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Xinzhi Ni
Montana State University, Bozeman, MT

Sharron S. Quisenberry
Montana State University - Bozeman

John P. Markwell
University of Nebraska-Lincoln, jmarkwell2@unl.edu

Tiffany Heng-Moss
University of Nebraska-Lincoln, thengmoss2@unl.edu

Leon G. Higley
University of Nebraska-Lincoln, lhigley1@unl.edu

See next page for additional authors

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In vitro enzymatic chlorophyll catabolism in wheat elicited by cereal aphid feeding

Xinzhi Ni[1], Sharron S. Quisenberry[1], John Markwell[2], Tiffany Heng-Moss[3], Leon Higley[3], Frederick Baxendale[3], Gautam Sarath[2] & Robert Klucas[2]

[1]*Department of Entomology, Montana State University, Bozeman, MT 59717, USA.* Tel.: (406)994-7626; Fax: (406)994-6029; E-mail: xni@montana.edu

[2]*Department of Biochemistry, and*

[3]*Department of Entomology, University of Nebraska–Lincoln, Lincoln, NE 68583, USA*

Abstract: Chlorophyll degradation is a complex phenomenon that often accompanies insect feeding damage to plants. Loss of chlorophyll can be initiated by several reactions, including oxidative bleaching, chlorophyllase activity, and Mg-dechelatase activity. Extracts from the Russian wheat aphid [*Diuraphis noxia* (Mordvilko)], the bird cherry-oat aphid [*Rhopalosiphum padi* (L.)], and aphid-infested and uninfested wheat plants were assayed *in vitro* for activities involved in chlorophyll degradation. Although the initial infestation was the same (10 apterous adults) for both aphid species, *D. noxia* weight was significantly higher than *R. padi* after feeding for 12 days. Consequently, *D. noxia* feeding caused greater fresh leaf weight reduction than *R. padi* feeding. Chlorophyll degradation assays showed no activity from either *D. noxia* or *R. padi* extracts. Plant extract assays showed a significant difference in Mg-dechelatase activity, while no difference was detected in either the chlorophyllase or oxidative bleaching pathways among the aphid-infested or uninfested plant extracts. *Diuraphis noxia*-infested leaf extracts showed a greater increase of Mg-dechelatase activity than either *R. padi*-infested or the uninfested plants. The findings suggest that leaf chlorosis elicited by *D. noxia* feeding is different from the chlorophyll degradation that occurs in natural plant senescence. Aphid-elicited chlorosis might be the result of a Mg-dechelatase-driven catabolism of chlorophyll in challenged wheat seedlings, however, the factor(s) from *D. noxia* that elicited the increase of Mg-dechelatase activity still remain to be determined.

Keywords: chlorophylls *a* and *b*, carotenoids, chlorophyllase, oxidative bleaching, Mg-dechelatase, Russian wheat aphid, bird cherry-oat aphid, Hemiptera, Aphididae

Introduction

It has been estimated that more than a billion tons of chlorophyll are degraded naturally every year (Tsuchiya et al., 1999). This natural breakdown of chlorophylls occurs at specific plant development stages, such as leaf senescence and fruit ripening. Although studies of chlorophyll degradation were conducted at the beginning of the 20th century, it has only sporadically received attention in the last century (Matile et al., 1999). In fact, there have only been a few reports on natural chlorophyll degradation until the last two decades. The chlorophyll degradation process in naturally senescing plants involves three enzymes in two pathways (Figure 1): the pheophorbide pathway (Matile et al., 1999) or the oxidative bleaching pathway (Janave, 1997).

For the most part, our understanding of the biochemical processes of seasonally occurring chlorophyll degradation has outpaced the acquisition of knowledge concerning chlorophyll degradation associated with biotic stresses like herbivory. Indeed, chlorophyll and other plant pigment losses caused by herbivore feeding are not clearly understood, although herbivory-elicited chlorophyll looses have been described in both aquatic (Spooner et al., 1994) and terrestrial ecosystems. In the terrestrial ecosystem, two well-known aphid species that

