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Comparison of Chlorophyll and Carotenoid Concentrations Among Russian Wheat Aphid (Homoptera: Aphididae)-Infested Wheat Isolines

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Comparison of Chlorophyll and Carotenoid Concentrations Among Russian Wheat Aphid (Homoptera: Aphididae)-Infested Wheat Isolines

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ABSTRACT Russian wheat aphid, *Diuraphis noxia* (Mordvilko), feeding injury on 'Betta' wheat isolines with the *Dn1* and *Dn2* genes was compared by assessing chlorophyll and carotenoid concentrations, and aphid fecundity. The resistant Betta isolines (i.e., Betta-*Dn1* and Betta-*Dn2*) supported similar numbers of aphids, but had significantly fewer than the susceptible Betta wheat, indicating these lines are resistant to aphid feeding. *Diuraphis noxia* feeding resulted in different responses in total chlorophyll and carotenoid concentrations among the Betta wheat isolines. The infested Betta-*Dn2* plants had higher levels of chlorophylls and carotenoids in comparison with uninfested plants. In contrast, infested Betta-*Dn1* plants had the same level of chlorophyll and carotenoid in comparison with uninfested plants. Our data provide essential information on the effect of *D. noxia* feeding on chlorophyll and carotenoid concentrations for Betta wheat and its isolines with *D. noxia*-resistant *Dn1* and *Dn2* genes.

KEY WORDS Russian wheat aphid, *Diuraphis noxia*, wheat, chlorophyll, carotenoid, plant resistance

THE RUSSIAN WHEAT APHID, *Diuraphis noxia* (Mordvilko), is a serious pest of cereal crops throughout the western United States, causing significant annual yield losses to wheat and barley (Webster et al. 1991, Burd and Burton 1992, Miller et al. 1994). *Diuraphis noxia* feeding results in destruction of plant chloroplasts that ultimately leads to reduced chlorophyll levels and photosynthetic activity (Burd and Elliott 1996, Rafi et al. 1996). It is not known, however, whether the aphid injects a phytotoxin during feeding that degrades chloroplasts or if the damage results from the plant's response to mechanical injury. Damage symptoms associated with aphid feeding include plant stunting, chlorosis, white streaking, and leaf rolling (Webster et al. 1987, Webster et al. 1991, Burd and Burton 1992, Burd et al. 1993).

Because the Russian wheat aphid was identified as an important pest of wheat and other cereal crops, extensive efforts have been undertaken to identify resistant wheat germplasm and to characterize their mechanisms of resistance (Burd and Elliott 1996, Rafi et al. 1996, Rafi et al. 1997, van der Westhuizen and Pretorius 1995, Haile et al. 1999). Various researchers (Burd and Elliott 1996, Rafi et al. 1996, Rafi et al. 1997, Miller et al. 1994, van der Westhuizen and Pretorius

1995) have also explored the influence of aphid feeding on chlorophyll loss in resistant and susceptible wheat, but these studies have often produced contradictory results. For example, Rafi et al. (1996) reported that susceptible plants have similar chlorophyll concentration levels as their respective uninfested plants after exposure to *D. noxia*, whereas resistant plants infested with *D. noxia* had reduced levels of chlorophyll when compared with uninfested plants. Conversely, Burd and Elliott (1996) found a significant decline in chlorophyll concentration in the infested leaf tissue of *D. noxia*-susceptible wheat and barley, whereas total chlorophyll concentration was not significantly affected by *D. noxia* in resistant wheat or barley. Thus, additional research is needed to confirm the effects of aphid feeding on chlorophyll loss in wheat with varying levels of aphid resistance.

The objective of this study was to determine the effects of *D. noxia* feeding on chlorophyll and carotenoid concentrations in resistant and susceptible wheat. Because no similar studies have been conducted to assess the effect of aphid feeding on carotenoid levels in resistant wheat, this research represents an initial effort to characterize the effects aphid feeding has on both chlorophyll and carotenoid loss in Betta wheat isolines.

