



Imidacloprid Has Little Effect on Growth or Drought Tolerance of Citrus Rootstock Seedlings without Pests

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Imidacloprid, a widely used systemic chloronicotinyl insecticide, has been credited with being able to increase plant growth and increase resistance to plant stress even in the absence of pest pressures. Greenhouse studies using imidacloprid and drought stress on 6-month-old seedlings of the citrus rootstocks Carrizo citrange (Carr) and Cleopatra mandarin (Cleo) were repeated in two consecutive years in the absence of pests. Imidacloprid was applied at the manufacturer's recommended rate either as a soil drench (Admire 2F) or as a foliar spray (Provado 1.6F). Half of the plants were drought stressed and the other half of the plants were kept well-irrigated. After 10 to 12 weeks, leaf N, plant water status, and growth were determined along with leaf photosynthesis and leaf water use efficiency (WUE) by gas exchange. In year 1, drought stress reduced leaf N but higher fertilizer rates in year 2 resulted in higher leaf N in drought stressed than in well-irrigated plants in both species. Imidacloprid had no effect on leaf N (percent dry weight), plant water status or total plant growth of either species in either year. Imidacloprid increased root growth but decreased total leaf area such that there was an increase in leaf thickness and an increase in leaf N when expressed on a leaf area basis. Imidacloprid increased leaf photosynthesis and WUE of both species in both years regardless of plant water status. Thus, imidacloprid changed growth allocation between roots and shoots and increased leaf dry weight per area, leaf photosynthesis, and WUE but these responses did not translate into any changes in drought tolerance or total plant growth.

Neonicotinoids or chloronicotinyls are an important class of synthetic insecticides that are commonly used for systemic control of plant-sucking insects (Tomizawa and Casida, 2003). Imidacloprid, a widely used chloronicotinyl insecticide for pest control in many crops including citrus, has good systemic action in plants and relatively low mammalian toxicity. Imidacloprid is effective when applied as a soil drench or as a foliar spray because it is readily translocated in the xylem (Buchholz and Nauen, 2001). The use of imidacloprid has become very widespread in Florida citrus to combat the insect vector of citrus greening, the Asian citrus psyllid (Boina et al., 2009; Rogers et al., 2008). Improvements in growth and yield in most crops after imidacloprid application has led to speculation that it may have some positive plant growth regulator effects or that imidacloprid can increase plant stress tolerance beyond that expected from insect pest control. There is little known about the effects of imidacloprid on plant stress tolerance but imidacloprid does not enhance growth and yield of muskmelon in the absence of whitefly (Palumbo and Sanchez, 1995). However, imidacloprid can alter plant nutrition and can even become phytotoxic to greenhouse tomatoes and cucumber (Ebel et al., 2000) but it may not alter growth and net gas exchange of leaves (Wallace et al., 2000).

In laboratory and greenhouse studies on the control of citrus root weevils on potted seedlings of citrus rootstocks, imidacloprid was shown to be an effective systemic insecticide when larvae fed on treated roots (McCoy et al., 1995). In addition, fibrous root growth in the imidacloprid treatments was more than 2.8-fold greater than in the untreated control seedlings without larval infestation. They speculated that either the imidacloprid applied as a soil drench or the early larval feeding prior to their

death stimulated root growth. McCoy et al. (1995) did not report any shoot growth data but since root growth often occurs at the expense of shoot growth (Eissenstat and Duncan, 1992) and roots and shoots do not grow at the same time (Bevington and Castle, 1985), any increase in root growth could have resulted in reduced shoot growth.

We designed two repeated greenhouse experiments to determine if imidacloprid, applied either as a soil drench or as a foliar spray, affected plant growth or nitrogen nutrition of two citrus rootstock seedlings without pests. We hypothesized that if imidacloprid affected plant growth allocation between shoots and roots, it should also affect plant water relations responses to drought stress, leaf N nutrition, and/or leaf photosynthesis.

Materials and Methods

Experiment 1, 2005

The study was carried out at the University of Florida/IFAS Citrus Research and Education Center, Lake Alfred, FL (28.09°N, 81.73°W; 51 m altitude). Uniform 6-month-old Carrizo citrange (Carr, *Citrus sinensis* L. Osb. × *Poncirus trifoliata* L.) and Cleopatra mandarin (Cleo, *C. reticulata* Blanco) seedlings were obtained from a commercial nursery and transplanted into 150-cc containers filled with native Candler fine sand and grown in an unshaded greenhouse from June through Sept. 2005. Forty-eight seedlings of each species were well-irrigated for a 2-week period to recover from transplant. Plants were then fertilized three times per week for 2 weeks with 20 mL each of a 10–2–12 (N–P–K from ammonium nitrate, potassium phosphate and potassium sulfate) nutrient solution at a concentration of 100 mg/L (ppm) of nitrogen.