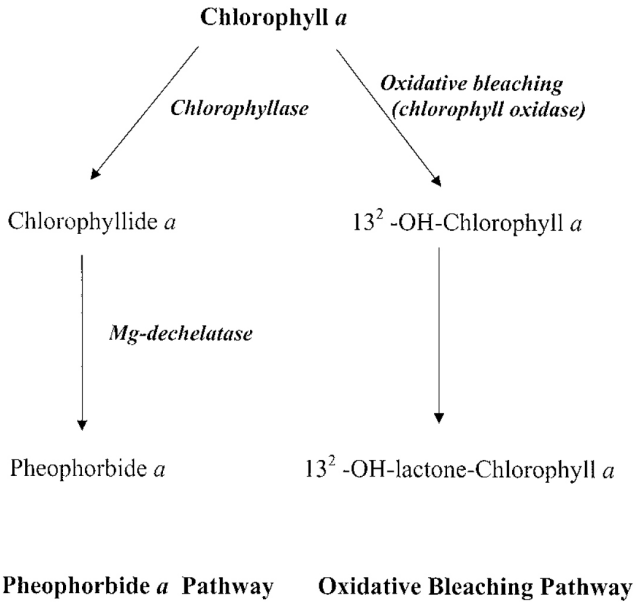

Figure 1. Chlorophyll a degradation pathways in naturally senescent plants described by Janave (1997) and Matile et al. (1999).

elicit chlorophyll loss are the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Hemiptera: Aphididae) on wheat, *Triticum aestivum* L. (Burd & Elliott, 1996; Burd et al., 1998), and the greenbug, *Schizaphis graminum* (Rondani) on sorghum, *Sorghum bicolor* (L.) Moench (Girma et al., 1998) and wheat. Leaf feeding by the Russian wheat aphid [*Diuraphis noxia* (Mordvilko)] (Hemiptera: Aphididae) and other sap-feeding insects elicits chlorosis and necrosis that cause significant crop losses worldwide. For example, the economic losses caused by *D. noxia* feeding in the U.S. have been estimated at $893.1 million between 1987 and 1993 (Morrison & Peairs, 1998). Significant chlorophyll loss elicited by *D. noxia* has been reported on wheat and barley (*Hordeum vulgare* L.), but not on triticale (× *Triticosecale* Wittmack) (Burd & Elliott, 1996). Furthermore, a significant discoloration measured by the red spot index and percentage of chlorophyll reduction elicited by *S. graminum* feeding, pectinases, and pectin fragments has been reported using plant chlorophyll meter (Ma et al., 1998). Unfortunately, no physiological mechanisms of chlorophyll loss elicited by these sap-feeding herbivores have been described.

The purpose of our current research is to establish baseline information necessary to understand *D. noxia*-elicited leaf chlorosis. We adopted techniques used in studying natural chlorophyll degradation in plants to examine herbivore-elicited chlorophyll degradation. The chlorosis-eliciting *D. noxia* and non-chlorosis-eliciting bird cherry-oat aphid [*Rhopalosiphum padi* (L.)] were used in this study. The objectives of this study were two-fold. One was to determine whether chlorophyll degradation differed between *D. noxia* (chlorosis-eliciting) and *R. padi* (non-chlorosis-eliciting), and among aphid-infested and uninfested wheat leaves by assaying chlorophyllase, oxidative bleaching, and Mg-dechelatase activities (Figure 1). The second objective was to determine which enzyme contributed the most to *D. noxia*-elicited leaf chlorosis.

Materials and methods

Plants and insects. 'Arapahoe' (*D. noxia* susceptible) wheat was used as the test plants in the experiment. Seeds of Arapahoe wheat were planted at the rate of three plants per Conetainer™ (3.81 cm diameter by 21 cm depth) (Stuewe & Sons, Inc., Corvallis, OR). Conetainers™ were filled with Sunshine™ soil mix No.1 (SunGro Horticulture, Bellevue, WA) and placed in Conetainer™ racks (61 × 30 × 18 cm), leaving a space between Conetainers™ to provide adequate light. Plants were watered uniformly from the bottom by placing a rack over a plastic tray (54 × 28 × 6 cm) filled with water. Before aphid infestation, plants were thinned to two plants per Conetainer™. The wheat plants were maintained in a growth chamber at L16:D8, and 21 °C.

Both aphid colonies were established using the field-collected aphids. The colony of chlorosis-eliciting *D. noxia* was established originally using aphids collected near Scottsbluff, NE, in 1994, while the non-chlorosis-eliciting *R. padi* colony was established using the aphids collected near Lincoln, NE, in 1996. The fodder plants (i.e., 'Stephens' wheat) and aphid colonies were maintained in Plexiglas cages (30 × 15 × 15 cm) in two separate Percival™ growth chambers (Percival Scientific, Boone, IA) at 21 ± 1 °C, a photoperiod of L16:D8, and 40–50% r.h. Voucher specimens of both aphid species have been deposited in the Entomology Collection at Montana State University, Bozeman, Montana, USA.

