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# Comparison of the Effects of Metamitron on Chlorophyll Fluorescence and Fruit Set in Apple and Peach

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Abstract. The effects of foliar applications of the photosystem II (PSII) inhibitor metamitron on chlorophyll fluorescence and fruit set were compared in peach and apple trees. Metamitron increased dark-adapted chlorophyll fluorescence, measured as a reduction in Fv/Fm values, in both peaches and apples. Maximum suppression of the normalized ratio of variable fluorescence to maximum fluorescence (Fv/Fm) in peaches occurred 1 to 2 days after application and Fv/Fm values recovered by 7 days after treatment. The effects of metamitron on chlorophyll fluorescence were more persistent in apples compared with peaches. Fv/Fm values in apple declined within 2 days of treatment and did not start recovering until 5 days after treatment or longer. Concentrations of metamitron greater than  $200~\text{mg}~\text{L}^{-1}$  were phytotoxic to peach leaves, reducing the leaf chlorophyll concentration as determined by SPAD measurements. At 300 mg·L<sup>-1</sup>, metamitron reduced fruit set in apple but not in peach. Inclusion of a non-ionic surfactant (Silwett L-77) with metamitron greatly increased its negative effect on Fv/Fm, quantum photosynthetic yield of PSII (ΦPSII), and relative electron transport rate (ETR). These results suggest that metamitron may be a useful thinner in apple but not in peach. Additional information is needed to understand how combining metamitron with existing thinning chemicals might enhance their activity. In particular, caution may be necessary if metamitron is applied as a tank mixture with commercial thinning products that have been formulated with a wetting agent.

Methods for regulating crop load to commercially acceptable levels that mitigate the need for hand-thinning remain a key challenge in apple (*Malus domestica* Borkh.) and peach (*Prunus persica* Batsch.) production systems worldwide. This challenge has become more acute given the uncertainty of the availability and cost of agricultural labor in the future and the increasing regulatory attention focused on products used for thinning of apples. Products currently registered for fruit thinning of apple in the United States include the carbamate

insecticide, 1-naphthyl methylcarbamate (carbaryl), the ethylene releasing agent, 2chloroethylphosphonic acid (ethrel, ethephon), the cytokinin, 6-benzyladenine (6-BA), and the synthetic auxins, 1-naphthaleneacetic acid (NAA) and naphthaleneacetamide. These products are often applied in different combinations and at different times during the 3-4 weeks after bloom to achieve more aggressive fruit abscission when compared with the application of any single product alone. Several of these compounds, notably carbaryl and ethrel, are coming under increasingly stringent regulatory pressures worldwide (Anon, 2006, 2009). The potential for loss of existing fruit thinning products, together with uncertainties about the cost and availability of agricultural labor for hand-thinning in the future, has provided focus for renewed efforts to identify alternative thinning materials for apple.

The imposition of shade treatments during or shortly after bloom stimulates fruit abscission in several crops including apples (Byers et al., 1985, 1990, 1991; McArtney et al., 2004; Zibordi et al., 2009), peaches (Byers et al., 1984), and grapes (*Vitis vinifera* L.) (Ferree et al., 2001). Shade treatments are presumed

to create a transient reduction in the supply of carbohydrates to developing fruit during a period when the fruit are sensitive to such a stress. In apple, shoot growth has priority over fruit growth for carbohydrate partitioning when light levels during the first 40 d after bloom are limiting (Bepete and Lakso, 1998). A carbon balance modeling approach was used to identify a high probability of fruit production being limited by the development of a carbohydrate deficit in the tree during the 2- to 3-week period after bloom (Lakso et al., 1999). Furthermore, application of the fruit thinner 6-BA to apple trees was recently shown to result in a carbohydrate deficit in the tree that was rapidly perceived in the fruit cortex (Botton et al., 2011). From gene expression studies it was hypothesized that embryo development was blocked by the severe carbohydrate deficit after 6-BA application, resulting in reduced polar auxin transport across the fruit pedicel and enhanced sensitivity of the abscission zone to ethylene, eventually leading to activation of the abscission zone (Botton et al., 2011).

