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Involvement of Ethylene in Aphid Infestation of Barley

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Abstract. The recently arrived Russian wheat aphid (RWA) is a major pest of wheat and barley in the United States. RWA induced ethylene production in Morex, a barley variety susceptible to RWA, but induced little ethylene production in PI 366450, a barley resistant to RWA. Greenbugs, another aphid pest, induced ethylene production in PI 366450 and Morex, both of which are susceptible to greenbugs. RWA infestation results in pronounced symptoms on barley including leaf streaking, stunting of growth, and rolled leaves. Incubation of barley in ethylene (5 and 50 μl/l) or other plant hormones (auxin, gibberellic acid, zeatin, kinetin, and abscisic acid at 10^{-4} M) failed to produce streaking or rolling in uninfested plants or to alter the production of these symptoms in infested plants. Incubation of Morex and PI 366450 in ethylene caused some stunting of the leaves and internodes that emerged during or soon after ethylene incubation. However, the leaf and internode that emerged 9 days after incubation showed some compensatory, or increased, growth.

The Russian wheat aphid (RWA) is a newly arrived pest to the United States. The aphid causes chlorotic streaks on leaves, a failure of new leaves to unroll, and stunted leaves in barley (Miller et al. 1994) and wheat (Burd et al. 1993). An understanding of the physiological mechanism(s) causing these symptoms would aid development of resistant plant varieties. A comparison of the physiological responses of susceptible and resistant barleys indicated no involvement of water stress in the infestation symptoms (Miller et al. 1994). Changes in protein patterns and chlorophyll fluorescence occurred but may not precede the appearance of damage symptoms (Miller et al. 1994).

Although electron and light microscopy studies have shown that the RWA probes intercellularly (Fouché et al. 1984), the stylet must penetrate the phloem cells and probably the bundle sheath cells to feed in the phloem. In fact, feeding monitor studies suggest mesophyll or bundle sheath cells are sometimes penetrated because ingestion by RWA occurs from nonphloem tissue as well as from phloem (Webster et al. 1993). Indeed, internal damage in the leaf occurs, as evidence by the rapid collapse of mesophyll and bundle sheath cells of RWA-infected barley leaves along the aphid's stylet pathway (Belefant-Miller et al. 1994). These collapsed cells are more numerous in resistant than susceptible plants. Greenbug infestation results in diseaselike symptoms in the leaves, i.e., necrotic lesions surrounded by chlorotic halos (Ryan et al. 1987). Plants undergoing infestation by either RWA or greenbugs are undoubtedly under stress and so may be producing elevated levels of ethylene (Hyodo 1991), which may have a role in the production of the symptoms of infestation.

The objective of this study was to determine if aphids—RWA and greenbug—induced ethylene production by barley. Then, if ethylene is produced, to determine if ethylene has a role in producing the visible symptoms of RWA damage, particularly leaf rolling, streaking, or stunting.

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Materials and Methods

Exposure of Plants to Hormones

Plant material and hormone treatments. Seeds of barley (*Hordeum vulgare* L.) were planted in pots under greenhouse conditions (Miller et al. 1994). The barley germplasm used were PI 366450, resistant to RWA [*Diuraphis noxia* (Mordvilko)] (Webster et al. 1991), and "Morex," susceptible to RWA (Webster et al. 1993). Solutions of indoleacetic acid (IAA), gibberellic acid (GA_3), zeatin, kinetin, and abscisic acid (ABA) (Sigma, St. Louis, MO) (10^{-4}) were sprayed on leaves when the third leaf was emerging. These hormones were applied daily over a 3-day period. Plants that were infested had five RWA placed in the whorl of each plant after the 3-day hormone treatment. The aphids were free to move around and reproduce during the course of the experiment. Hormones were reapplied to each plant every third day thereafter. Observations were made at 23 days after the initial hormone treatment.

Ethylene treatment. Plants were exposed to the ethylene gas for 48 h when their second leaf was partially emerged. Plants were placed in large glass containers of known volume (5–10 l) and the tops were sealed with covers having silicone rubber gaskets to prevent gas leakage (Ketring and Morgan 1970). A known volume of pure ethylene gas (Matheson Co., Atlanta, GA) was injected with a gas-tight syringe through rubber septa to bring the container to the desired ethylene concentration (50, 100, 500, and 1000 μl/l). Only four plants in their pots could fit at one time in the containers, so each test was limited to two Morex and two PI 366450 for each ethylene level. Tests were carried out twice at 50 and 100 μl/l and twice at 500 and 1000 μl/l. Results are shown for one of 50- and 100-μl/l tests, but similar patterns were observed among leaf ages for all tests.