Aphid infestation and plant sample collection. There were three levels of aphid infestations per plant: i.e., 0 aphid, 10 *R. padi* adults, or 10 *D. noxia* adults. The experiment was initiated when plants were at the 3leaf stage or stage 13 of the Zadoks' scale (Zadoks et al., 1974). All plants were caged using polyethylene tube-

cages (30-cm length by 4-cm diameter) and maintained in a Percival™ growth chamber under the conditions described previously. Wheat leaves from the experimental plants were collected 3, 6, 9, and 12 days after the initial aphid infestation. Three Conetainers™ or six wheat seedlings were used for each treatment on each sampling date. Because of the relative small size of wheat leaf-blades, we combined leaves from six plants as one sample to be processed. The leaf samples were weighed to quantify protein content.

Extraction of plant and aphid enzymes. The enzyme extractions from both wheat and aphids were conducted according to Mihailović et al. (1997) modified using Ellsworth (1971), Drąkiewicz (1994), and Janave (1997). The plants (1.0 to 3.0 g) were ground with liquid nitrogen in mortars and pestles, while aphids (0.1 g) were homogenized in micro-centrifuge tubes using micro-centrifuge pestles. Because peroxidase and catalase activities from whole aphid extracts mimicked the enzyme activities from aphid salivary gland extracts (Ni et al., 2000), we used the whole aphid extract in this study. Chilled extraction buffer (5 ml) containing 0.1 M potassium phosphate buffer (pH 6.2), 1% NaCl, 1% Triton X-100, and 1% polyvinylpyrrolidone (PVP) were used for all plant materials, while only 1 ml buffer was used for aphid enzyme extractions. Plant homogenates were filtered through miracloth. Chlorophylls or aphid pigments were then removed twice using one volume of *n*-butanol and centrifuged at 3000×*g* for 3 min. Because the literature search yielded no specific absorption coefficients for the photosynthetic pigments (i.e., chlorophylls *a* and *b* and carotenoids) in butanol, and further pigment extraction from the butanol layer would introduce experimental error, the pigments in the butanol layer were not quantified in the current study.

The protein in the lower (aqueous) layer was then collected and precipitated with three volumes of cold (4 °C) acetone. After the addition of acetone, the samples were swirled briefly and allowed to stand for 5 min on ice. The samples were then centrifuged at 10 000 × *g* for 5 min, and the precipitate was normalized by re-suspending it in 1 ml of 0.1 M potassium phosphate buffer (pH 7.0), and then kept at 4 °C for 2 h before the initiation of enzyme assays. Only fresh enzyme samples were used for the assays.

Preparation of enzyme assay substrates. In order to assay chlorophyll degradation enzyme activities from wheat, quantified substrates (i.e., chlorophyll and chlorophyllin) were prepared using spinach leaves. Fresh spinach leaves were used for chlorophyll extraction according to Janave (1997). After spinach leaves were macerated in chilled acetone, the extracts were filtered through Whatman No. 2 filter paper, and then centrifuged at 6000 × *g* for 10 min to remove insoluable materials in the filtrates. The supernatant was then purified twice by dioxane precipitation (Iriyama et al., 1974; Janave, 1997). The ratio of dioxane:acetone was 1:7 (v/v), and distilled water was added drop-wise, with stirring, until the precipitate formed. The precipitated chlorophyll was then centrifuged at 3000 × *g* for 3 min, and resuspended in acetone. The concentration of chlorophyll a was determined by diluting the original chlorophyll solution with 80% acetone, and calculated according to the formula described by Bertrand & Schoefs (1997) using A_{646} and A_{663}.The chlorophyll solution was stored in the dark at −20 °C until used as a substrate for chlorophyll degradation and chlorophyllase activity assays.

Chlorophyllin was prepared from chlorophyll by Molisch conversion as described by Vicentini et al. (1995). Spinach leaves (approximately 6 g) were macerated in 80% acetone. Chlorophyll concentration was then determined according to Bertrand & Schoefs (1997). The chlorophyll was then partitioned in petroleum ether in a ratio of 1:1 (v/v). The petroleum ether phase was washed twice with distilled water. One hundred μl of 30% KOH in methanol was then added to 12 ml of chlorophyll solution in petroleum ether. The precipitated chlorophyllin was centrifuged at 3000×g for 5 min and dissolved in 10 ml of distilled water. The pH of the chlorophyllin solution was adjusted to pH 9 by adding tricine. Chlorophyllin solution was stable at pH 9 when stored in the dark at −20 °C. Chlorophyllin was used as the substrate for the Mg-dechelatase assay.