Foliar application of photosynthetic inhibitors has been used to stimulate fruit abscission in fruit crops, although none are currently registered for this purpose. Lime sulfur reduced leaf photosynthesis (Hoffman, 1935; Hyre, 1939; Palmer et al., 2003) and fruit set (McArtney et al., 2006) in apple. The PSII inhibitor terbacil reduced fruit set of peaches (Byers et al., 1984; Del Valle et al., 1985), apples (Byers et al., 1985, 1990), and grapes (Lopez et al., 2004). More recently, the PSII inhibitor metamitron has been shown to reduce fruit set in apples (Clever, 2007; Deckers et al., 2010; Dorigoni and Lexxer, 2007; Lafer, 2010). Photosynthetic inhibitors might also be used to enhance the activity of existing chemical thinning agents in apples (Byers et al., 1984). Before adopting such an approach, it may be necessary to account for increased activity of PSII inhibitors if they are applied in combination with commercial formulations of existing thinning chemicals that include a surfactant.

The triazinone herbicide metamitron is a systemic, xylem-translocated PSII inhibitor that acts by blocking electron transfer between the primary and secondary quinones of PSII (see Abbaspoor et al., 2006, and references cited therein). Interruption of photosynthetic electron transport inhibits adenosine 5'-triphosphate production and carbon fixation. If this interruption is permanent, plant death is caused by lipid peroxidation and proteolysis and dissociation of the protein-pigment complexes of PSII as a result of light-induced oxidative stress (Abbaspoor et al., 2006). The photosynthetic response to metamitron was described in a number of plant species (Van Oorschot and Van Leeuwen, 1979). Complete recovery of photosynthesis in sugar beet (Beta vulgaris L.) occurred within 2 h after a spray application to the leaves or withdrawal of metamitron from the rooting medium. Recovery of photosynthesis was slower and incomplete in perennial ryegrass (Lolium perenne L.) and undetectable in maize (Zea mays L.) and Portulaca oleracea L. Differences in the rate of photosynthetic recovery of different plant

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We express our appreciation to Makhteshim Agan of North America, Inc. for providing metamitron. Mention of a trademark, proprietary product, or vendor in this publication does not constitute a guarantee or warranty of the product and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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species after exposure to metamitron are the result of the rate of inactivation through an enzymatic, light-independent deamination (Schmidt and Fedtke, 1977). The effects of metamitron on the photosynthetic activity of tree fruits such as apple and peach have not been described.

Photochemistry, chlorophyll fluorescence, and heat dissipation represent three competing de-excitation pathways for the light energy absorbed by chlorophyll in plant leaves (Maxwell and Johnson, 2000). A reduction in the efficiency of photochemistry can be measured as an increase in chlorophyll fluorescence or heat dissipation. The normalized ratio of variable fluorescence to maximum fluorescence represents the maximum potential quantum efficiency of PSII if all capable reaction centers are open. Changes in Fv/Fm were used to study the recovery process after the addition of root-absorbed PSII inhibitors to the nutrient solution of sugar beets growing in hydroponic culture (Abbaspoor et al., 2006).

The objectives of the present study were to 1) compare the thinning responses of apple and peach to different concentrations of foliar-applied metamitron; 2) use the dark-adapted chlorophyll fluorescence parameter Fv/Fm to describe the effects of this PSII inhibitor on the photosynthetic apparatus in these two species; and 3) use various chlorophyll fluorescence parameters to describe the effects of a non-ionic surfactant on the activity of metamitron in apple.