Materials and Methods

Interactions between Betta (susceptible parent) wheat (Du Toit 1988) and its two isogenic lines (Betta-

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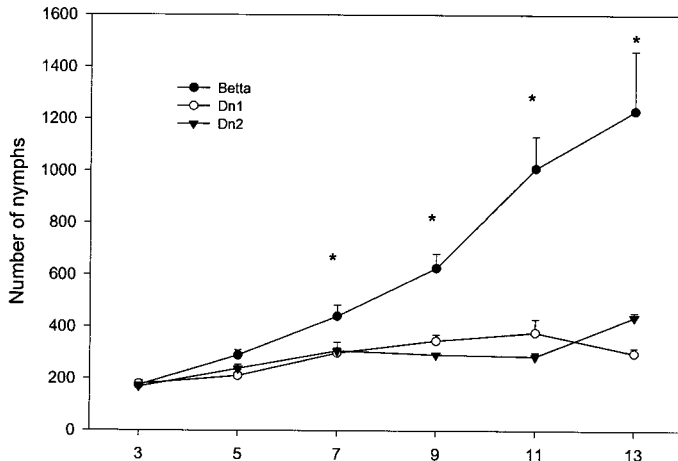


Fig. 1. Mean number of aphids produced on each wheat isolate. *Significantly different at $P < 0.05$, LSD.

Dn1, antibiosis and Betta-*Dn2*, tolerance) (Du Toit 1989, Budak et al. 1999) challenged by two *D. noxia* densities (0 and 20 aphids) were evaluated in this study. Plants from each of the three wheat lines were infested with 20 *D. noxia* at 14 d after planting. The *D. noxia* colony used in this study was collected from wheat fields near Scottsbluff, NE, in 1994, and maintained in the laboratory on 'Stephens' (susceptible) wheat cultivar. Aphids were introduced onto the second fully expanded leaf blade using a camel hair brush. Tubular, Plexiglas cages (33.5 cm diameter \times 8.5 cm height) served to confine aphids on the seedlings. Noninfested seedlings were also caged to ensure that all plants received the same microenvironmental conditions, especially light. Experiments were conducted in a growth chamber that was maintained at $22 \pm 2^\circ\text{C}$ with a 16:8 (L:D) h photoperiod and 40–50% RH. The experimental design was a randomized complete block design with six replications.

The concentrations of total chlorophyll, chlorophylls *a* and *b*, and carotenoids were measured at 3, 5, 7, 9, 11, and 13 d after aphid introduction to assess the effect of aphid feeding on chlorophyll and carotenoid loss in Betta wheat and resistant isolines. In addition, the number of aphids was recorded on each evaluation date to determine the level of antibiosis for Betta-*Dn1* and Betta-*Dn2*.

Chlorophyll measurements were performed to determine the chlorophyll concentrations in both the aphid-injured portion of the leaf blade and the entire infested leaf blade. The chlorophyll concentration in the injured portion of the infested leaf blade was measured at three locations per leaf blade (2.5, 7.5, and 12.5 cm from the leaf sheath) using a chlorophyll meter (model Spad-502, Minolta, Japan). Chlorophyll concentration was also measured from the same location on the noninfested control leaf blade to permit comparison between infested and uninfested. Total leaf chlorophyll, chlorophylls *a* and *b*, and carotenoid concentrations were quantified using the biochemical extraction methods described by Arnon (1949) and Snell and Snell (1937), respectively.

Mixed model analysis (PROC MIXED, SAS Institute 1997) was conducted for each measurement to detect differences in aphid numbers, total chlorophyll, chlorophylls *a* and *b*, and carotenoid concentrations among wheat lines and aphid infestation levels (Littell et al. 1996). Block and block X treatment were the random effects in the model. When appropriate, means were separated using Fisher least significant difference (LSD) procedure.