Chlorophyll degradation assay. The disappearance of chlorophyll a was measured according to the method used by Janave (1997). The reaction mixture (1.0 ml) contained 0.36 ml 0.1 M potassium phosphate buffer (pH 7.0), 0.288 ml of acetone (to make 30% in final reaction mixture), 12 μl of chlorophyll in acetone (to make 10 μM of chlorophyll a in final reaction mixture), and 0.34 ml of enzyme extract. The mixture was incubated at 30 °C in a water bath under dark for 30 min. The control incubation did not contain the enzyme extract. The reaction was stopped by adding 0.1 ml of 1 N NaOH followed by 3ml acetone/hexane mixture (1/2, v/v). The reaction mixture was then vortexed vigorously

until emulsion formation, allowed to stand for 10 min, and centrifuged at 3000 × g for 5 min. The absorbance of hexane layer at 663 nm was recorded. Chlorophyll a concentration was determined by employing the specific absorption coefficient of 94.5/M·cm. Activity was expressed by μmol of chlorophyll a degraded $min^{-1} \cdot g^{-1}$ leaf fresh weight. The activities determined by this procedure include both chlorophyllase and chlorophyll oxidase bleaching pathways (Janave, 1997).

Chlorophyllase activity. We modified the procedure reported on cavendish banana (Janave, 1997) according to a procedure on rye (*Secale cereale* L.) seedlings (Tanaka et al., 1982). The enzyme solution was incubated in 1.0 ml of reaction mixture containing 0.35 ml of 0.1 M potassium phosphate buffer (pH 7.0), 0.288 ml of acetone (to make 30% in final concentration), 0.01 ml of 0.1 M ascorbate, and 12 μl chlorophyll (for final concentration of 10 μM). The reaction was initiated by adding 0.34 ml enzyme extract. After 30 min at 30 °C, 0.1ml of 1 NNaOH was added to stop the reaction. Then, 3 ml of acetone/n-hexane (1:2 v/v) was added to the reaction mixture. The mixture was then vigorously shaken so that the chlorophyllide formed by the enzymatic reaction was partitioned into the lower aqueous layer. The mixture was then centrifuged at 3000 × *g* for 5 min. The enzyme activity was determined by measuring the decrease of chlorophyll a using the absorbance changes at 663 nm.

Oxidative chlorophyll bleaching activity. The presence of oxidative enzymes catalyzing chlorophyll degradation was examined by conducting an enzyme assay using ascorbate as an inhibitor for oxidative bleaching (Janave, 1997). Degradation of chlorophyll without the inhibitor indicates both chlorophyllase and oxidative bleaching pathways. In the presence of 2 mM ascorbate, only the chlorophyllase pathway is indicated because the ascorbate in the reaction mixture totally inhibits the oxidative bleaching pathway (Janave, 1997). The contribution of the oxidative bleaching pathway to overall chlorophyll degradation was calculated by subtracting the absorbance changes with the ascorbate inhibitor from the absorbance changes without the inhibitor.

Mg-dechelatase activity. The dechelation of magnesium from chlorophyllin (or chlorophyllide) to form pheophorbide was determined by monitoring the change in absorbance with time at 686 nm according to Vicentini et al. (1995) and Janave (1997). The assay mixture was comprised of 830 μl 50 mM Tris-Tricine (pH 8.0), 50 μl chlorophyllin (A_{686} nm = 0.1), 100 μl 1% Triton X-100, and 20 μl of enzyme extract. The control treatment did not contain enzyme extract. The reaction was carried out at 25 °C and activity was expressed as $A_{686}/min^{-1}g^{-1}$ fresh weight. The decrease of substrate chlorophyllin and the increase of pheophorbide were confirmed on a spectrophotometer (Model Genesys 5, Spectronic Instruments, Rochester, NY) according to the protocol described by Janave (1997). The identity of chlorophyllin and pheophorbide was further confirmed using a reverse phase high performance liquid chromatograph system with V4 detector (System 5004, Isco, Lincoln, NE) according to the protocol described by Almela (2000).