## **Materials and Methods**

Response of peaches to metamitron and terbacil. Twenty-five uniform 'Contender' peach trees were selected within a mature orchard at the Mountain Horticultural Crops Research Station in Mills River, NC, in Apr. 2011. Five fully guarded whole trees were sprayed with 100, 200, or 400 mg·L<sup>-1</sup> metamitron (Goltix; Makhteshim Agan of North America, Inc., Raleigh, NC), five trees were left as an unsprayed control, and five trees were sprayed with 200 mg·L<sup>-1</sup> terbacil (Sinbar; Tessenderlo Kerley, Inc., Phoenix, AZ). The treatments were applied to fully guarded singletree plots with an airblast sprayer calibrated to deliver a water volume of 1496 L·ha<sup>-1</sup>. The treatments were first applied on 5 May [30 d after bloom (DAB)] when the mean fruit diameter was 18.8 mm and then reapplied on 20 May (45 DAB) when the mean fruit diameter was 32.3 mm. Treatments were arranged in a randomized complete block design experiment with five replications.

Shoot length and initial fruit number were counted on 10 sample shoots selected from shoulder height around the periphery of each tree on 5 May and the final fruit number on each shoot was counted again on 2 June. Fruit set was expressed as the final fruit number per centimeter shoot length and as percent fruit set, i.e., the final fruit number expressed as a percent of the initial fruit number on each shoot. All of the trees were hand-thinned to a commercial crop load on 7 June to leave 15–20 cm between fruit on a branch.

Chlorophyll fluorescence measurements were carried out on four recently fully expanded leaves from each tree daily during the 10 d after each treatment application to provide an indication of the effects of metamitron on the maximum potential quantum efficiency of PSII (Fv/Fm). Fluorescence measurements were made between 0900 HR and 1100 HR each day. Individual leaves were marked and subsequent measurements were made on the same leaves so that differences in absorbance were likely to be insignificant. A different group of leaves was selected for fluorescence measurements after each application date. Fluorescence was measured using a portable chlorophyll fluorometer (OS1p; Opti-Sciences, Hudson, NH) with a modulated light source of 0.2  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> at 660 nm and a saturation pulse from a white light light-emitting diode with an intensity of 7700 µmol·m<sup>-2</sup>·s<sup>-1</sup> for a duration of 0.8 s. Leaves were darkadapted for 30 min before measurement to ensure that all capable PSII reaction centers were fully oxidized.

Leaf chlorophyll content was estimated using a chlorophyll meter (SPAD 502; Spectrum Technologies Inc., Plainfield, IL). Measurements were taken on 31 May, 26 d after the initial application, on the sample leaves used for fluorescence measurements after the second treatment application date.

Response of apples to metamitron. A second study was undertaken using 16 5-year-old 'SunCrisp'/M.7 apple trees. Four trees were sprayed with metamitron (Goltix; Makhteshim Agan of North America, Inc., Raleigh, NC) at 100, 200 or 300 mg·L<sup>-1</sup> and four trees were left as unsprayed controls. The spray treatments were applied to the apple trees on the same two dates in 2011 as for peaches (5 May, 23 DAB, mean fruit diameter 19.6 mm; 20 May, 38 DAB, mean fruit diameter 26.5 mm) using the same equipment and water rate as in the previous study. Fruit set was recorded by counting the number of flower clusters at bloom and the final number of fruit on two sample limbs in each tree. Chlorophyll fluorescence and leaf chlorophyll content were measured as previously described. All of the trees were handthinned to a commercial crop load on 7 June to leave 15-20 cm between fruit on a branch. Trees sprayed with 300 mg·L<sup>-1</sup> metamitron required no additional hand-thinning since they were overthinned.