Ethylene Production by Barley

Tube culture of barley. Seeds of barley were dropped onto wet vermiculite in an 80-ml glass test tube, one seed per tube. Tubes were capped with a cotton plug. Seeds were watered with a dilute (one-half of the recommended strength) commercial nutrient solution (Peters: Grace-Sierra Horticultural Products, Milpitas, CA) every 4 or 5 days. Attempts to produce healthy seedlings under sterile conditions were unsuccessful and found to be unnecessary for obtaining zero ethylene production before infestation. Therefore, no attempt was made to obtain or maintain a sterile environment other than rolling the seeds in Arasan 50 (tetramethylthiuram disulfide: du Pont de Nemours, Wilmington, DE) fungicide before planting.

Aphid material and infestation of tube culture. Stocks of RWA and greenbug [*Schizaphis graminum* (Rondani) biotype E] were raised on barley cv. "Wintermalt" under greenhouse conditions (Starks and Burton 1977). One hundred aphids were counted into glass vials and then emptied into the test tubes containing the barley plants. Each day, the tubes were sealed with a rubber septum, and measurements were made every 4 h. Tubes were plugged with cotton the rest of the time. The aphids remained in the tubes for 4 days to measure ethylene production as they fed.

Ethylene measurement. Ethylene production was measured by removing 5 ml from the headspace of the sample tube. The sample was injected onto a 1/8″ × 5′ stainless steel column packed with activated alumina (60/80 mesh). The column was installed in a Hewlett-Packard model 5840 gas chromatograph with an oven temperature of 90°C isothermal, injector temperature of 100°C, and flame ionization detector at 150°C with helium as a carrier gas flowing at 30 ml/min. The amount of ethylene produced was determined by comparing the peak height of sample peaks with a standard curve generated from using different concentrations of an ethylene standard (Neogen, Inc., Lansing, MI). The experiment was carried out initially with 50 and 150 aphids per plant and then again with 100 per plant. Means are reported as an average of ethylene production by three plants ± the standard error. The presence of ethylene was verified by the method of Sanders et al. (1989).

Results and Discussion

The Effect of RWA and Greenbug on Ethylene Production by Barley

During RWA infestation, Morex (susceptible to RWA) showed increased ethylene production, whereas PI 366450 (resistant to RWA) showed little ethylene production (Fig. 1). Both Morex and PI 366450 produced ethylene during infestation by greenbugs, to which both barleys are susceptible. Maximum production of ethylene occurred 2 days after infestation for both aphids and then declined to baseline levels by 4 days after infestation. The lag period for ethylene production suggests that ethylene production is not due to a wound response. Uninfested barley produced no detectable levels of ethylene over the 4-day period.

Reports on ethylene induction by aphid feeding include wheat infested with greenbug (Anderson and Peters 1994) and alfalfa infested with spotted alfalfa aphid (Dillwith et al. 1991, Neese 1990). In these studies, susceptible genotypes also produced more ethylene than corresponding resistant genotypes during infestation. Alfalfa explants susceptible to spotted alfalfa aphid produced more ethylene than uninfested explants or explants infested with a pea aphid that does not accelerate senescence (Neese 1990).

Aphid saliva contains numerous substances, the buildup of which may either induce or repress a stress response (Miles 1990). Furthermore, it is possible the aphids may transfer microorganisms which themselves also induce ethylene production, as happens with the sucking insect, cotton fleahopper, on cotton (Martin et al. 1988). Ethylene production does not appear to be related to internal cellular

