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Effect of Spotted Tentiform Leafminer Injury on Ethylene Production and ACC Content in Apple Leaves

Frank Kappel
Agriculture Canada, Research Station, Harrow, ON N0R 1G0, Canada

J.T.A. Proctor and D.P. Murr
Department of Horticultural Science, University of Guelph, Guelph, ON N1G 2W1, Canada

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Abstract. **Apple leaves (*Malus domestica* Borkh.) injured by spotted tentiform leafminer (*Phyllonorycter blancardella* F.) released significantly higher levels of ethylene than control leaves. Leaves with tissue-feeding mines released the most ethylene, about eight times as much as control leaves. Leaves with tissue-feeding mines did not have increased levels of ACC, while some leaves with sapfeeding mines had increased levels of ACC. Chemical name used: 1-aminocyclopropane-1-carboxylic acid (ACC).**

One of the reported effects of spotted tentiform leafminer (STLM) injury to apple trees is premature fruit and leaf drop (7, 9, 10, 12). The cultivar McIntosh is particularly prone to premature fruit drop under a number of adverse conditions. Possible causes of premature fruit drop associated with STLM injury include changes in nutritional status (1), changes in levels of growth regulators other than ethylene (14), reduced photosynthetic capacity due to reduced leaf area (11), or increased ethylene from leaf wounding. Many tissues that normally evolve little or no ethylene produce large amounts when either stressed or wounded (17). There are many reports of increased ethylene production by plants injured by fungal, bacterial, and viral diseases (4, 13, 15). This increased ethylene production may be involved in wound-healing, production of phytoalexins, shedding of injured parts, increase in disease resistance, or promotion of plant growth under stress (17). Some reported responses of apple trees to STLM injury (premature ripening, fruit drop, and leaf drop) may be explained by the increased ethylene production from STLM-injured leaves.

The purpose of this study was to determine the relationship among STLM injury, ACC levels in apple leaves, and ethylene release from leaves injured by STLM.

Ethylene assays. The leaves for ethylene assay and ACC extraction were collected from either 40-year-old 'McIntosh' trees on seedling rootstock from a commercial orchard or from 13-year-old 'McIntosh' trees on M.26 rootstock in a research orchard. The mine stages included various stages of sapfeeding (SF) and tissue feeding (TF) mines. Control leaves were uninjured leaves from the same tree as the leaves with mines.

Two leaves were placed in each of six or ten 50-ml test tubes with petioles immersed in 2 ml of distilled water. The test tubes were left open for 1 hr, then flushed with ethylene-free air for 1 min and sealed with a rubber serum stopper. After a 4-hr incubation in ambient room light (14 $\mu mol \cdot s^{-1} \cdot m^{-2}$) and at room temperature (22° ± 2°C), 3 ml of head space gas was withdrawn with a syringe and analyzed for ethylene (C_2H_4) using a Hewlett Packard gas chromatograph (Model 5880A) (8).

The same leaves were used for the ethylene release and ACC data in Table 2. Once the air sample was taken for the ethylene assay, the leaves were weighed quickly and then frozen with liquid N_2 and placed in the freezer until ACC determinations were completed.

ACC extraction. The extraction method was modified from that of Mikitzel (8). Leaf samples (about 5 g, fresh weight) were frozen in liquid N_2 and held at −12°C until the extraction of ACC. The frozen leaves were then extracted in 95% ethanol.

ACC assay. ACC in apple leaf extracts was assayed by chemical conversion to ethylene in the presence of $HgCl_2$. A modified method (8) of the Lizada and Yang (6) assay was performed.

ACC was quantified using internal standards. For each extract tested, two equal volumes were taken, and one was spiked with 500 pmol of ACC. From the difference in ethylene produced, the yield of ethylene from the added ACC was calculated, and this amount used to determine efficiency of ethylene production from ACC in the unspiked sample. The mean conversion efficiencies for the two ACC extractions were 64% ± 1.7% and 56.2% ± 1.0%.

The Student *t* test or an analysis of variance was performed on the data. When the F test was significant, mean separation was by LSD.

Ethylene release. 'McIntosh' leaves injured by second generation sap-feeding (SF) or tissue-feeding (TF) mines released higher amounts of ethylene than control leaves (Table 1). Leaves with SF mines produced about twice as much ethylene as the control, whereas leaves with TF mines produced about eight times as much ethylene as the control.

Leaves with third generation TF mines from the research orchard released almost four times more ethylene than controls (Table 2). Leaves

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Table 1. Ethylene release from 'McIntosh' apple leaves in a commercial orchard, injured by second-generation sap-feeding and tissue-feeding spotted tentiform leafminer mines.