Effect of surfactant on metamitron activity on apple. The effect of a non-ionic surfactant on activity of metamitron was investigated in a randomized complete block design experiment using five year old 'Cameo'/M.7 apple trees. In addition to an unsprayed control, metamitron (Metamitron 150 SG, Makhteshim Agan of North America, Inc.) was applied on 30 June 2011 at 200 mg·L<sup>-1</sup> alone or in combination with Silwet L-77 at a final concentration of 0.05% (v/v). The treatments were applied to fully guarded single tree plots with five replications in a spray volume of 1496 L·ha<sup>-1</sup>. The chlorophyll fluorescence parameters (Fv/Fm), ΦPSII, and relative ETR were measured on four leaves per tree before the treatments were applied (Day 0) and again on the same leaves

1, 3, 5, and 7 d later. Fv/Fm was measured as previously described. The leaves selected for ΦPSII and ETR measurements were in full sunlight at the time of measurement, i.e., under steady-state photosynthesis conditions. ΦPSII and ETR measurements were made between 1100 HR and 1300 HR each day when the incident photosynthetically active radiation (PAR) was greater than 1500  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. Leaf temperature (°C) and the level of PAR (µmol·m<sup>-2</sup>·s<sup>-1</sup>) incident on each measurement leaf were recorded with a leaf PAR clip (Opti-Sciences) that held the fiberoptic probe of the fluorometer at a constant 45° to the leaf surface. ETR was calculated using the formula ETR =  $\Phi PSII \times PAR \times leaf$  absorption coefficient  $\times$ fraction of light absorbed by the PSII antennae. Average plant values of 0.84 and 0.50 were used for the leaf absorption coefficient and the fraction of light absorbed by PSII, respectively.

Statistical analysis. The data were analyzed using analysis of variance (ANOVA) and mixed model procedures in SAS software (SAS Institute Inc., Cary, NC). Treatment effects on fruit set, total yield, chlorophyll content, and mean fruit weight were analyzed with the proc ANOVA command and mean separations by LSMEANS. Differences between treatment means were assessed by Duncan's multiple range test at the 0.05 P level for these response variables. Univariate analysis of repeated measures was performed on Fv/ Fm data from the 'Contender' peach and 'Suncrisp' apple studies with the proc MIXED command specifying a compound symmetry covariance structure.

#### Results

Response of peaches to metamitron and terbacil. Foliar application of metamitron to 'Contender' peaches reduced the maximum potential quantum efficiency of PSII 1 d after treatment, measured as an increase in darkadapted chlorophyll fluorescence (syn. reduction in Fv/Fm values) relative to the untreated control. The reduction in Fv/Fm reached a maximum within 1-2 d after treatment (Fig. 1) and Fv/Fm slowly recovered to control values by 6-8 d after treatment (Fig. 1; Table 1). Curiously, 100 mg·L<sup>-1</sup> metamitron was without effect on Fv/Fm after the first spray application, but when this concentration of metamitron was reapplied to the same trees 15 d later, Fv/Fm was reduced relative to the control

Terbacil (200 mg·L<sup>-1</sup>) appeared to have a more negative effect on Fv/Fm compared with 300 mg·L<sup>-1</sup> metamitron at both spray timings (Fig. 1). Leaf chlorophyll content was significantly lower in trees sprayed with metamitron at 200 or 300 mg·L<sup>-1</sup> compared with the control (Table 2) and could be observed as mild chlorosis. Metamitron and terbacil showed no effect on fruit set, total yield, or mean fruit weight of 'Contender' peaches (Table 2).

Response of apples to metamitron. Fv/Fm declined 2 d after the first foliar application of metamitron to 'SunCrisp' apple trees, and Fv/Fm values on sprayed trees remained

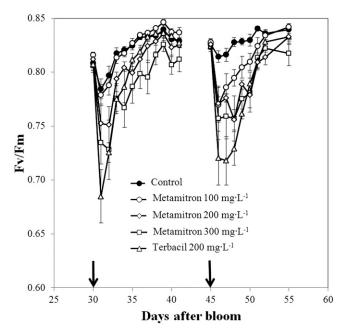


Fig. 1. Effects of foliar applications of the photosystem II (PSII) inhibitors metamitron and terbacil on dark-adapted chlorophyll fluorescence (Fv/Fm) in leaves of 'Contender' peaches. Metamitron and terbacil were applied on 5 May [30 d after bloom (DAB)] and reapplied on 20 May (45 DAB) in a spray volume of 1496 L·ha<sup>-1</sup>. Arrows indicate application dates. Vertical bars indicate se of the means; n = 5.

