Acetolactate Synthase Inhibitors Increase Ethylene Production and Cause Fruit Drop in Citrus

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Abstract. The abscession action of two sulfonyleureas and one imidazolone was evaluated in laboratory studies with harvested orange (Citrus sinensis L. cv. Valencia) fruit and greenhouse studies with orange (cv. Hamlin) and grapefruit (Citrus paradisi Macf. cv. Marsh) trees. Dipping harvested fruit in 90 mg·L–1 imazameth, 2 mg·L–1 metsulfuron-methyl, or 30 mg·L–1 prosulfuron solutions increased levels of internal ethylene. Internal ethylene concentration was higher when fruit were dipped in 2 mg·L–1 metsulfuron-methyl solutions at low pH. Fruit retained on trees and dipped in 2 mg·L–1 metsulfuron-methyl solutions produced more ethylene than control fruit. Drop of treated fruit began when ethylene production was at a maximum. High temperatures (average 33 °C) suppressed ethylene production and fruit drop of metsulfuron-methyl–treated fruit. The results indicate the importance of environmental conditions in evaluating the potential of sulfonyleureas and imidazolones as abscession agents for citrus. Chemical names used: ±2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid (imazameth); methyl 2-[[4-(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] amino] sulfonyl] benzoate (metsulfuron-methyl); 1-(4-methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl) phenylsulfonyl] urea (prosulfuron); N-(phosphonomethyl) glycine (glyphosate); 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinoxlinecarboxylic acid (imazaquin).

Sulfonyleurea and imidazolone herbicides are widely used to control broadleaf weeds in agronomic crops. These herbicides inhibit acetolactate synthase (ALS), an enzyme involved in the biosynthesis of branched chain amino acids (LaRossa and Schloss, 1984). In addition to their activity as herbicides, sulfonyleureas and imidazolones promote abscission in citrus (Wilcox and Taylor, 1996, 1997). When sprayed on fruited branches, some compounds induced fruit drop of mature oranges but did not affect leaves. In general, rates of application for citrus fruit abscission were at least an order of magnitude lower than those recommended for herbicidal activity. Many sulfonyleurea and imidazolone compounds are known, and their herbicidal activities differ with various substitutions made on each toxophore (Hay, 1990; Ladner, 1990). Thus, abscission activity could differ as well. The purpose of this study was to test the abscission activity of these compounds and identify conditions that affect response. We applied commercial formulations of two sulfonyleureas, metsulfuron-methyl and prosulfuron, and one imidazolone, imazameth, to either harvested fruit or fruit maintained on greenhouse-grown trees, and measured fruit ethylene production and fruit drop. Ethylene production is highly correlated with abscission in plant organs (Brown, 1997). We, therefore, used ethylene accumulation or production in fruit as a basis for evaluating the abscission potential of these compounds.

Materials and Methods

Studies with harvested fruit. Mature fruit from field-grown ‘Valencia’ trees at the Citrus Research and Education Center, Lake Alfred, Fla., were harvested by clipping the peduncle 1 cm above the fruit calyx. Fruit (20 fruit/treatment) were dipped in the peduncle in 90 mg·L–1 a.i. imazameth (‘Cadre’; American Cyanamid, Parsippany, N.J.); 2 mg·L–1 a.i. metsulfuron-methyl (‘Ally’; DuPont Chemical Co., Wilmington, Del.); or 30 mg·L–1 a.i. prosulfuron (‘Peak’; Novartis, Basel, Switzerland) solutions for 10 s. The dipping solution also contained an organosilicone adjuvant (0.125% Kinetic; Setre Chemical Co., Memphis, Tenn.) and a buffer (0.02% Buffer Xtra Strength; Setre Chemical Co.) to adjust the pH to 6.5. Control fruit were dipped in solutions containing adjuvant and buffer only. To determine the effect of solution pH on internal ethylene accumulation, fruit (20 fruit/treatment/time period) were dipped in solutions of metsulfuron-methyl (2 mg·L–1 a.i.) containing adjuvant and either 100 mm citrate, 2-(N-morpholino) ethanesulfonic acid (MES; Sigma, St. Louis) or 3-(N-morpholino) propanesulfonic acid (MOPS; Sigma) adjusted to pH 4.5, 5.5 (citrate), 6.5 (MES), or 7.5 (MOPS) with HCl or NaOH. Control fruit were dipped in buffered solutions containing adjuvant without metsulfuron-methyl. Fruit were placed in a storage room held at 24 °C and 92% relative humidity (RH). After various times up to 10 d after dipping, internal ethylene accumulation was measured by sampling the evacuated fruit airspace. Fruit were submerged in water and vacuum applied (60 mm Hg) for 10 s. Evacuated fruit airspaces were collected in the neck of an inverted funnel placed over the fruit. For ethylene determinations, 1 mL headspace was injected into a gas chromatograph. Studies with harvested fruit were repeated twice. Internal ethylene concentrations are presented as the means of 40 fruit replicates with standard error of the mean. Sulfonyleureas and imidazolones are known, and their herbicidal activities differ with various substitutions made on each toxophore (Hay, 1990; Ladner, 1990). When sprayed on fruited branches, some compounds induced fruit drop of mature oranges but did not affect leaves. In general, rates of application for citrus fruit abscission were at least an order of magnitude lower than those recommended for herbicidal activity. Many sulfonyleurea and imidazolone compounds are known, and their herbicidal activities differ with various substitutions made on each toxophore (Hay, 1990; Ladner, 1990). Thus, abscission activity could differ as well. The purpose of this study was to test the abscission activity of these compounds and identify conditions that affect response. 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Results and Discussion