Protein determination. Protein concentration of all enzyme samples was determined according to the dye-binding assay (Bollag & Edelstein, 1991), using bovine serum albumin as a standard. The enzyme extracts used in protein determination had been treated with butanol (to remove pigments) and acetone (to concentrate protein) for chlorophyll catabolism enzyme assays.

Experimental design and data analysis. The assay for chlorophyll degradation enzyme activities from aphid extracts was a completely randomized design. The two aphid species were treatment factors, while the three aphid samples (0.1 g each) were used as replications for the experiment. The experiment of aphid-infested versus uninfested leaf extracts was a two-factor experiment (i.e., infestation types and sampling time) with repeated measures on one factor (i.e., the sampling time). This type of experiment also called a special type of split-plot design with split in time (Neter et al., 1985). The experiment had three treatments (three infestation levels) of Arapahoe wheat and four successive sampling dates (i.e., 3, 6, 9, and 12 days after infestation). Six plants were used for each treatment on each sampling date. Because of the relatively small size of wheat leaf blades, six plants were combined as a sample. The experiment was repeated six times. In total, 36 plants were used per treatment per sampling date. The data from both aphid and plant extracts were analyzed using the PROC GLM procedure of the SAS software followed by TEST statements to assure correct error terms used in assessing main effect of experiment factors (Cochran & Cox, 1957; SAS Institute, 1989). The means were separated using the least significant difference (LSD) test ($\alpha = 0.05$).

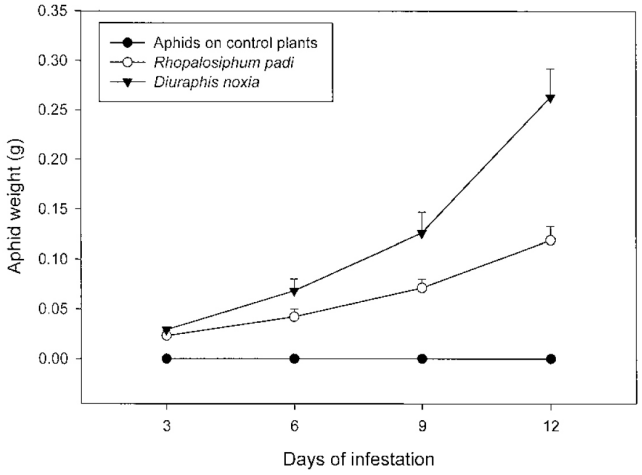

Figure 2. Aphid weight (g) from four sampling dates. Each data point represents the mean calculated from six replications, and a replication was comprised of all aphids from six wheat seedlings. Error bar indicates the standard error of the mean.

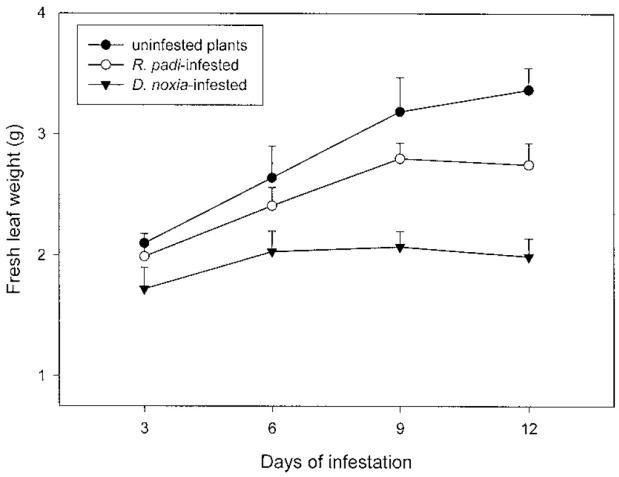

Figure 3. Fresh leaf weight (g) from four sampling dates. Each data point represents the mean calculated from six replications, and a replication was comprised of wheat leaves from six seedlings. Error bar indicates the standard error of the mean.

Results

Aphid and plant weight. Although 10 apterous adult aphids of both species were used for the initial infestation, *D. noxia* weight was significantly higher than *R. padi* ($F = 20.30$, $df = 1, 5$, $P = 0.0064$) (Figure 2). Aphid weight was significantly affected by aphid species-sampling date interaction ($F = 15.80$, $df = 3, 30$, $P < 0.0001$) and the sampling dates ($F = 87.06$, $df = 3, 30$, $P < 0.0001$). The finding suggested that *D. noxia* had higher reproductive potential than *R. padi* on Arapahoe wheat.

