationship between aggregation and aphid fecundity has also been shown for aphid species that, under natural conditions, form compact aggregations (Way and Cammell 1970, Hayamizu 1984).

The most obvious characteristics of *D. noxia* injury symptoms are leaf rolling and chlorosis (Walters et al. 1980, Hewitt et al. 1984) which can lead to plant stunting, prostrate growth or even sterility, and finally yield loss. The prevention of new leaf unfolding and reduction in leaf size are resulted from loss of leaf turgor below the threshold for elongation and cell-wall extensibility (Burd and Burton 1992, Burd et al. 1993, Bradford and Hsiao 1992).

Chlorosis indicates the loss of chlorophylls (i.e., chlorophylls *a* and *b*) caused by *D. noxia* feeding. Although *D. noxia*-elicited chlorophyll losses and their effects on plant's photosynthetic efficiency have been studied extensively (Hewitt et al. 1984, Riedell 1989, Burd and Todd 1992, Miller et al. 1994, van der Westhuizen and Pretorius 1995, Burd and Elliott 1996, Ni et al. 2002, Burd 2002, Macedo et al. 2003, Heng-Moss et al. 2003), the biochemical mechanism of chlorosis induced by infestation of *D. noxia* is still unknown.

The toxic effect of *D. noxia* feeding on cereal crops resembles the symptoms of plant diseases. Particularly, the chlorotic streaks resemble plant viral infestation symptoms. Thus, it is not by accident that *D. noxia* was discovered for the first time in several areas by plant pathologists, and that the toxic effects of feeding have been thought to be associated with transmission of plant pathogens (e.g., viruses). Hewitt et al. (1984) concluded that the injury symptoms caused by *D. noxia* infestation were not caused by phytopathogen infection. Fouche et al. (1984) also indicated that the typical symptoms associated with *D. noxia* feeding were induced by a toxin injected during aphid salivation and not by a virus or viruses. Kruger and Hewitt (1984) showed that *D. noxia* extracts adversely affected photosynthetic rates of spinach chloroplasts *in vitro*. They speculated that impact on photosynthetic rates could be secondary to the degradation of the chloroplast membrane or *vice versa*.

Diuraphis noxia is a phloem-feeding aphid whose stylets typically follow an intercellular route through the leaf mesophyll until phloem contact is achieved (Fouche et al. 1984, Girma et al. 1992, Ni and Quisenberry 1997). Early symptom development in susceptible barley indicates that localized changes in the ultrastructure and photosynthetic function of the chloroplast occur adjacent to the stylet paths (Belefant-Miller et al. 1994). Ultrastructural analysis of *D. noxia* injury to wheat by Fouche et al. (1984) described sequential events occurring at the cellular level and demonstrated the involvement of the chloroplast membrane and photosynthetic pigments as primary sites of action in the injury response. In their study, the initial response to *D. noxia* feeding was the retraction and convolution of the plasmalemma, followed closely by the distention of the chloroplast granal and stromal lamellae. The result was a substantial increase in the volume of plastoglobuli. Subsequent degeneration of the chloroplast envelope was followed by the disintegration of other cell organelle membranes that culminated in cell bleaching. Burd and Elliott (1996) compared the chlorophyll content kinetics between susceptible and resistant plants and found *D. noxia* feeding caused significant reductions in chlorophyll *a*, chlorophyll *b*, and total chlorophyll content in susceptible wheat ('Pavon' and 'TAM W-101') and barley ('Wintermalt') but not in resistant plants. Likewise, *D. noxia* infestation resulted in a significant increase of nonvariable fluorescence (F_0), a decrease of maximal fluorescence (F_m), and a variable fluorescence (F_v) in susceptible plants. The photochemical efficiency of photosystem II (F_v/F_m) and the half-rise time from F_0 to F_m ($t_{1/2}$) were reduced significantly in the *D. noxia* infested susceptible entries but remained relatively unchanged in the resistant cultivars. They concluded that *D. noxia* injury went beyond the simple removal of photosynthates from the plant because of the substantial decrease in F_v/F_m following aphid infestation for both susceptible wheat and barley showed a significant decrease in the capacity and efficiency of the primary photochemistry of photosystem II. Haile et al. (1999) compared the changes in CO_2 exchange rates from susceptible and resistant (i.e., antibiotic or tolerant) wheat lines elicited by *D. noxia* infestation. There was significant permanent reduction of photosynthetic rates, especially in PI 137739. Nevertheless, the impact of *D. noxia* feeding on the photosynthetic rates of PI 262660 (tolerance) was less than on PI 137739 (*D. noxia*-antibiotic) and 'Arapahoe' (*D. noxia*-susceptible) wheat. They suggested that this phenomenon was caused by different resistance mechanisms that led to different photosynthetic compensation abilities after aphid feeding.