Studies with harvested fruit. Dipping harvested fruit in solutions of either imazameth, metsulfuron-methyl, or prosulfuron increased internal ethylene concentration (Fig. 1), making these chemicals potential candidates for abscission agents. Significant increases in internal ethylene production occurred 2 d after dipping fruit in imazameth or prosulfuron and 3 d after dipping fruit in metsulfuron-methyl. Dipping harvested fruit in metsulfuron-methyl solutions at pH 4.5 caused the largest increase in internal ethylene production 6 d after application (Fig. 2). High ethylene production levels were also induced (sustained) in fruit treated with metsulfuron pH 4.5. In contrast, treatment with metsulfuron-methyl solutions between pH 5.5 and 7.5 was less effective, and internal ethylene concentrations declined after 10 d. Reducing the pH of solutions containing sulfonylureas or imidazolinones increases compound lipophilicity, promoting greater uptake across cellular membranes (Hay, 1990). However, hydrolysis of sulfonylurea increases as pH is reduced, limiting the use of low pH to increase uptake.

Greenhouse studies. Metsulfuron-methyl increased ethylene production in fruit of trees held in the 18°C greenhouse between 2 and 5 d after treatment. Fruit drop of both ‘Hamlin’ orange and ‘Marsh’ grapefruit began as ethylene production peaked (Fig. 3). Total fruit drop was 72% and 56% after 10 d in oranges and grapefruit, respectively. In contrast, an average greenhouse temperature of 33°C markedly reduced ethylene production and fruit drop of mature orange and grapefruit treated with metsulfuron-methyl. Fruit ethylene production was low and did not significantly change during the course of the study, and cumulative fruit drop was <7% in both species. High temperatures reportedly reduce herbicidal activity of sulfonylurea and imidazolinone herbicides severely because of the rapid rate of detoxification in treated tissues (Hay, 1990; Ladner, 1990). Temperatures >30°C also reduce ethylene production (Abeles et al., 1992). Temperatures >33°C can occur during the citrus harvesting season that typically begins in October and ends in May. Application of sulfonylureas or imidazolinones to stimulate fruit abscission should be avoided during periods of high temperature.

Application of sulfonylureas or imidazolinones to citrus caused ethylene production and fruit drop, but the reason for increased ethylene production after application remains unclear. Treatment of bean (Phaseolus vulgaris L.) seedlings with glyphosate increased ethylene and CO2 production, suggesting that chemical wounding had a role in the response (Abu-Irmaileh et al., 1979). Chemical wounding of peel in response to abscission materials was considered to be the cause of ethylene production in whole citrus fruit (Evensen et al., 1976; Holm and Wilson, 1977), and ethylene may originate from wound-specific up-regulated ethylene biosynthetic genes (Morgan and Drew, 1997). However, Risley (1986) showed that imazaquin reduced wound ethylene production in leaf discs. Metsulfuron-methyl (2 mg·L⁻¹) also reduced ethylene production in discs (1 cm diameter × 4 mm thick) of orange peel (Burns, Hartmond, and Kender, unpublished), indicating that wounded tissue responds differently than do whole fruit. In whole fruit or tissues treated with acetolactate synthase inhibitors, increased ethylene production may not be a direct consequence of wounding. Amino acid metabolism can be greatly altered as a consequence of acetolactate synthase inhibition. Transgenic potato plants with repressed ALS activity have reduced levels of valine and leucine, but greatly elevated levels of several other amino acids, including a 5-fold increase in methionine (Höfgen et al., 1995). Methionine originating from this source may boost the normally low endogenous pool of methionine (Abeles et al., 1992) and be utilized by the plant to synthesize ethylene coincident with the abscission process.

We have confirmed that sulfonylurea and imidazolinone compounds show potential as abscission materials for citrus. Although these compounds caused variable peel pitting in whole fruit, there were no detrimental effects on internal quality (data not shown), indicat-
Fig. 3. Fruit ethylene production and percent cumulative fruit drop in ‘Hamlin’ orange and ‘Marsh’ grapefruit trees held in greenhouses at average temperatures of 18 °C (●) or 33 °C (○). Fruit were dipped in metsulfuron-methyl (2 mg·L⁻¹) and maintained on the tree. Open bars indicate percent cumulative fruit drop at 18 °C, and solid bars indicate fruit drop at 33 °C. Vertical lines through points indicate SE. Control fruit ethylene production (not shown) at both temperatures remained below 0.25 µL·h⁻¹·kg⁻¹ fresh weight throughout the duration of the study.

Florida citrus industry, an abscission material must: 1) be selective for mature fruit; 2) loosen fruit uniformly; 3) not affect subsequent yield; 4) not be phytotoxic; and 5) have a high probability of registration (Kender, 1998). We are currently evaluating these abscission agents at various application rates for their ability to loosen Florida citrus under commercial conditions.

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