Fresh leaf weight was significantly affected by the infestation-sampling date interaction ($F = 3.24$, $df = 6, 45$, $P = 0.0098$) (Figure 3). The fresh leaf weight was also affected by aphid infestation types ($F = 42.21$, $df = 2, 10$, $P < 0.0001$), and sampling dates ($F = 23.77$, $df = 3, 45$, $P < 0.0001$). An initial infestation of 10 *D. noxia* per plant was enough to stop normal plant growth because *D. noxia*-infested wheat showed no increase in fresh leaf weight over the four sampling dates ($F = 2.21$, $df = 3, 15$, $P = 0.1291$). In contrast, *R. padi*-infested wheat leaves showed no significant reduction of growth in comparison with the uninfested plants irrespective of sampling date ($F = 3.67$, $df = 1, 10$, $P = 0.1312$). Thus, *D. noxia* infestation caused greater fresh weight reduction than *R. padi* infestation.

Total protein content per gram of fresh leaf weight was not affected by the infestation by sampling date interaction ($F = 1.04$, $df = 6, 44$, $P = 0.4133$). Nor did aphid infestation ($F = 0.75$, $df = 2, 10$, $P = 0.4954$) affect leaf protein content. However, protein content decreased significantly during the 12-d infestation period ($F = 4.14$, $df = 3, 44$, $P = 0.0114$).

Total chlorophyll a degradation. Aphid extracts were found to have no influence on chlorophyll a degradation ($F = 0.29$, $df = 1,4$, $P = 0.6165$). Similarly, aphid-infested and uninfested leaf extracts did not cause significant ($F = 2.88$, $df = 2, 10$, $P = 0.0874$) chlorophyll a degradation (Figure 4). Furthermore, neither infestation by sampling date interaction ($F = 0.50$, $df = 6, 43$, $P = 0.8079$), nor did the sampling dates significantly affect ($F = 1.36$, $df = 3, 43$, $P = 0.2677$) chlorophyll a degradation. The results demonstrated that neither *D. noxia* nor *R. padi* feeding caused direct chlorophyll catabolism.

Chlorophyllase activity. Assays of the aphid extracts showed no significant difference ($F = 1.30$, $df = 1, 4$, $P = 0.3173$) in chlorophyllase-caused chlorophyll a degradation. Aphid-infested and uninfested leaf extracts showed no difference in chlorophyllase activity ($F = 2.59$, $df = 2, 10$, $P = 0.1237$) (Figure 5); however, the chlorophyllase activity was significantly different among sampling dates ($F = 7.55$, $df = 3, 44$, $P = 0.0004$). The aphid infestation by sampling date interaction showed no significant effect on chlorophyllase activity ($F = 0.40$, $df = 6, 44$, $P = 0.8752$. Chlorophyllase activity was not detected from either aphid extracts or aphid-infested plant extracts.

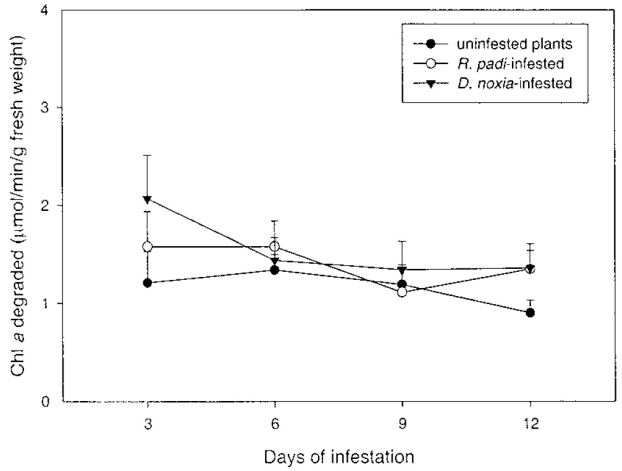

Figure 4. Total chlorophyll a degradation (μmol min^{-1}g^{-1} fresh weight) caused by aphid-infested and uninfested wheat leaf extracts. Each data point was calculated from six replications, and a replication was comprised of all leaves from six wheat seedlings. Error bar indicates the standard error of the mean.