Table 1. P values from mixed model univariate analysis of repeated measurements of dark-adapted chlorophyll fluorescence (Fv/Fm) after application of photosystem II inhibitors to 'Contender' peaches.

Source of variation	df	Pr > F	Time after application (d)	Treatment effect $(Pr > F)$	
				First spray	Second spray
Treatment	4	< 0.001	0	0.910	0.998
Spray	1	0.143	1	< 0.001	< 0.001
Treatment*spray	4	0.136	2	< 0.001	< 0.001
Day (spray)	19	< 0.001	3	< 0.001	< 0.001
Treatment*day (spray)	76	< 0.001	4	< 0.001	< 0.001
			5	0.006	< 0.001
			6	0.023	0.007
			7	0.048	0.213
			8	0.488	
			9	0.641	
			10	0.258	0.479
			11	0.476	

Table 2. Effects of the photosystem II inhibitors metamitron and terbacil on fruit set, leaf chlorophyll content, total yield per tree, and mean fruit weight at harvest of 'Contender' peaches.<sup>z</sup>

	Fruit set		Chlorophyll	Total yield	Mean fruit
Treatment	Fruit/cm	Percent	content (SPAD)	(kg fruit per tree)	wt (g)
Control	0.29	77	44.4 a <sup>y</sup>	54.9	174
Metamitron 100 mg·L <sup>-1</sup>	0.26	69	42.4 a	50.2	190
Metamitron 200 mg·L <sup>-1</sup>	0.24	66	38.2 b	53.2	180
Metamitron 300 mg⋅L <sup>-1</sup>	0.25	74	38.8 b	47.5	178
Terbacil 200 mg·L <sup>-1</sup>	0.25	73	42.1 a	48.8	185
Significance	NS <sup>x</sup>	NS	***	NS	NS

<sup>&</sup>lt;sup>z</sup>Treatments were applied on 5 May and reapplied on 30 May.

suppressed 11 d after treatment when applied at 300 mg·L<sup>-1</sup> (Fig. 2; Table 3). In contrast to peaches in which the maximal effect of metamitron on Fv/Fm lasted until only 1–3 d after treatment (Fig. 1), Fv/Fm was suppressed for 5–6 d after treatment to apples before it started to recover (Fig. 2). Significant

effects of treatment on Fv/Fm persisted until at least 11 d after the first spray application to 'SunCrisp' apples; however, these effects had already disappeared 8 d after the second spray application (Table 3). Metamitron was without effect on leaf chlorophyll content (Table 4), indicating there were no phytotoxic effects at concentrations up to 300 mg·L<sup>-1</sup>. Metamitron reduced fruit set of apples in a concentration-dependent manner {fruit set [%] =  $70.2 - [0.055 \times (\text{mg·L}^{-1} \text{ metamitron})] - [0.00019064 \times (\text{mg·L}^{-1} \text{ metamitron})^2]; r^2 0.69, P < 0.001$ }. There was a negative linear relationship between metamitron concentration and fruit yield per tree at harvest [yield (kg) = 47.3 - ppm metamitron;  $r^2 = 0.39$ ; P < 0.01]. There was no effect of metamitron on mean fruit weight at harvest (Table 4).