Four weeks after transplanting, imidacloprid (Admire 2F, Bayer CropScience, Kansas City, MO) was applied once at the

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beginning of the experiment to one-third of the seedlings as a soil drench at the manufacturers recommended rate of 0.02 mL/L of soil (0.66 mL/ft³). A foliar imidacloprid (Provado 1.6F) treatment was applied to another third of the seedlings at the manufacturer's recommended rate of 0.546 mL/L (7 fl oz/100 gal) of water plus Kinetic organosilicone adjuvant at 56 mL/3.78 L (2 fl oz/gal of water) every 10 to 12 d throughout the experiment. The Provado was thoroughly sprayed onto each seedling to run-off (approximately 8 mL per seedling) while the soil surface was covered to prevent contamination. The remaining one-third of the seedlings served as untreated controls. Half of the seedlings in each treatment were watered 3 times per week while half were allowed to become drought stressed by withholding fertigation until the youngest leaves at the growing tip began to wilt, about every 4 to 6 d. This resulted in an experimental design of 2 species × 3 imidacloprid × 2 drought levels with 8 replicate seedlings in each treatment. Midday photosynthetically active radiation was about 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; maximum/minimum day/night temperatures were 40/26 °C and relative humidity varied from 40% to 100%.

Since fertilizer was only added in solution, seedlings that had different water use rates received different amounts of N. On 13 Sept. 2005 the fertilizer concentration was doubled to 200 mg/L nitrogen, which was applied for the remainder of the experiment when irrigation was required. With the exception of the Provado treatments, weekly applications of either 1% Safer soap or 1% petroleum spray oil were alternated for pest control. No pest damage or leaf phytotoxicity was observed on any seedlings.

At the end of the experiment, leaf photosynthesis and water relations were measured before fertigation on clear days using a fully expanded mature leaf from the mid-shoot area of six replicate plants from each treatment. Net assimilation of CO₂ (A_{CO_2}), stomatal conductance (g_s) and leaf transpiration were determined with a Li-Cor portable photosynthesis system (LI-6200; Li-Cor Inc., Lincoln, NE) in the morning from 9:30 to 11:30 when leaf temperature in the cuvette was 31 to 33 °C. Leaf water use efficiency (WUE) was calculated as $A_{\text{CO}_2}/\text{transpiration}$. Drought stress was assessed by measuring stem water potential (SWP) in the mid afternoon on the same day as gas exchange measurements using a Scholander-type pressure chamber (PMS instrument, Corvallis, OR; Scholander et al., 1965). Six similar leaves as those used for gas exchange measurements, were enclosed for about 3 h in aluminum foil-covered plastic bags to allow leaf water potential to equilibrate to stem water potential (McCutchan and Shackel, 1992).

At the end of 12 weeks, during the last week in September, plants were harvested. The leaves were removed and measured for total leaf area for each plant using a leaf area meter (Li-3000; Li-Cor). The leaves, stems and washed roots and were separated, dried in an oven at 60 °C for 3 d and their dry weights determined. Dried leaves were ground to a powder and total nitrogen (N) was analyzed by a commercial lab.

Experiment 2, 2006

Experiment 2 repeated the 2005 experiment using similar seedlings and the same treatments during the summer of 2006. However, the fertilizer concentration was increased to 260 mg/L of nitrogen and N rates were adjusted for irrigation frequency so all seedlings received a similar total amount of N per plant over the course of the experiment. The treatments were begun during the second week in July and continued for 10 weeks. Leaf gas exchange and water relations characteristics were measured as in 2005 but average leaf temperatures were 28 to 31 °C.

STATISTICS. Data were analyzed using analysis of variance on the 2 species × 3 imidacloprid × 2 drought treatments with 8 replicate seedlings in each treatment. When treatments were significant, means were separated using Duncan's multiple range test at $P < 0.05$.