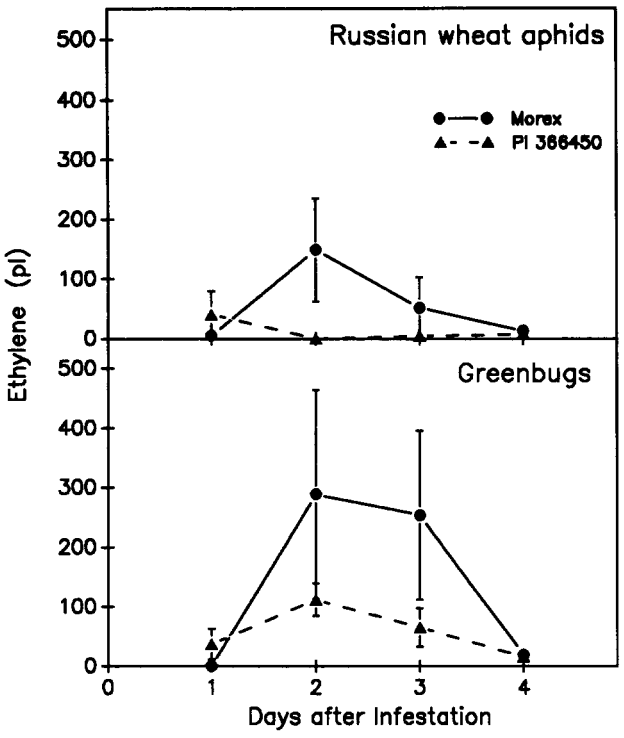

Fig. 1. Ethylene production by barley resistant (PI 366450) or susceptible (Morex) to the RWA. Plants were infested by introducing 100 RWA or 100 greenbugs into the test tubes when plants were at the second-leaf stage.

damage because higher levels of a hypersensitive-like cell collapse occur in PI 366450 than in Morex (Belefant-Miller et al. 1994).

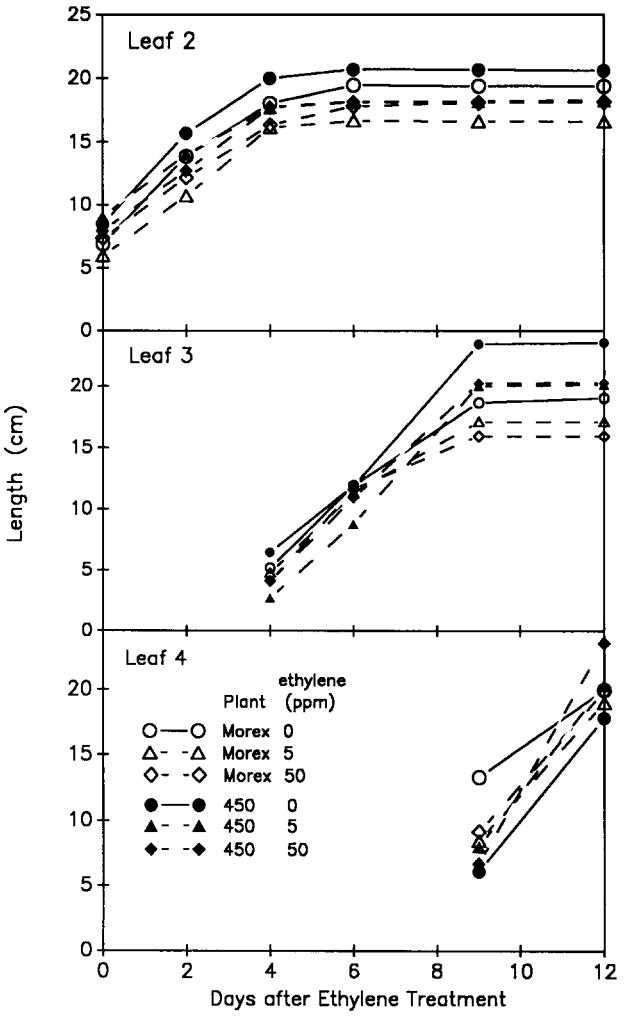

Fig. 2. Effect of ethylene on leaf length of barley.

The Effect of Ethylene on Barley Leaves

The effects of RWA on susceptible barley include reduced height and leaf length, leaf rolling (Miller et al. 1994) and chlorotic leaf streaks (Miller et al. 1995). Incubation of both a resistant (PI 366450) and susceptible (Morex) barley in ethylene concentrations up to 1000 µl/l for 2 days failed to produce either rolling or streaking, although streaking can be induced by only 1 day of feeding by one RWA (Miller et al. 1995). These streaks extend into younger tissue the aphid has not fed in, but no chlorosis was observed at any incubation level of ethylene.