Effect of surfactant on metamitron activity. Although metamitron (200 mg·L<sup>-1</sup>) alone was without effect on Fv/Fm in leaves of 'Cameo' apple, addition of Silwet L-77 at a final volume of 0.05% significantly reduced Fv/Fm 1 d and 3 d after treatment compared with the control (Fig. 3). ΦPSII and ETR were more sensitive than Fv/Fm to metamitron, which significantly reduced these parameters by  $\approx 20\%$  and 15% 1 d and 3 d after treatment, respectively. ΦPSII and ETR were not significantly different between the control and the metamitron alone treatment when measured 5 d after treatment (Fig. 3). Addition of the non-ionic surfactant Silwet L-77 significantly increased the negative effect of metamitron on  $\Phi$ PSII and ETR; these parameters were  $\approx 60\%$ , 48%, and 30% lower than the controls when measured 1, 3, and 5 d after treatment, respectively.

### Discussion

Optimal values of the fluorescence parameter Fv/Fm are ≈0.83 for most plant species with lower values indicating photoinhibitory stress (Maxwell and Johnson, 2000). During the course of measurements, Fv/Fm values in untreated peach and apple trees ranged from 0.78–0.83 and 0.76–0.82, respectively. These values, obtained from field-grown trees, are consistent with values previously reported for container-grown apple trees under similar light levels (Cheng et al., 2001). Dark-adapted values of Fv/Fm declined 1 d after application of 200 mg·L<sup>-1</sup> terbacil to peach trees, indicating that this PSII inhibitor had an immediate negative effect on the photosynthetic efficiency in peach leaves. Furthermore, Fv/Fm values in the peach leaves did not recover until 6-7 d after application of the PSII inhibitors. The fluorescence responses of peaches to terbacil in the present study were consistent with previous reports describing the effects of this compound on the rate of leaf photosynthesis in peaches (Del Valle et al., 1985). Reduced values of Fv/Fm were also measured immediately after a foliar application of metamitron to 'SunCrisp' apple trees, although recovery of Fv/Fm appeared to be more rapid in leaves of peach compared with apple. These data indicate that apples, but not peaches, are able to recover from the negative effects of metamitron at rates up to 300 mg·L<sup>-1</sup> before permanent damage to the photosynthetic apparatus occurs. The reduction in Fv/Fm values in response to foliar applications of metamitron in the present study were relatively minor compared with the response of sugar beets to continuous exposure of 140 mg·L<sup>-1</sup> metamitron in nutrient solution, in which Fv/Fm levels

<sup>&</sup>lt;sup>y</sup>Mean in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

 $<sup>^{</sup>x}NS$ , \*\*\*Nonsignificant or significant at  $P \le 0.001$ , respectively.

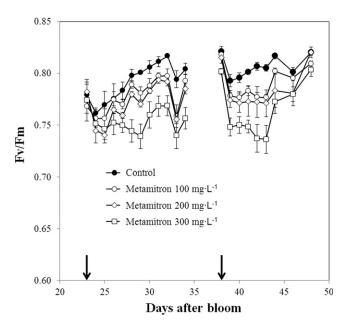


Fig. 2. Effects of foliar applications of the photosystem II (PSII) inhibitor metamitron on dark-adapted chlorophyll fluorescence (Fv/Fm) in leaves of 'SunCrisp'/M.7 apples. Metamitron was applied on 5 May [23 d after bloom (DAB)] and reapplied on 20 May (38 DAB) in a spray volume of 1496 L·ha<sup>-1</sup>. Arrows indicate application dates. Vertical bars indicate se of the means; n = 4.

Table 3. *P* values from mixed model univariate analysis of repeated measurements of dark-adapted chlorophyll fluorescence (Fv/Fm) after application of the photosystem II inhibitor metamitron to 'SunCrisp' apples.