Results and Discussion

Aphid Fecundity. No significant differences were detected in numbers of nymphs among the three wheat lines at 3 and 5 d after aphid introduction (day 3: $F = 0.1$; $df = 2, 8$; $P < 0.91$; day 5: $F = 2.9$; $df = 2, 8$; $P < 0.12$) (Fig. 1). However, the total number of nymphs among the three wheat lines was significantly different starting at 7 d (day 7: $F = 4.5$; $df = 2, 8$; $P < 0.04$; day 9: $F = 24.7$; $df = 2, 8$; $P < 0.0004$; day 11: $F = 25.5$; $df = 2, 8$; $P < 0.0003$; day 13: $F = 15.8$; $df = 2, 8$; $P < 0.002$). The greatest number of aphids was recorded on Betta wheat, indicating this line is the most suitable host for *D. noxia* reproduction. In contrast, Betta-*Dn1* and Betta-*Dn2* supported similar numbers of aphids, but had significantly fewer than Betta. This study demonstrates that the two Betta isolines are antibiogenic to *D. noxia*. Studies by Du Toit (1989) and Ni and Quisenberry (1997) have previously characterized the *Dn1* gene as antibiogenic to *D. noxia*. Haile et al. (1999) reported that PI 262660 (tolerance, *Dn2* gene) also possesses antibiosis. Our data concur that the *Dn2* gene also may be antibiogenic, because *D. noxia* population levels were significantly lower on Betta-*Dn2* than on the Betta parent (Fig. 1).

Chlorophyll Concentration. Chlorophyll concentration, as determined by the chlorophyll meter, was significantly different among treatments (day 3: $F = 4.1$; $df = 5, 20$; $P < 0.01$; day 5: $F = 29.0$; $df = 5, 20$; $P < 0.0001$; day 7: $F = 7.9$; $df = 5, 20$; $P < 0.0003$; day 9: $F = 12.3$; $df = 5, 20$; $P < 0.0001$; day 11: $F = 12.9$; $df = 5, 20$; $P < 0.0001$; day 13: $F = 8.3$; $df = 5, 20$; $P < 0.0003$)

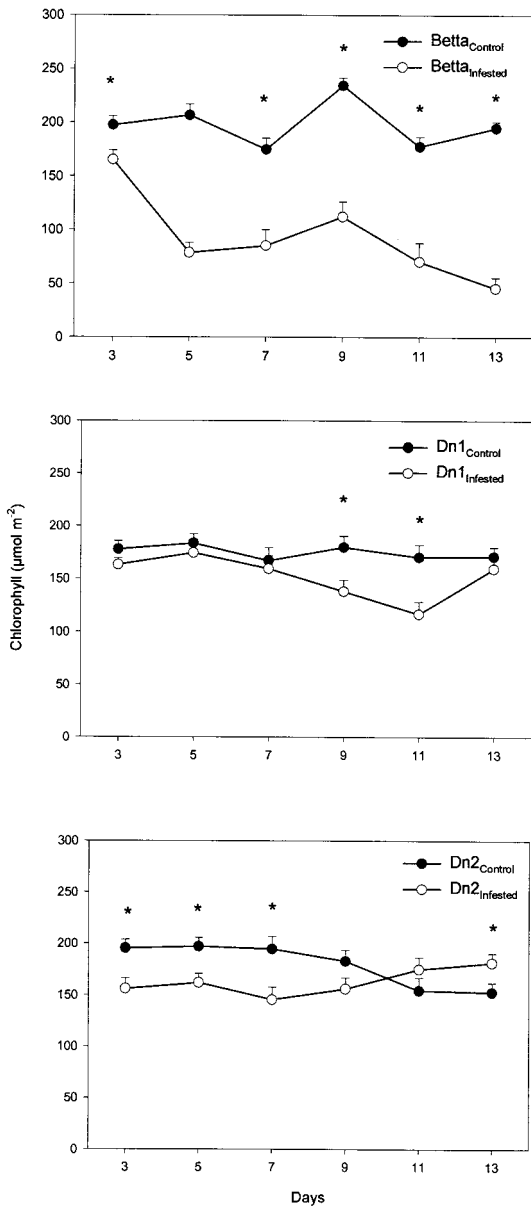


Fig. 2. Mean chlorophyll content as determined by the chlorophyll meter. *Significantly different at $P < 0.05$, LSD.