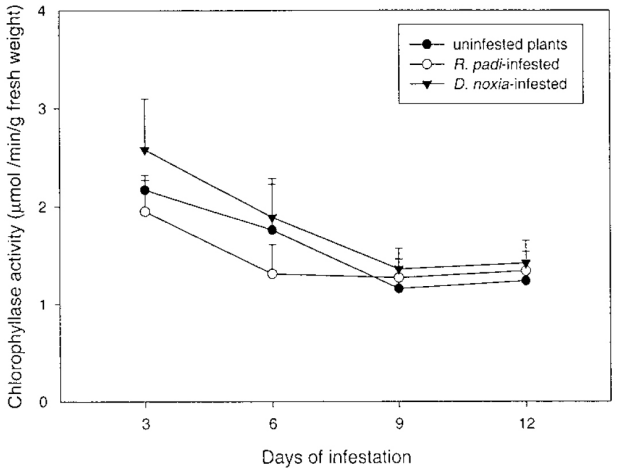

Figure 5. Chlorophyllase activity (μmol min^{-1}g^{-1} fresh weight) from aphid-infested and uninfested wheat leaf extracts. Each data point was calculated from six replications, and a replication was comprised of all leaves from six wheat seedlings. Error bar indicates the standard error of the mean.

Oxidative bleaching. The oxidative bleaching pathway was not detected in either aphid or plant samples. No differences in oxidative bleaching activity was found between the aphid extracts (F = 0.74, df = 1, 4, P,= 0.4395), nor among the aphid-infested and uninfested leaf extracts (F = 2.14, df = 2, 10, P = 0.1524). Furthermore, no differences in oxidative bleaching activity were found among sampling dates (F = 2.68, df = 3, 43, P = 0.0588) nor the aphid infestation by sampling date interaction (F = 0.81, df = 6, 43, P = 0.5686). Thus, oxidative bleaching did not play a direct role in chlorophyll degradation.

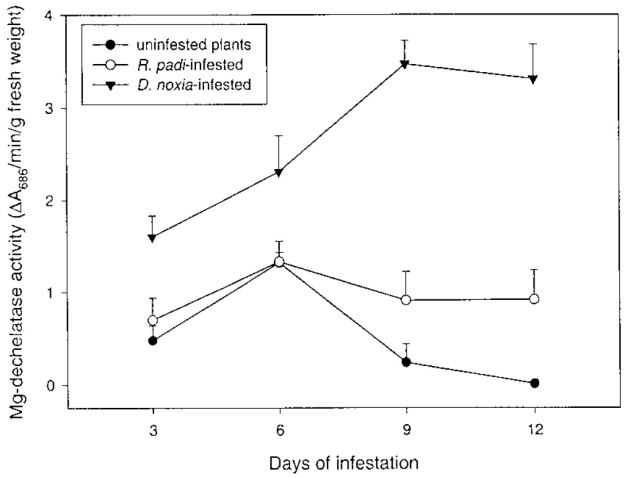

Figure 6. Mg-dechelatase activity (A_{686} min^{-1}g^{-1} fresh weight) from aphid-infested and uninfested wheat leaf extracts. Each data point was calculated from six replications, and a replication was comprised of all leaves from six wheat seedlings. Error bar indicates the standard error of the mean.

Mg-dechelatase activity. While Mg-dechelatase activity was not detected in the two aphid species (F = 3.59, df = 1, 4,P = 0.1311), plant extracts showed significant different amounts of enzyme activity (Figure 6). Mg-dechelatase activities were affected by the type of aphid infestations (F = 81.54, df = 2, 10, P < 0.0001) and sampling date (F = 5.47, df = 3, 44, P = 0.0028). The aphid infestation type and sampling date interaction also showed a significant effect on Mg-dechelatase activity (F = 7.52, df = 6, 44, P < 0.0001). Although *D. noxia*-infested wheat leaf weight did not increase through the experiment period (Figure 2), Mg-dechelatase activity increased as high as five-fold (or 5.21 times) in comparison with the uninfested leaves on the last sampling date (Figure 6). Furthermore, although *R. padi*-infested leaves showed higher Mg-dechelatase activity than in the uninfested leaves (F = 59.63, df = 1, 5, P = 0.006), the increase of Mg-dechelatase activity caused by *R. padi* infestation was less than double (i.e., 1.88 times) of the activity recorded in the uninfested leaves.