Chlorophyll breakdown *in vitro* results from the activities of a series of degradative enzymes (e.g., chlorophyllase, Mg-dechelatase, and chlorophyll oxidase) in two degradation pathways (i.e., pheophorbide *a* and oxidative bleaching pathways) (Vicentini et al. 1995, Janave 1997, Matile et al. 1999, Takamiya et al. 2000, Dangl et al. 2000). Commonly, the hydrolysis of chlorophyll into chlorophyllide and phytol is regarded as the initial step of degradation by chlorophyllase in the pheophorbide *a* pathway (Matile et al. 1999). Without any inhibitor (i.e., ascorbate), enzymatic chlorophyll degradation may go through both pheophorbide and oxidative bleaching pathways (Janave 1997). Ni et al. (2001b, 2002) reported a significantly higher Mg-dechelatase activity in *D. noxia*-infested susceptible wheat plant extracts when compared with the *R. padi*-infested and the uninfested controls. However, neither chlorophyllase nor oxidative bleaching activity, described by Janave (1997) and Matile et al. (1999) in naturally senescing plants, was detected in their experiment. They suggested that the increase of Mg-dechelatase activity indicated that chlorosis formation on *D. noxia*-infested plants was possibly an Mg-dechelatase-driven physiological phenomenon and the elicitor for leaf chlorosis might not be the direct result of aphid salivary secretion.

CHAPTER 3

ASSESSMENT OF APHID RESISTANCE IN WHEAT NEAR-ISOGENIC LINES

Introduction

Chlorophyll catabolism can be differentiated into two types. One is senescent chlorophyll catabolism associated with normal progressive senescence and the other is leaf chlorosis in growing plants elicited by herbivore feeding, nutritional deficiencies, or pathogen-infections (Ni et al. 2002). Although the chlorophyll degradation *in vitro* has been well documented (Janave 1997, Matile et al. 1999, Takamiya et al. 2000, Dangl et al. 2000), the mechanism of chlorosis caused by herbivore infestation remains unclear.

The Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae), is an important pest of cereal crops that causes chlorosis, which indicates the loss of photosynthetic pigments (e.g., chlorophyll *a*, chlorophyll *b*, and carotenoids). Photosynthetic pigments are vital for plant growth and without them, light cannot be absorbed and therefore energy cannot be stored. All photosynthetic organisms contain one or more organic pigments capable of absorbing visible radiation that will initiate the photochemical reactions of photosynthesis (Blankenship 2002). Four major classes of pigments found in plants, bacteria, and algae are the chlorophylls (e.g., chlorophyll *a*, *b*, *c* and *d*), the bacteriochlorophylls (e.g., bacteriochlorophyll *a*, *b*, *c*, *d*, *e*, *f* and *g*), the carotenoids (e.g., β-carotene, α-carotene, luteol, violaxanthol and fucoxanthol) and the phycobilins (e.g., Phycoerythrins, phycocyanins and allophycocyanins) (Hall and Rao

1992, Biswal 1995). The pigments in higher plants mainly consist of chlorophyll *a*, chlorophyll *b*, and most of the carotenoids (Blankenship 2002).

Chlorophylls *a* and *b* have the absorption maximum at 663 and 645 nm respectively (in acetone) that gives them characteristic green color. They act as primary light harvesters in plant photosynthesis. Carotenoids, which are responsible for the orange-yellow colors observed in the leaves of plants, absorb light between 400 and 500 nm, a range in which absorption by chlorophylls is relatively weak. As such, carotenoids play a minor role as accessory light-harvesting pigments, absorbing and transferring light energy to chlorophyll molecules (Malkin and Niyogi 2000). Most importantly, carotenoids function in a process called photoprotection. Under the high light intensities often found in nature, plants may absorb more light energy than they can actually use for photosynthesis. This excessive excitation of chlorophylls can result in increased formation of the singlet oxygen that is detrimental to plant photosynthesis (Malkin and Niyogi 2000, Blankenship 2002). Carotenoids are able to accept excitation energy and prevent singlet oxygen formation (Malkin and Niyogi 2000, Blankenship 2002). Therefore, reduction of chlorophylls and/or carotenoids in plants induced by herbivore infestation will negatively affect the photosynthetic capacity of plants.