(Fig. 2). Although all three infested wheat lines exhibited aphid injury, the amount of chlorophyll loss in the injured portion of the leaf blades differed dramatically. The chlorophyll concentration in the uninfested Beta leaves was significantly higher than aphid-infested Beta leaves. This decline in chlorophyll indicated that aphid feeding was adversely affecting the plant and directly impacting chlorophyll content. The chlorophyll concentrations in the aphid-infested resistant isolines were similar to levels observed in their respective uninfested plants. This indicates that aphid feeding may have less effect on chlorophyll loss in resistant wheat.

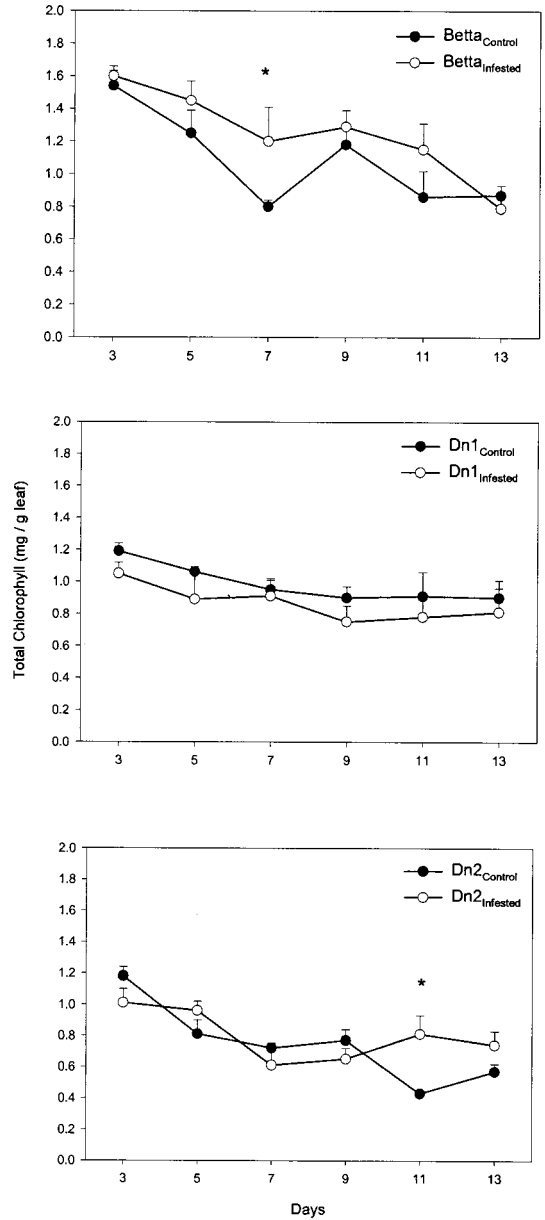


Fig. 3. Mean chlorophyll content as determined by the biochemical extraction method described by Arnon (1949). *Significantly different at $P < 0.05$, LSD.