Discussion

The current examination of chlorophyll degradation in wheat seedlings elicited by cereal aphid feeding demonstrated that herbivore-elicited chlorosis differed from the chlorophyll degradation in naturally senescing plants. Neither chlorophyllase nor oxidative bleaching pathways, described by Janave (1997) and Matile

et al. (1999) in naturally senescing plants, was detected in aphid-infested wheat seedlings. However, *D. noxia*-infested wheat leaves showed significantly (over five times) higher Mg-dechelatase activity than uninfested leaves, even though leaf fresh weight did not increase and total protein content decreased. However, our previous work (Ni et al., 2001) reported an increase of total protein content in aphid-infested cereal leaves in comparison with the control plants. The variation between the results of total protein content assays from the present study in comparison with our previous work on oxidative enzymes (Ni et al., 2001) was likely caused by the experimental protocols we used. While total protein content in oxidative response study had been assayed using the plant extract directly, the protein content in the current study was assayed after butanol treatment and cold-acetone precipitation as described in the previous section. Such treatments of the enzyme extracts were necessary to remove the contamination of pigments and to concentrate the enzyme samples for chlorophyll degradation enzyme assays.

The increase of Mg-dechelatase activity indicates that chlorosis formation on *D. noxia*-infested plants is possibly a Mg-dechelatase-driven physiological phenomenon. Because our assays for chlorophyllase and oxidative bleaching suggested that neither enzyme was involved in increased chlorophyll degradation, the substrate (i.e., chlorophyllide) that produced higher Mg-dechelatase activity might be provided by other pathways. Possible pathways could be either enzymatic or non-enzymatic chlorophyll degradations, such as photochemical degradation of chlorophylls. Another intriguing possibility might be that Mgdechelatase used the chlorophyllide accumulated by active chlorophyll biosynthesis in growing leaf tissue, and degraded the chlorophyllide into pheophorbide instead of becoming chlorophylls catalyzed by chlorophyll synthase. Furthermore, pheophorbide is not a suitable substrate for chlorophyll synthase in the chlorophyll biosynthesis process (Rüdiger, 1997). Therefore, the dynamics of normal chlorophyll biosynthesis might be interrupted and further degraded, which contributed to the development of leaf chlorosis. The *D. noxia*-elicited leaf chlorosis process in the young growing wheat seedlings, therefore, would differ greatly from the chlorophyll degradation processes in naturally senescing plant leaves. Previous work has demonstrated that *D. noxia* infestation elicits the increases in amino nitrogen levels, but decreases in fructan, sucrose, and hexose levels (Burd et al., 1998). *Diuraphis noxia* prefers to feed on young growing cereal leaves that are physiological sinks for phloem-mobile nutrients (e.g., amino compounds and carbohydrates). The aphid feeding alters normal plant carbohydrate partitioning and delays the sink-to-source transition in the aphid-infested leaves (Burd et al., 1998). Furthermore, oxidative responses of *D. noxia*-infested wheat, barley, and oat (*Avena sativa* L.) plants are different (Ni et al., 2001). While *D. noxia*-infested resistant 'Halt' wheat has a three-fold increase in peroxidase activity in comparison with its control, *D. noxia*-infested susceptible 'Morex' barley has a nine-fold increase. *Diuraphis noxia* infestation has no effect on peroxidase activity in either 'Arapahoe' wheat or 'Border' oat plants. *Diuraphis noxia*-elicited leaf chlorosis is probably a much more complex process in comparison with the previously described chlorophyll degradation pathways in senescing plants. The changes of Mg-dechelatase and peroxidase activities and aphid salivary factors merit further studies.

This is the first study to show *in vitro* evidence of enzymatic chlorophyll catabolism elicited by a piercing-sucking insect, although the herbivory-elicited chlorophyll loss has been described in both aquatic and terrestrial ecosystems previously (Spooner et al., 1994; Burd & Elliott, 1996). In the aquatic ecosystem, chlorophyll degradation (or 'defunctionalisation') during zooplankton herbivory was considered to be the result in part from endogenous enzyme activities of the ingested alga; however, no specific enzymes were determined (Spooner et al., 1994). Because oxidative bleaching and chlorophyllase activities were not detected from either symptomatic *D. noxia* or asymptomatic *R. padi* extracts in the current study, we concluded that the elicitor for leaf chlorosis might not be the direct result of aphid salivary secretion. The enzyme assay of plant samples showed that Mgdechelatase activity in *D. noxia*-infested plants was significantly higher than either *R. padi*-infested or uninfested plants (Figure 6), although chlorophyllase and oxidative bleaching activities were not different. Our results indicate that *D. noxia*-elicited chlorosis is the result of increased Mg-dechelatase and possibly other plant physiological and/or biochemical changes. Based on the data presented herein, an array of questions can be asked in relation to aphid resistance on wheat and other crop plants. This study will serve as baseline information for further investigations on *D. noxia*- and other sap-feeding insect-elicited chlorophyll losses.

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