Many researchers have assessed the impact of sap-feeding herbivore-elicited photosynthetic pigment changes and their effects on plant photosynthetic efficiency (Kruger and Hewitt 1984, Riedell 1989, Burd and Todd 1992, Miller et al. 1994, van der Westhuizen and Pretorius 1995, Burd and Elliott 1996, Ni et al. 2002, Macedo et al. 2003, Heng-Moss et al. 2003). The objectives of this study were to assess the resistance of Tugela and three wheat near-isogenic lines (or isolines) (i.e., Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*) to cereal aphids [i.e., *D. noxia* and the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae)] through comparisons of aphid and plant biomass; to quantify changes of photosynthetic pigment concentrations (i.e., chlorophylls *a* and *b*, and carotenoids) in Tugela wheat lines with three aphid infestation treatments (i.e., *D. noxia*, *R. padi*, and control); to elucidate the effects of photosynthetic pigment changes on photosynthetic capacities among the aphid-infested plants; and to correlate photosynthetic pigment variations with the particular genes among the Tugela and Tugela *Dn* plants that confer aphid resistance.

Materials and Methods

Insects and Plants

Diuraphis noxia, a chlorosis-eliciting species, and *R. padi*, a non-chlorosis-eliciting species used in the experiment were from the colonies established from field-collected aphids. The colony of *D. noxia* was established originally using aphids collected near Scottsbluff, NE in 1994; *R. padi* colony was established using the aphids collected near Lincoln, NE in 1996 (Ni et al. 2001a). Aphids were maintained on 'Stephens' (*D. noxia* susceptible) wheat in Plexiglas cages (30 x 15 x 15 cm) in a Percival^{TM} growth chambers (Percival Scientific, Boone, IA) at 21°C, with a photoperiod of 16:8 (L: D) h and 40-50% RH.

Tugela (susceptible), Tugela-*Dn1* (antibiosis), Tugela-*Dn2* (tolerance), and Tugela-*Dn5* (antibiosis and antixenosis) wheat lines were used in the experiment. Seeds of the four wheat lines were planted at the rate of three plants per Conetainer^{TM} (3.81 cm diameter by 21 cm depth) (Stuewe and Sons, Inc., Corvallis, OR). Conetainers^{TM} were filled with Sunshine™ soil mix No.1 (SunGro Horticulture, Bellevue, WA) and placed in Conetainer™ racks (61 x 30 x 18 cm), leaving a space among Conetainers™ to provide adequate light. Plants were watered uniformly from the bottom by placing a rack over a plastic tray (54 x 28 x 6 cm) filled with water. Before aphid infestation, plants were thinned to two seedlings per Conetainer™. Experiments were maintained in a growth chamber at 21°C with a photoperiod of 16:8 (L: D) h and 40-50% RH.

Aphid Precondition and Infestation

Aphids were preconditioned on Stephens wheat caged with polyethylene tubes (30-cm length by 4-cm diameter) in a Percival™ growth chamber (Percival Scientific, Boone, IA) at 21°C with a photoperiod of 16:8 (L: D) h and 40-50% RH (Schotzko and Smith 1991). The adults were placed on Stephens wheat plants with three leaves at growth stage 13 of Zadoks scale (Zadoks et al. 1974) and removed after three days. Nymphs were maintained on the plants for approximately 10 days before infestation. Thus, the protocol provided us with age-specific aphids with a 3-day age variation.

There were three types of aphid infestations on plants: 0 aphid, 10 *R. padi* adults, and 10 *D. noxia* adults. The experiment was initiated when plants were at Zadoks stage 13. All plants were caged using polyethylene tubes and maintained in a Percival™ growth chamber under the conditions described previously.

Collection of Aphid and Plant Samples and Chlorosis Evaluation

Wheat leaves and aphids from the experimental plants were collected on the 3rd, 6th, 9th, and 12th days after initial aphid infestation. Aphids (i.e., *D. noxia* and *R. padi*) were weighed to evaluate effects of Tugela wheat lines. Chlorotic injuries of wheat leaves caused by *D. noxia* infestation were qualitatively evaluated by rating the relative amount of chlorosis based on the report by Webster (1990) and Burd et al. (1993). Chlorosis was quantified using a nine point scale define the chlorosis area on leaves: 1, plants appear healthy and have scattered yellow spots; 2, isolated chlorotic spots obvious; 3, chlorosis ≤15% of total leaf area, chlorotic lesions coalesced; 4, chlorosis >15% but ≤25% of total leaf area, leaf streaks appear; 5, chlorosis >25% but ≤40% of total leaf area, obvious streaks; 6, chlorosis >40%, but ≤55% of total leaf area; 7, chlorosis >70%, but ≤85% of total leaf area; 9, plant appears dead or beyond recovery (Webster 1990).