Total chlorophyll concentration, as determined by chlorophyll extraction, was significantly different among the wheat lines examined on each evaluation date, except at 13 d after aphid infestation (day 3: $F = 10.5$; $df = 5, 20$; $P < 0.0001$; day 5: $F = 5.3$; $df = 5, 20$; $P < 0.003$; day 7: $F = 3.1$; $df = 5, 20$; $P < 0.03$; day 9: $F = 9.4$; $df = 5, 20$; $P < 0.0001$; day 11: $F = 3.4$; $df = 5, 20$; $P < 0.02$; day 13: $F = 1.4$; $df = 5, 20$; $P < 0.3$) (Fig. 3). Although chlorophyll meter readings indicated that aphid feeding directly impacted chlorophyll concentration in the injured portion of the

infested Betta leaf, the entire leaf chlorophyll concentration showed the ability of the infested leaf to maintain a chlorophyll concentration similar to an uninfested leaf. Infested Betta plants had a higher chlorophyll concentration than the leaves from the uninfested plants at each evaluation date except day 13. Possible explanations for the ability of infested plants to maintain chlorophyll concentrations similar to their uninfested controls include delayed chlorophyll degradation, increased chlorophyll production, and resource allocation of chlorophyll. However, by day 13, infested plants may no longer be able to compensate for chlorophyll loss.

The infested Betta-*Dn1* leaves had a lower chlorophyll concentration when compared with the leaves of the Betta-*Dn1* uninfested plants, suggesting an aphid-induced loss of chlorophyll and an inability of this antibiotic line to compensate for chlorophyll loss in the leaf blades infested with aphids. Haile et al. (1999) found a significant decline in photosynthetic rate in aphid-injured leaves of PI 137739 (antibiotic wheat line, *Dn1* gene) and speculated that this decline in photosynthetic rate may have resulted from increased synthesis of chemical defense compounds in response to herbivory. Thus, the decline in chlorophyll concentration in the Betta-*Dn1* may also be attributed to increased production of defensive compounds.

The chlorophyll concentrations in infested and uninfested Betta-*Dn2* leaves were similar at each evaluation date. The greatest difference in chlorophyll concentration between infested and uninfested plants was observed at 11 and 13 d after aphid infestation. The ability of infested Betta-*Dn2* plants to maintain a chlorophyll concentration in the infested leaf blade similar to or greater than the uninfested leaf blade suggests that Betta-*Dn2* is able to compensate for aphid feeding. The increased concentration of chlorophyll at 11 and 13 d after infestation may have contributed to the increased level of tolerance for this line. These results are consistent with studies conducted by Burd and Elliott (1996) and van der Westhuizen and Pretorius (1995). Haile et al. (1999) reported photosynthetic compensation in the *D. noxia* tolerant plant introduction line PI262660. Photosynthetic measurements of the tolerant plant introduction line PI262660 began recovering 3 d after aphid removal and achieved complete photosynthetic recovery 7 d after aphid removal. This gradual photosynthetic compensation did not occur in Arapahoe (susceptible wheat) or the plant introduction line PI137739 (antibiosis).

Concentrations of chlorophylls *a* and *b* were similarly impacted by *D. noxia* feeding (chlorophyll *a*: day 3: $F = 7.5$; $df = 5, 20$; $P < 0.0002$; day 5: $F = 4.3$; $df = 5, 20$; $P < 0.006$; day 7: $F = 3.3$; $df = 5, 20$; $P < 0.02$; day 9: $F = 8.2$; $df = 5, 20$; $P < 0.0001$; day 11: $F = 2.7$; $df = 5, 20$; $P < 0.05$; day 13: $F = 1.4$; $df = 5, 20$; $P < 0.3$ and chlorophyll *b*: day 3: $F = 12.3$; $df = 5, 20$; $P < 0.001$; day 5: $F = 3.6$; $df = 5, 20$; $P < 0.01$; day 7: $F = 3.0$; $df = 5, 20$; $P < 0.03$; day 9: $F = 7.8$; $df = 5, 20$; $P < 0.0002$; day 11: $F = 2.6$; $df = 5, 20$; $P < 0.05$; day 13: $F = 0.9$; $df = 5, 20$; $P < 0.5$). Infested *Dn1* plants had lower concentrations of chlorophyll *a* and *b* when com-