A literature search failed to uncover studies of the effects of applied ethylene on barley leaves or stems. However, applications of ethylene-producing compounds decreased barley plant height: ethephon decreased height 2 weeks after application (Foster et al. 1992) and CEPA decreased height at maturity (Wünsche 1977). Since RWA infestation shortened Morex height to 21% of control plants and PI 366450 height to 67% of control plants (Miller et al. 1994), we measured the effect of ethylene on leaf length and stem internode length. The results indicated a reduction in the lengths of leaves and internode that were developing at the time of ethylene treatment or emerging soon after ethylene treatment (Figs. 2 and 3). Both Morex and PI 366450 were similarly affected; the lengths of the second and third leaves and the second internode of ethylene-treated plants were 84–94% (leaves) and 44–60% (internode) the lengths of those of the control plants (Figs. 2 and 3). In contrast, the lengths of the fourth leaf and of the third internode, emerging over a week after ethylene treatment, were longer on the ethylene-treated plants than on the control plants (Figs. 2 and 3). The stunting effect of ethylene does not appear to be long-lived and the post-ethylene treatment increases in leaf and internode

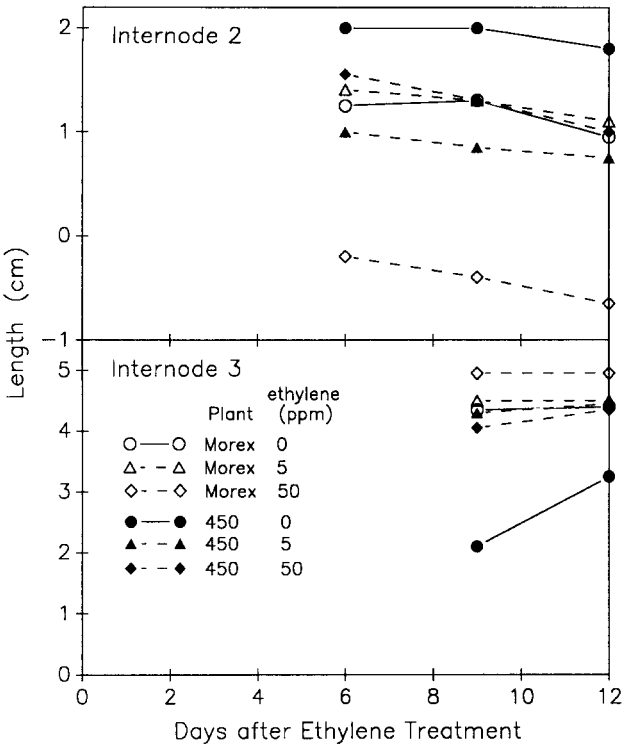

Fig. 3. Effect of ethylene on internode length of barley. Negative numbers occurred when the node was visible but was just below the previous node. Note the different scales on the y axis.

length may be a result of compensation (Dale 1986, Trewavas 1986). Leaf size has been shown to be adjusted to maximize whole plant carbon gain (Givnish 1986).

Hormone Involvement in RWA Damage Symptoms

We tested other plant hormones for either induction or alleviation of RWA-type damage. Application of the plant hormones or regulators IAA, GA_3, zeatin, kinetin, and ABA at 10^{-4} M to uninfested barley leaves failed to produce the RWA-induced damage symptoms of rolling or streaking. Furthermore, the application of these regulators to infested plants did not affect the development of RWA-induced damage.

Ethylene production by barley is increased by aphid infestation, by either RWA or greenbug, if the plant is susceptible to the aphid. However, exposure of barley to ethylene or other plant hormones did not result in rolling or streaking. Our results showed no clear detectable relationships between plant hormones, including ethylene, and RWA symptoms on barley. Although ethylene production is induced by susceptible plants during infestation, the lack of production of typical RWA-induced infestation symptoms by ethylene, other than short-term inhibitory effects on barley growth, appears to reduce the possibility of ethylene involvement in the development of the visible symptoms, at least of streaking and rolling. This does not exclude the possibility that ethylene is in some way involved in successful feeding by RWA or greenbug. The occurrence of wounding or the contents of the saliva may also be necessary to produce the damage symptoms.

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