After the evaluation of chlorosis, leaf samples were excised and weighed. A small part (0.3 g) of the entire leaf samples was randomly selected for pigment assay, the rest was analyzed later for chlorophyll degradation enzyme activities. All leaf samples were stored in a -20°C freezer until final analysis.

Photosynthetic Pigment Measurement

Control and aphid-infested plant leaves (about 0.3 g) were ground with liquid nitrogen in a mortar and pestle under low light condition. Acetone (3 ml of 80%) was added to extract photosynthetic pigments (i.e., chlorophyll *a*, chlorophyll *b*, and total carotenoids). About 1.5 ml of the mixture was aspirated by polyethylene pipette (Fisher Scientific, Pittsburgh, PA) and centrifuged at 6000 x g for 10 min to remove insoluble plant tissues. Final supernatant was diluted with 80% acetone to ensure the absorbance readings at 663 nm were lower than 1.5. The absorbance of pigment extracts were measured in a spectrophotometer respectively at wavelength of 470 nm, 646 nm, and 663 nm. Concentration of the three types of pigments was obtained following the equation described by Bertrand and Schoefs (1997):

$$C_a = 12.21\ A_{663} - 2.81\ A_{646}$$

$$C_b = 20.13\ A_{646} - 5.03\ A_{663}$$

$$C_c = (1000\ A_{470} - 3.27\ C_a - 104\ C_b)/198$$

C_a, C_b, and C_c are the concentrations in μg / ml of chlorophyll *a*, chlorophyll *b*, and total carotenoids, respectively. A_x represents the absorbance at x nm. Final concentrations of the pigments were determined in microgram per gram of fresh wheat leaf tissue (μ g/g). Thus, $C_{a,\ b,\ c\ (final)} = (C_{a,\ b,\ c} \times r \times v)/w$ (r is the dilute ratio of pigment measurement, v and w are the volume of 80% acetone to extract the pigments and the weight of sample plant leaves respectively). C_a / C_b and C_{a+b}/ C_c also were calculated to determine the photosynthetic efficiency.

Experimental Design and Data Analysis

The experiment was a split-split plot design. The experiment was repeated six times. Four sampling dates (3rd, 6th, 9th, and 12th) were the main plots within each trial. Three aphid treatments (control, *D. noxia,* and *R. padi*) were the sub-plots within each main plot (sampling dates) and the four wheat lines (Tugela, Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5*) were the sub-sub-plots within each sub-plot (aphid treatments). Six plants were used for each treatment on each sampling date and therefore, 36 plants in total were used per treatment per sampling date. Data were analyzed using the PROC GLM procedure of the SAS software followed by TEST statements to ensure correct error terms were used in assessing main effects of experimental factors (Cochran and Cox 1957, SAS Institute 1989). The means were separated using the Fisher's LSD test ($\alpha = 0.05$).