	STLM larval stage			
	Sap-feeding[z]		Tissue-feeding[y]	
Treatment	Ethylene released ($nl \cdot g^{-1} \cdot hr^{-1}$)	Ethylene release (% control)	Ethylene released ($nl \cdot g^{-1} \cdot hr^{-1}$)	Ethylene release (% control)
Control	0.22 ± 0.06[x]	100	0.55 ± 0.20	100
Mined	0.47 ± 0.09	214	4.44 ± 0.60	807
t (n = 18)	2.398	---	6.184	---
P	0.05	---	0.001	---

[z]Measured 13 Aug. 1984.
[y]Measured 31 Aug. 1984.
[x]Mean ± SE for 10 replications.

Table 2. Ethylene release and ACC levels in 'McIntosh' apple leaves from the Research Station orchard injured by third-generation spotted tentiform leafminer mines.

	Ethylene release		ACC	
Mine stage	($nl \cdot g^{-1} \cdot hr^{-1}$)	Percent of control	($nmol \cdot g^{-1}$ fresh wt)	Percent of control
Control	0.31 ± 0.01 a[zy]	100	14.26 ± 5.38 a	100
Sap-feeding	0.65 ± 0.21 ab	210	14.36 ± 2.40 a	101
Tissue-feeding	1.15 ± 0.24 b	371	17.26 ± 3.72 a	121
Empty[x]	1.11 ± 0.21 b	358	13.22 ± 3.25 a	93

[z]Mean ± SE for six replications.
[y]Means separated by LSD, $P = 5\%$. ($LSD_{0.05} = 0.58$).
[x]Second-generation mines.

Table 3. ACC levels in 'McIntosh' apple leaves from a commercial orchard injured by third-generation spotted tentiform leafminer mines.

	ACC ($nmol \cdot g^{-1}$ fresh wt)	
Mine level	Sap-feeding (7 Sept. 1983)	Tissue-feeding (28 Sept. 1983)
Control	3.89 ± 0.91[z]	3.17 ± 0.43
Low[y]	5.25 ± 0.89	2.66 ± 0.22
High[x]	6.04 ± 1.07	3.04 ± 0.15
Main effect (mine stage)	5.65	2.85
	$P = 0.01$	

[z]Mean ± SE with four replications.
[y]Sap-feeding low = 1 to 3 mines/leaf and tissue-feeding low = 1 to 2 mines/leaf.
[x]Sap-feeding high = 5 to 8 mines/leaf and tissue-feeding high = 4 to 8 mines/leaf.

with SF mines also released increased amounts of ethylene, but the increase was not significant compared to the control leaves. Leaves with empty mines released higher amounts of ethylene than the control leaves. These mines were from the second generation, and all that remained in the mines after the adult emerged was insect feces.

The ethylene release data (Tables 1 and 2) were used to determine the relationship between the number of mines in an assay tube and the amount of ethylene released. As mine number increased, ethylene release increased, with TF mines having a slightly better relationship than SF mines, but the correlation coefficients for both types of mines were not significant (data not shown). In one assay, the amount of ethylene actually decreased with increasing SF mine number. When the size of leaves (fresh weight) was included and multiple correlation was used, the relationship was improved slightly for both the SF and TF data, but it still was not significant (data not shown).

ACC content of leaves. The differences in ACC content of leaves between control and the mine types (SF, TF, or empty) were not significant (Table 2). In Table 3, the mine type (either SF or TF) was the only significant component, with the SF mines having two times more ACC than the TF (5.65 vs. 2.85 $nmol \cdot g^{-1}$).

There was a poor relationship between the amount of ACC in the leaf and the amount of ethylene released ($r = 0.25$).

Leaves injured by the TF stage of the STLM produced much more ethylene than control leaves or leaves injured by the SF stage. The TF stage is the first stage in which the insect actually consumes leaf tissue (9). The injury becomes evident on the adaxial surface of the leaf during this stage. Ethylene production due to plant diseases either corresponded to lesion formation (4) or to symptom development for wilt diseases (15). It is difficult to compare directly the amounts of ethylene produced because of differences of injury, plant material, and amount of inoculum, but the STLM-injured leaves produced ethylene in amounts similar to diseased leaves (3).

The differences in the amount of ethylene produced by the two mine stages is likely due to the different types of injury. Pottinger and LeRoux (9) described the feeding injury of both stages. The SF larvae shears the spongy mesophyll cells adjacent to the lower epidermal cells and then imbibes the released cell contents, while the fourth and fifth instars (TF) chew out irregular sections of palisade cells up to the upper epidermis, which is left intact.