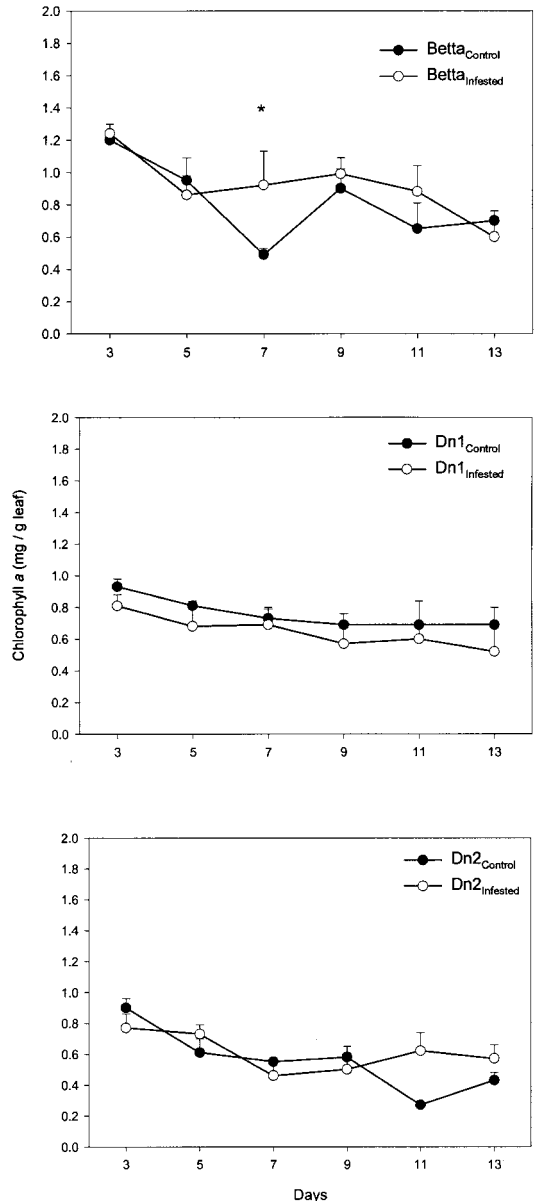


Fig. 4. Mean chlorophyll *a* content as determined by the biochemical extraction method described by Arnon (1949). *Significantly different at $P < 0.05$, LSD.

pared with uninfested plants on all evaluation dates, whereas infested Betta and *Dn2* plants had similar concentrations or slightly higher concentrations of chlorophyll *a* and *b* when compared with uninfested plants (Figs. 4 and 5). Chlorophyll *a:b* ratios for *D. noxia* infested plants were not significantly different from uninfested plants, suggesting that both chlorophyll *a* and *b* concentration levels declined proportionately.

Carotenoid Concentration. The level of carotenoids among the three wheat lines was significantly different