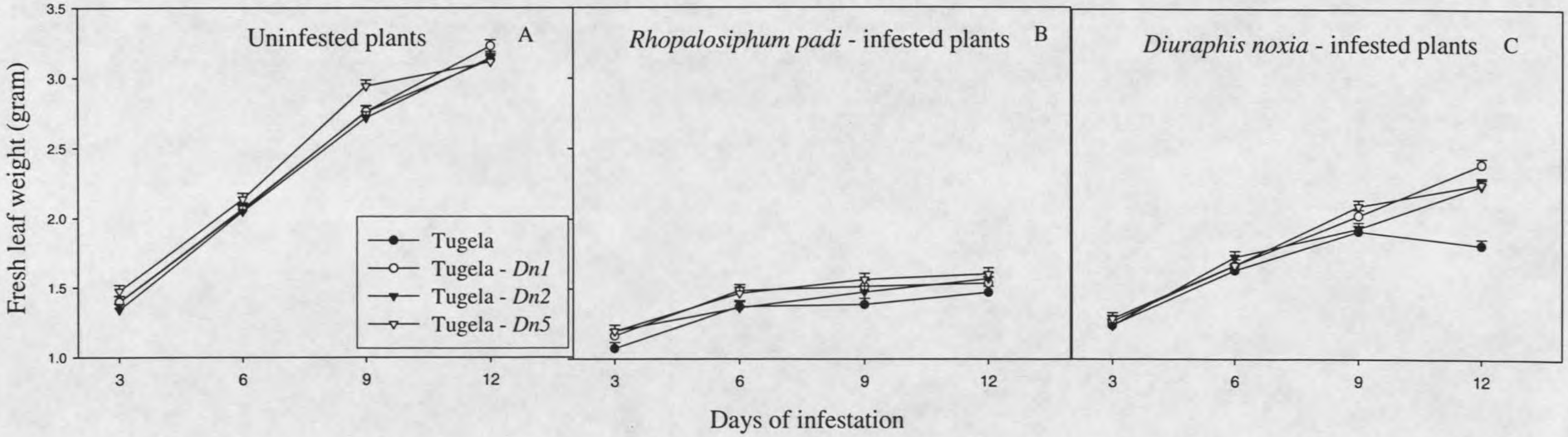
Results

Plant Biomass

Plant weight of the Tugela wheat lines was significantly affected by the three-way (isoline by date by aphid) interaction ($F = 2.41$, df = 18, 90, $P = 0.0034$). Also, plant weights were significantly affected by aphid-date interaction ($F = 94.15$, df = 6, 30, $P < 0.0001$), and isoline-date interaction ($F = 2.96$, df = 9, 45, $P = 0.0075$), but not by isoline-aphid interaction ($F = 1.59$, df = 6, 30, $P = 0.1835$). Significantly different plant weight was detected in the four wheat lines ($F = 3.88$, df = 3, 15, $P < 0.0310$), the four sampling dates ($F = 254.74$, df = 3, 15, $P < 0.0001$), and the three aphid infestation treatments ($F = 179.34$, df = 2, 10, $P < 0.0001$). Because the three-way interaction significantly affected plant weight, plant weights were further analyzed within each aphid treatment.

Plant weight of the uninfested plants was higher than aphid-infested plants (Fig. 1). Although injury symptoms (e.g., chlorosis and leaf rolling) appeared only on *D. noxia*-infested plants, plant weights of all *D. noxia*-infested wheat lines were greater than those of *R. padi*-infested plants in all sampling dates (Fig 1). Therefore, Tugela isolines that had been reported to be resistant to *D. noxia* were not resistant to *R. padi*. We found that when infested by *D. noxia*, all Tugela *Dn* plants sustained growth throughout the infestation durations (Fig. 1C). However, plant weight of Tugela decreased on day 12.

Figure 1. Temporal changes of plant biomass of the four wheat lines with three aphid treatments A: Uninfested Tugela plants; B: *R. padi*-infested Tugela plants; C: *D. noxia*-infested Tugela plants (n = 18). Each data point represents the mean on each sampling date. Error bar indicates the standard error of the mean.



This finding confirmed that Tugela is susceptible to *D. noxia* infestation, while Tugela-*Dn1*, Tugela-*Dn2*, and Tugela-*Dn5* are resistant. No statistical difference in plant weight was detected among *R. padi*-infested and uninfested Tugela wheat lines on all sampling dates (Figs. 1A and 1B).

Aphid Biomass

Biomass of aphids collected from Tugela wheat lines was significantly affected by the three-way (isoline by date by aphid) interaction ($F = 4.39$, df = 9, 45, $P = 0.0004$). Aphid biomass also was significantly affected by all the two-way interactions (P-values < 0.0001). Significantly different aphid biomass was detected in the four isolines ($F = 72.92$, df = 3, 15, $P < 0.0001$), the four sampling dates ($F = 650.20$, df = 3, 15, $P < 0.0001$), and between the two type of aphids ($F = 504.32$, df = 1, 5, $P < 0.0001$). Because the three-way interaction was significant, temporal developments of *R. padi* and *D. noxia* biomass were analyzed.

Rhopalosiphum padi biomass was not affected by date-isoline interaction ($F = 0.63$, df = 9, 45, $P = 0.7686$). No significant difference was detected in aphid biomass of *R. padi* collected from different wheat lines ($F = 2.51$, df = 3, 15, $P = 0.0983$) (Fig. 2A). However, significant difference was found in biomass of *R. padi* collected at different dates ($F = 178.82$, df = 3, 15, $P < 0.0001$).

Biomass of *D. noxia* was significantly affected by date-isoline interaction ($F = 10.05$, df = 9, 45, $P < 0.0001$) (Fig. 2B), which indicated different impacts on *D. noxia* growth rate among the four wheat lines. Significantly different aphid biomass was detected in *D. noxia* collected from different wheat lines ($F = 202.09$, df = 3, 15, $P <$