Results

In the 2005 experiment, the Cleo seedlings were about half the size of the Carr seedlings based on total plant dry weight (Table 1). Cleo seedlings required less water so they received less N than the Carr seedlings which resulted in the Cleo seedlings having lower leaf N levels than Carr. Entire Cleo leaves had more leaf dry weight per area (LDW/A) and so were thicker than the trifoliate Carr leaves. The well-irrigated treatment received more water than the drought stressed treatment and thus more N per plant over the course of the 12-week experiment. This was reflected by more growth and higher leaf N in the well-irrigated than drought stressed seedlings which allocated relatively more growth to roots than shoots as measured by the higher root/shoot dry weight ratio (RT/SH) in the drought stressed treatment. Both imidacloprid treatments increased root growth, so RT/SH was increased but imidacloprid also reduced leaf area growth such that total plant growth was not affected. Imidacloprid had no effect on leaf N expressed on a percentage dry weight basis but soil applied Admire increased leaf N when expressed on a leaf area basis due to the consistent increases (though non-significant) in LDW/A.

In 2006, Cleo seedlings were again smaller, had greater LDW/A and lower leaf N (percent dry weight) than Carr seedlings even though all seedlings received equal amounts of N (Table 1). Differences in leaf N disappeared, however, when expressed on a leaf area basis. Drought stress decreased plant growth but increased LDW/A, RT/SH and leaf N even though leaf N was relatively high. Imidacloprid treatments did not affect root growth but decreased leaf area growth which was reflected in increased LDW/A. Total plant growth was not affected by imidacloprid treatments but foliar Provado reduced shoot growth such that RT/SH was increased. Again, imidacloprid treatments had no effect on leaf N on a percent dry weight basis but it did increase leaf N when expressed on a leaf area basis due to the increase in LDW/A.

In both 2005 and 2006, Carr seedlings experienced more drought stress (more negative SWP) than Cleo and drought stressed seedlings consequently had lower SWP than well-irrigated seedlings (Table 2). In 2005, Cleo leaves had higher rates of net gas exchange than Carr leaves but in 2006, Cleo leaves had lower A_{CO_2} and g_s but higher WUE than Carr leaves. Drought stress reduced A_{CO_2} and leaf WUE in both years. Imidacloprid treatments did not affect plant water status or stomatal conductance in either year so drought tolerance was not affected. Imidacloprid treatments increased A_{CO_2} and leaf WUE in both years.

Discussion

Although all leaf N values were above optimum (2.6% dry weight) for bearing trees (Obreza and Morgan, 2008), Carr leaves had higher leaf N concentration when expressed on a dry weight basis than Cleo leaves in both years. This was likely attributable to the higher efficiency of N uptake in Carr than Cleo (Syvertsen and Graham, 1985) as this difference disappeared in 2006 when higher fertility rates were used. Drought stress reduced leaf N concentration in 2005 but the higher fertilizer rates in year 2 re-

Table 1. Effects of total nitrogen applied, drought stress and imidacloprid treatments on plant growth characteristics and leaf N of Carrizo citrange (Carr) and Cleopatra mandarin (Cleo) seedlings (n = 8) harvested on 29 Sept. 2005 and 18 Sept. 2006.

Treatment	N applied (g)	Leaf area (cm ²)	Leaf DW (g)	LDW/A (g/m ²)	Root DW (g)	TPDW (g)	RT/SH	Leaf N (% dry wt)	N (mmol/m ²)
2005									
Carrizo	139 a ^z	144 a	1.16 a	80.8 b	1.66 a	4.64 a	0.58 b	3.06 a	176 a
Cleo	99 b	87 b	0.72 b	83.5 a	0.93 b	2.20 b	0.74 a	2.80 b	166 b
Well-irrigated	135 a	140 a	1.13 a	81.7 NS	1.41 a	3.99 a	0.58 b	3.03 a	176 a
Drought	102 b	91 b	0.75 b	82.6	1.18 b	2.85 b	0.74 a	2.83 b	166 b
Control	119 NS	122 a	0.97 NS	80.7 NS	1.23 b	3.43 NS	0.63 b	2.91 NS	167 b
Admire 2F	119	113 b	0.92	83.2	1.30 ab	3.36	0.69 a	2.96	176 a
Provado 1.6F	119	112 b	0.92	82.5	1.35 a	3.47	0.67 ab	2.91	171 ab
2006									
Carrizo	192 NS	132 a	1.01 NS	78.1 b	1.47 a	3.71 a	0.66 a	4.35 a	244 NS
Cleo	192	115 b	1.02	89.2 a	0.99 b	2.77 b	0.56 b	3.98 b	253
Well-irrigated	192 NS	141 a	1.10 a	79.7 b	1.34 a	3.65 a	0.58 b	3.94 b	222 b
Drought	191	106 b	0.93 b	87.6 a	1.11 b	2.83 b	0.65 a	4.39 a	275 a
Control	192 NS	131 a	1.02 NS	79.6 b	1.21 NS	3.26 NS	0.59 b	4.18 NS	237 b
Admire 2F	192	122 ab	1.03	85.2 a	1.21	3.25	0.59 b	4.18	253 a
Provado 1.6F	192	118 b	0.99	86.1 a	1.27	3.21	0.66 a	4.14	254 a

^zValues within a column (within a year) followed by unlike letters are significantly different at $P < 0.05$ as tested by Duncan's multiple range test. NS = nonsignificant.