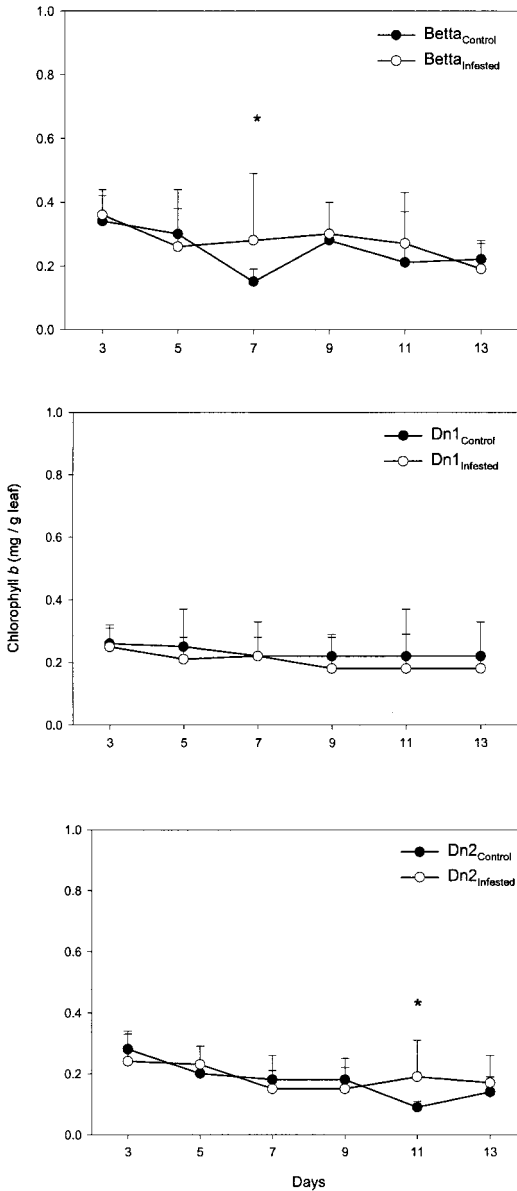


Fig. 5. Mean chlorophyll *b* content as determined by the biochemical extraction method described by Arnon (1949). *Significantly different at $P < 0.05$, LSD.

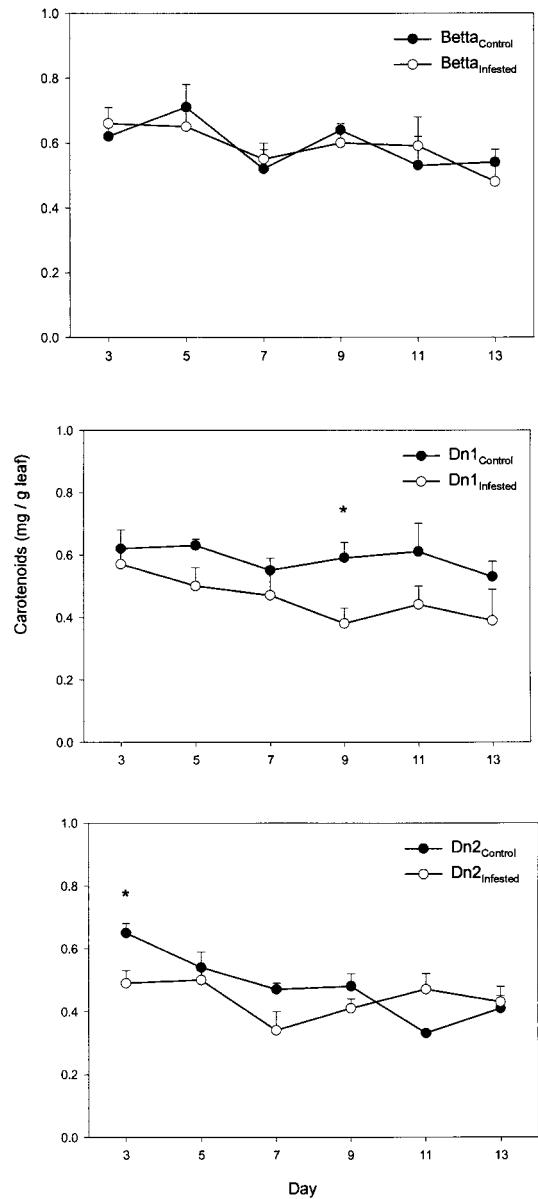


Fig. 6. Mean carotenoid content as determined by the biochemical extraction method described by Snell and Snell (1937). *Significantly different at $P < 0.05$, LSD.