			Time after	Treatment effect (Pr > F)	
Source of variation	df	Pr > F	application (d)	First spray	Second spray
Treatment	3	< 0.001	0	0.745	0.317
Spray	1	0.005	1	0.566	0.001
Treatment*spray	3	0.968	2	0.093	0.001
Day (spray)	19	< 0.001	3	0.187	< 0.001
Treatment*day (spray)	57	< 0.001	4	0.044	< 0.001
			5	< 0.001	< 0.001
			6	< 0.001	< 0.001
			7	0.003	
			8	0.006	0.139
			9	0.002	
			10	< 0.001	0.355
			11	0.001	

Table 4. Effects of the photosystem II inhibitor metamitron on fruit set, leaf chlorophyll content, total yield per tree, and mean fruit weight at harvest of 'SunCrisp' apples.<sup>2</sup>

	Fruit set		Chlorophyll	Total vield	Mean fruit
Treatment	Fruit/cm <sup>2</sup>	Percent	content (SPAD)	(kg fruit per tree)	wt (g)
Control	7.4 a <sup>y</sup>	71.1 a	50.7	46.9 a	199
Metamitron 100 mg·L <sup>-1</sup>	8.0 a	59.9 a	51.6	42.9 a	215
Metamitron 200 mg·L <sup>-1</sup>	8.3 a	54.2 a	52.5	32.5 b	208
Metamitron 300 mg·L <sup>-1</sup>	4.5 b	35.1 b	52.0	29.5 b	216
Significance of regression					
Linear	*x	***	NS	**	NS
Quadratic	***	***	NS	*	NS

<sup>&</sup>lt;sup>z</sup>Treatments were applied on 5 May and reapplied on 30 May.

declined to less than 0.10 3 d after treatment (Abbaspoor et al., 2006). Whereas complete recovery of Fv/Fm to pre-treatnent levels occurred just 4 d after removal of metamitron from the nutrient solution in sugar beets (Abbaspoor et al., 2006), recovery in peach and apple trees in the present studies was slower, indicating that apples and peaches

were unable to recover from sublethal doses of this compound as rapidly as sugar beets.

Despite having a negative effect on Fv/Fm in both peach and apple trees, fruit set was reduced by foliar applications of metamitron in apple only. It has previously been suggested that terbacil concentrations less than 500 ppm would be effective for thinning

peach trees (Del Valle et al., 1985), yet foliar sprays of 200 mg·L<sup>-1</sup> terbacil were without effect on fruit set of 'Contender' peaches in the current study. In contrast, the concentrationdependent negative effect of metamitron on Fv/Fm in 'SunCrisp' apples paralleled a negative linear effect of metamitron on fruit set. Presumably, the transient reduction in PSII efficiency after foliar application of metamitron to 'SunCrisp' apple trees created a transient carbohydrate deficit in the tree that was severe enough to result in activation of the fruit abscission zone (Botton et al., 2011). Generation of a carbohydrate deficit in 'Contender' peach trees did not result in fruit abscission, suggesting that young peach fruits may be stronger carbohydrate sinks compared with young apple fruits.

The reduction in ΦPSII and ETR after application of metamitron to 'Cameo' apples was greater when a non-ionic surfactant was included in the spray. The increased activity of metamitron when applied with a surfactant indicates potential for increased thinning activity if metamitron is applied in combination with thinning products that are formulated with a wetting agent. It was suggested that photosynthetic inhibitors should be investigated for their potential to enhance the activity of chemical thinning agents such as carbaryl or NAA in apples (Byers et al., 1984). Application of a PSII inhibitor such as metamitron to apple trees can result in a transient carbohydrate stress that may increase the sensitivity of the fruit to a chemical thinner application. In addition to this direct effect, if metamitron is applied in combination with a chemical thinner that has been formulated with a wetting agent, then the resulting carbohydrate stress may result in aggressive thinning compared with a chemical thinner that does not have a wetting agent included in its formulation.