Fourth or fifth instar larvae and feces were enclosed in separate assay tubes for 4 hr and no ethylene was detected (data not shown). Also, leaves with empty mines produced more than three times as much ethylene as the control leaves, which is at the rate of ethylene production by leaves containing TF mines (Table 2). Most of the work done with plant diseases suggests that the ethylene is produced by the living host cells and not by the pathogen or the dead host cells within the lesions (4), but the effect of secondary microbial contamination in the mines was not investigated.

Stress or wound ethylene biosynthesis is believed to follow the same ethylene biosynthetic pathway as in most other systems (16). SF-injured leaves from the commercial orchard had significantly higher ACC content than the TF-injured leaves, even though leaves with TF mines produced more ethylene than SF-injured leaves in all our assays. Leaves

from the research orchard injured by STLM did not have increased ACC content, but the amounts of ACC in these leaves were higher than in those injured leaves from a commercial orchard. The differences in apple leaf ACC content may be due to different orchard management practices and orchard environment, and the fact that data were accumulated in different years. The higher ACC levels in SF-mined leaves compared with TF-mined leaves also may be due to differences in development of the SF mine or to differences in the rate of ACC metabolism. De Laat and van Loon (3) suggested that, for tobacco leaves infected with tobacco mosaic virus, the final step requiring the ethylene-forming enzyme is rate-limiting. Therefore, ACC levels may increase without an increase in ethylene production.

The signal from the STLM injured leaves to the fruit abscission zone still has not been determined. Large increases in ACC levels in SF-injured leaves and in ethylene release from TF-injured leaves have been measured. There are conflicting reports of ethylene movement within plants, suggesting ethylene is moved throughout the plant (5) or that ethylene does not move in the plant (18). There are also reports of the translocation of ACC and the release of ethylene at a site distant from where ACC production occurs (2). Either ACC or ethylene or both may be sending the signal to the abscission zone, causing premature fruit drop.

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Ethylene Evolution by Tomato Plants Under Stress of Ammonium Toxicity

K.A. Corey, A.V. Barker, and L.E. Craker
Department of Plant and Soil Sciences, University of Massachusetts, Amherst, MA 01003

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Abstract. **Tomato (*Lycopersicon esculentum* Mill. cv. Heinz 1350) plants grown in soil with N supplied from $(NH_4)_2SO_4$ solutions showed a morphological disorder characterized by leaf epinasty. The development of this disorder was accompanied by an increase in the rate of ethylene evolution from whole plants. Ethylene evolution from plants supplied with 0.04 M NH_4-N increased to a peak of 112 $nl \cdot g^{-1} \cdot hr^{-1}$ at ≈2 weeks following the start of fertilization compared to 11 $nl \cdot g^{-1} \cdot hr^{-1}$ from plants supplied with 0.04 M NO_3-N. Fertilization with KCl in molar equivalency to the supply of NH_4-N prevented epinasty and the burst in ethylene evolution. Ethylene evolution from plants of the *yellow-green*-5 and *neglecta*-1 mutants did not increase in response to NH_4-N fertilization. Potassium concentrations in shoots of 'Heinz 1350', *yellow-green*-5, and *neglecta*-1 were 2.10, 2.53, and 3.22% (dry weight), respectively, if plants were supplied with NH_4-N and no additional K, suggesting that tolerance to NH_4 toxicity may be explained in part by differences in K accumulation.**

Tomato plants grown in soil culture often develop brown, sunken lesions on the stems if supplied with fertilizer solutions containing high concentrations of NH_3 (8). Lesion development can be prevented if K is supplied in molar equivalency to the supply of NH_4 (1, 2). Considerable genotypic variation in tolerance to toxic levels of NH_4-N has been demonstrated for tomato (7), with the mutant lines *yellow-green*-5 (*yg*-5) and *neglecta*-1 (*neg*-1) exhibiting nearly complete resistance and the cultivar Heinz 1350 showing high susceptibility (3). Genetic tolerance has been attributed to accumulation of greater quantities of K (3). Ammonium nutrition combined with deficient K levels in plant tissue leads to increased activity of enzymes for the synthesis of polyamines such as putrescine (5, 13, 14), which in turn may have a role in the development of NH_4 toxicity of K deficiency symptoms.

Symptoms of plants under the stress of NH_3 toxicity also may include chlorosis and necrosis of leaves and leaf epinasty (9). Leaf epinasty has been reported to be caused by ethylene (6, 10, 11), and may occur as a result of a range of plant stresses. Poinsettias mechanically stressed by sleeving often produce increased quantities of ethylene accompanied by leaf epinasty (12). Tomato plants subjected to prolonged anoxia in the root zone

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