Table 2. Effects of drought and imidacloprid treatments on midday stem water potential (SWP), leaf photosynthesis (A_{CO_2}), stomatal conductance (g_s), and water use efficiency ($WUE = A_{CO_2}/transpiration$) of Carrizo citrange (Carr) and Cleopatra mandarin (Cleo) seedlings (n = 6 leaves) measured on 26–27 Sept. 2005 and 11–13 Sept. 2006.

Treatment	SWP (MPa)	A_{CO_2} ($\mu\text{mol}/\text{m}^2/\text{s}$)	g_s ($\text{mmol}/\text{m}^2/\text{s}$)	WUE ($\mu\text{mol}/\text{mmol}$)
2005				
Carrizo	-1.39 b ^z	4.39 b	139 b	2.08 b
Cleo	-0.89 a	6.18 a	166 a	2.91 a
Well-irrigated	-0.82 a	6.35 a	163	2.99 a
Drought	-1.46 b	4.71 b	148 NS	2.22 b
Control	-1.20 NS	4.48 b	147 NS	2.13 b
Admire 2F	-1.16	6.24 a	160	2.93 a
Provado 1.6F	-1.09	5.51 ab	157	2.58 ab
2006				
Carrizo	-2.03 b	5.05 a	415 a	0.87 b
Cleo	-1.14 a	3.60 b	162 b	1.42 a
Well-irrigated	-0.96 a	5.57 a	318 a	1.38 a
Drought	-2.23 b	3.08 b	259 b	0.91 b
Control	-1.62 NS	3.26 b	284 NS	0.83 b
Admire 2F	-1.60	5.04 a	285	1.35 a
Provado 1.6F	-1.44	4.68 a	296	1.25 a

^zValues within a column (within a year) followed by unlike letters are significantly different at $P < 0.05$ as tested by Duncan's multiple range test. NS = nonsignificant.

sulted in higher leaf N in drought stressed than in well-irrigated plants in both species.

Imidacloprid had no effect on the concentration of leaf N (percent dry weight) in either year but it did increase leaf N on a leaf area basis in both years due to the increase in leaf thickness (LDW/A). Imidacloprid had no effect on plant water status or total plant growth of either species in either year. Imidacloprid increased root growth in both years supporting results of McCoy et al. (1995). Based on the current data, the increase in root growth, however, occurred at the expense of shoot growth as supported by the decreased total leaf area and an increase in LDW/A. Thus, imidacloprid increased the RT/SH ratio in both years. These data can be used to interpret previous results of McCoy et al. (1995) where imidacloprid apparently increased fibrous root growth in the greenhouse without pests, but may have done so at the expense of shoot growth. These observations may have implications for current interests in controlling shoot flush growth to facilitate Asian citrus psyllid control for greening management.

Imidacloprid also increased leaf photosynthesis and WUE of both species under both drought stress and well-irrigated conditions in both years. Since this was not due to enhanced water relations or stomatal conductance, it could have been due to the thicker or more dense leaves per unit area as supported by the increased LDW/A. In addition, there could have been a stimulatory effect on photosynthesis from increased root growth demands for photosynthates (Mashela and Nthangeni, 2002). To the extent that leaf WUE from gas exchange ($A_{CO_2}/transpiration$) can be a surrogate for yield per water use (Liu et al., 2005), the imidacloprid-induced increases in RT/SH and WUE might have implied an increase in drought tolerance (Shahnazari et al., 2007) but there was no imidacloprid effects on total plant growth or water status.

In summary, despite experiencing lower SWP at midday, Carrizo seedlings grew more and accumulated more N than Cleo seedlings. Drought stress decreased total plant growth and decreased growth of roots more than shoots as RT/SH ratio increased. Imidacloprid affected growth allocation as RT/SH was increased by increasing root growth and decreasing leaf area. Imidacloprid also increased leaf dry weight per area which increased leaf N when expressed on a leaf area basis and may have contributed to the higher rates of leaf photosynthesis in imidacloprid-treated leaves. However, the changes in growth allocation patterns and increases in leaf photosynthesis and water use efficiency that were attributable to imidacloprid treatments were not translated into increases in drought tolerance or total plant growth.

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