The fluorescence parameters ΦPSII and ETR can provide useful information concerning photosynthetic performance of field-grown plants, particularly when measurements are made on homogeneous samples through time (Maxwell and Johnson, 2000). In these experiments, individual leaves for fluorescence measurements were marked and subsequent measurements made on the same leaves throughout each series of measurements, ensuring that leaf-to-leaf differences in absorbance were minimized. Cheng et al. (2001) reported a curvilinear relationship between ΦPSII and the true quantum yield for CO<sub>2</sub> assimilation in apple leaves with the relationship being linear up to a quantum yield of ≈0.05 mol CO<sub>2</sub>/mol quanta (corresponding to a  $\Phi$ PSII value of  $\approx$ 0.5). Because  $\Phi$ PSII values in the current experiments were less than 0.5, we have assumed that differences in ΦPSII values are linearly related to differences in the true quantum yield for CO<sub>2</sub> assimilation. ΦPSII values in control plants in the current study were within the range typical of apple trees grown under similar light intensities (Cheng et al., 2001). Metamitron reduced ΦPSII in 'SunCrisp' apple leaves by 20% and 15% measured 1 d and 3 d after application,

<sup>&</sup>lt;sup>y</sup>Mean in the same column followed by the same letter are not significantly different at  $P \le 0.05$ .

 $<sup>^{</sup>x}NS$ , \*, \*\*, \*\*\* indicates nonsignificant or significant at  $P \le 0.05$ , 0.01, or 0.001, respectively.

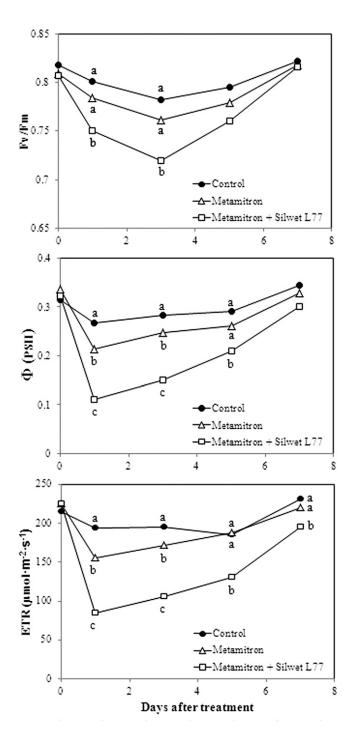


Fig. 3. Effects of a non-ionic surfactant (Silwet L-77) on the fluorescence responses of 'Cameo'/M7 apple leaves to  $200~{\rm mg\cdot L^{-1}}$  metamitron. The maximum potential quantum efficiency of photosystem II (PSII) (Fv/Fm), quantum photosynthetic yield of PSII ( $\Phi$ PSII), and estimated relative electron transport rate (ETR) were measured on four leaves on each tree. Lower case letters indicate significant differences between treatment means on a given day; days after treatment without letters are not significantly different (Duncan multiple range test,  $P \le 0.05$ , n = 5).

respectively. Inclusion of a non-ionic surfactant to metamitron reduced ΦPSII by 60%, 47%, and 28% measured 1 d, 3 d, and 5 d after application, respectively. Thus, it appears that application of metamitron to apple trees can interfere with photosynthetic electron transport, creating a transient carbohydrate stress in the tree that can result in activation of the fruit abscission zone. An increase in the activity of the fruit abscission zone can increase

the sensitivity of the young fruit to a chemical thinner application. If activation of the fruit abscission zone is triggered by a critical threshold level of carbohydrates within the fruit cortex, as proposed by Botton et al. (2011), then the efficacy of metamitron as a fruit thinner will be dependent on a number of factors, including carbohydrate balance in the tree at the time of application, daily level of carbon assimilation, and allocation of

assimilated carbohydrates between competing sinks such as shoots, fruit, and respiration. Characterization of the effects of metamitron on whole-tree carbohydrate assimilation in apple trees, together with the adoption of a carbon balance modeling approach to the fruit abscission process in apple (Lakso, 2011; Robinson and Lakso, 2011), may ultimately provide a practical way to achieve predictable and consistent chemical thinning responses in apples.

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