at 5, 9, and 11 d after aphid infestation (day 5: $F = 4.6$; $df = 5, 20$; $P < 0.007$; day 9: $F = 8.9$; $df = 5, 20$; $P < 0.0002$; day 11: $F = 3.0$; $df = 5, 20$; $P < 0.04$) (Fig. 6). However, no significant differences in carotenoid concentrations among the six treatments were detected at 3, 7, and 13 d after aphid infestation (day 3: $F = 2.5$; $df = 5, 20$; $P < 0.06$; day 7: $F = 1.8$; $df = 5, 20$; $P < 0.16$; day 13: $F = 0.8$; $df = 5, 20$; $P < 0.56$). In general, the carotenoid concentration of the three wheat lines followed a trend similar to that observed for the chlorophyll, suggesting chlorophyll and carotenoid

concentrations of resistant and susceptible wheat may be similarly affected by *D. noxia* feeding. Infested and uninfested Beta plants had similar levels of carotenoids at each evaluation date. Carotenoid concentrations were lower for infested Beta-*Dn1* plants when compared with uninfested plants. Initially, infested Beta-*Dn2* plants also had a lower carotenoid concentration than the uninfested plants; however, at day 11, carotenoid concentrations were higher in the infested plants than their respective control plants. Because carotenoids serve as protective agents of cellular membranes, the removal of

carotenoid pigments may result in the degradation of the membranes (Timko 1998). This may help explain similarities in the patterns of carotenoid and chlorophyll loss in the susceptible and resistant wheat.

Despite the fact that we did not observe significant differences in chlorophyll *a:b* ratios between infested and uninfested plants, strong indications, such as reductions in chlorophyll *a* and *b* and carotenoid contents, suggest that *D. noxia* feeding negatively impacts the stacked region of the thylakoid membranes (Fouche et al. 1984). However, the exact site of damage is still unknown. One potential site for *D. noxia* damage is the light harvester complex II, in which chlorophylls (*a* and *b*) and carotenoids (luteins) play important roles as chromophores (Kühlbrandt 1994). Carotenes are also found in the antenna and act by protecting the photosynthetic apparatus from light damage generated by excessive excitation of the photosystem that could result in the excessive reduction in electron transport components. β -carotene, for example, is able to convert back products from this excessive excitation, such as the triplet state of chlorophyll and the singlet state of oxygen, to the corresponding ground state, dissipating this excessive energy as heat and, thus, plants can keep pace of their metabolism. The exact mechanism by which *D. noxia* affects plant metabolism is not fully understood at this time, but we speculate that, by feeding mainly on phloem tissue, *D. noxia* elicits a change in the pH either in the luminal side of the thylakoid membrane avoiding the formation of zeaxanthin, or in the stromal side where the regeneration of violaxanthin takes place. Both of these carotenoids are responsible for the nonphotochemical quenching of exciton energy (Heldt 1997). The reduction in chlorophyll *a* is also an indication that the other potential site for *D. noxia* damage is the photosystem II reaction center in which a special pair of chlorophyll molecules, chlorophyll *a*, is responsible for the transfer of electrons inside of the reaction center (Heldt 1997). Burd and Elliott (1996) reported that the primary site for the damage may be at the reaction center protein, the D1 protein, which even in normal photosynthetic conditions has a high turnover rate (Heldt 1997). *Diuraphis noxia* feeding could reduce protein synthesis making the photo-inhibition irreversible in addition to the blockage in electron transport on the acceptor site of the photosystem II reaction center causing an over-reduction in the system (Burd and Elliott 1996).

Our data provide essential information on the effect of *D. noxia* feeding on chlorophyll and carotenoid concentrations for Betta wheat and its isolines with *D. noxia*-resistant *Dn1* and *Dn2* genes. Changes in total leaf chlorophyll and carotenoid concentrations in response to *D. noxia* feeding suggest a feeding-induced stress response in both resistant and susceptible wheat. However, the resistant isolate Betta-*Dn2* showed minimal loss of chlorophylls and carotenoids even after 13 d of aphid feeding. This suggests Betta-*Dn2* can compensate for aphid feeding damage. Further research is needed to investigate the mechanisms of resistance for the Betta-*Dn2* isolate and explore the

potential use of photosynthetic pigments (e.g., chlorophylls and carotenoids) and other plant pigments as markers for identifying *D. noxia* and other chlorosis-eliciting insect resistant germplasm.

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