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Whole-plant Ontogenic Pattern of Flush Phenology and Plant Growth Regulator Responses

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Flushing phenology in citrus is important to integrated management of multiple insect pests, which thrive and reproduce on new flush, but it has been described as irregular. The pattern of new flush emergence in citrus is modulated by external factors and internal signals. We initiated trials of plant growth regulators (PGRs) in the greenhouse to manipulate plants to produce a more synchronized flushing pattern, with naphthalene acetic acid (NAA) to suppress and gibberellic acid (GA_3) to accelerate. In the greenhouse experiments, 21.6 ppm foliar GA_3 application induced more flushes on the 1-year-old potted plants, compared with other rates that are lower than 21.6 ppm. NAA application delayed first flush up to 20 days but the optimum rate depended on soil vs. foliar application rates were shown a varied suppressing effect in sprouting, depending on foliar vs. soil application. Furthermore, observation of the pattern of emergence regardless PGR treatment revealed clusters of first flush emergence. We hypothesized this was controlled by bud age. In the field trial, mature trees were pruned to induce a synchronized flush, then GA_3 was applied foliarly at various times after flush budbreak to test whether bud age affected the flushing response to GA_3. There was a significant response to application at 8 weeks after budbreak, where there was not to application at 4, 6, 8, or 10 weeks. However, we observed flushing induced on mature branches rather than on the most recent flush. Results suggest that the ontogenic response of flushing to exogenous GA_3 application is on the whole-tree level response rather than individual bud age.

Flush patterns establish the canopy structure and may influence the possible inflorescence shoot in the following year. Flush management may contribute to current citrus cultivation systems in the context of huanlongbing (HLB) disease. New flush is central to the spread of HLB, and manipulation of flush phenology could lead to more effective coordination of vector management. Citrus flush is likely controlled by internal factors, such as endogenous hormone level and bud age (Krajewski and Rabe, 1995). For example, auxins maintain apical dominance which inhibits axillary sprouting. A synthetic auxin was utilized as a shoot growth inhibitor to improve shoot management in pruned citrus trees (Lundberg and Smith, 1974; Phillips and Tucker, 1974).

In comparison, gibberellic acid (GA_3) enhances shoot growth and is associated with bud break (Altman and Goren, 1974). We hypothesize that these can be utilized to delay (auxin) and trigger new flush (GA_3). In this study, flush response to a synthetic auxin, naphthalene acetic acid (NAA) and GA_3 is evaluated to determine whether the flush response is affected by bud age or not.

Materials and Methods

We performed three experiments: In the first greenhouse experiment, NAA and GA_3 were applied at 5 rates in both soil and foliar application. All potted plants, 1-year-old 'Hamlin' (*Citrus sinensis*) grafted on 'Swingle' citrumelo (*C. paradisi* × *Poncirus trifoliata*) were tipped before treating with NAA and GA_3 to either soil or canopy in a randomized complete block arrangement. The dosage information is shown in Table 1. The second study was initiated to assess the effects of higher rates of NAA application, 0, 12, 60, 300, 1000 ppm to soil or canopy to the same variety and ages as Experiment 1. In both studies, the date of first flush emergence was observed. In the third experiment, conducted in the field, 60 sweet orange (*C. sinensis*) field-grown trees, 'B6-68' sweet orange grafted onto either 'C-35' citrange (*C. sinensis* × *C. trifoliata*) or 'Swingle' citrumelo rootstock, of similar size and vigor and with few HLB-symptoms were selected. The field study was conducted at the experimental grove (28°05'10.3"N 81°36'52.2"W), in Haines City, FL, managed by Citrus Research and Education Center.

A randomized complete block design was adopted with 5 trees per block and 12 blocks coupled with 4 treatment groups of trees with the application to accorded flush shoot age and the control group. In the beginning of the trial all trees were manually pruned to trigger a coordinated new flush. After the first flush was observed, we labelled 20 pruned shoots per tree around the canopy. GA_3 was applied to the canopy on the subsequent flush shoot at 1) 4 weeks after flush initiation (WAF), 2) 6 WAF, 3) 8 WAF, 4) 10 WAF (Table 1 and Fig. 1), or the control (CTL), which was

Table 1. The plant growth regulators (PGRs) and application methods assigned to the citrus plants in greenhouse experiments.

	Experiment 1		Experiment 2
Condition	Greenhouse, potted		
Plant age	1-year-old		
Scion/Rootstock	Hamlin/Swingle citrumelo		
PGR	NAA & GA_3		NAA
Application method	Foliar & soil application		
Application rate (ppm)	0.04	0.02	0
	0.17	0.09	12
	0.87	0.47	60
	4.33	2.33	300
	21.6	11.7	1000

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*Corresponding author. Email: civince@ufl.edu pruned and did not receive any GA_3 application. New sprouting on the first 5 nodes of each labeled shoot was observed at 4 weeks after GA_3 application for each treatment, as well as in the control. We count that the flush shoot has the response to gibberellin application if any one of 5 nodes sprouted. Most of the sprouting buds were observed to be the terminal ones. Some field trees lost a few labelled flush shoots due to the psyllid nymph feeding. On these trees only 10–15 shoots were valid for evaluation.

All data were analyzed by using linear mixed effects models (package: nlme) in R (version 3.6.0), in which rootstock cultivars and grove management practices and their interaction were considered as fixed affect, while the experimental repeats and the interaction effect between experimental repeat and rootstock variety were considered as random effects. The flush shoot count is expressed as new shoots per node on the five most distal nodes of labelled shoots. These were calculated as a mean per plant. Flush count data collected from a few plants were discarded and regarded as missing data due to the sever psyllid damage or off-type scion-rootstock combination. Tukey least significant differences comparisons were performed using the agricolae package in R, and *P*-values < 0.05 were considered significant.

Results

PGRs serve a role as flush suppressor and inducer. In the first greenhouse experiment, 11.7 ppm, the highest dosage, of NAA showed a delaying effect on subsequent flush. No significant differences were observed in inducing flush among the rates of GA_3 in soil application (data not shown). The highest foliar rate, 21.6 ppm, induced higher proportion of plants to flush. This result indicated that 21.6 ppm is sufficient to induce the flush in young citrus plants.

Comparison of NAA application methods in delaying effect. In the both soil and foliar applications, all rates delayed the subsequent flush. The effectiveness of each application method with the specific rate indicates that foliar application results in delaying effect with lower rates, 60 to 300 ppm (Fig. 1). The delaying effect can last up to 40 days after tipping. Comparing to soil application method, the same delaying effect requires higher rates, 300 to 1000 ppm to achieve the similar delaying effect.

Bud age and foliar GA_3 application effect on flushing response of HLB-affected plant. In the greenhouse experiment, GA_3 at 21.6 ppm was preliminarily confirmed to induce flushes in young citrus plants. A similar rate, 20 ppm of GA_3 was foliar-applied to field plants which are affected by HLB. The sprouting nodes were recorded at 4 weeks after GA_3 application to the trees with corresponded flush shoot age. Most sprouting nodes were the terminal ones from the flush shoot and shown the longer flush growth (data not shown). No sprouting node on the recent flush shoot was shown in the CTL plants (Fig. 2). A few sprouting nodes were observed on flush shoots in the plants with 4 WAF, 6 WAF, 8 WAF, and 10 WAF separately. However, only the plants with 8 WAF had the significantly higher sprouting rate, 10.7% than the control plants. Meanwhile, more sprouting nodes were shown on the previous branches and shoots than the most recent ones which were induced by pruning.

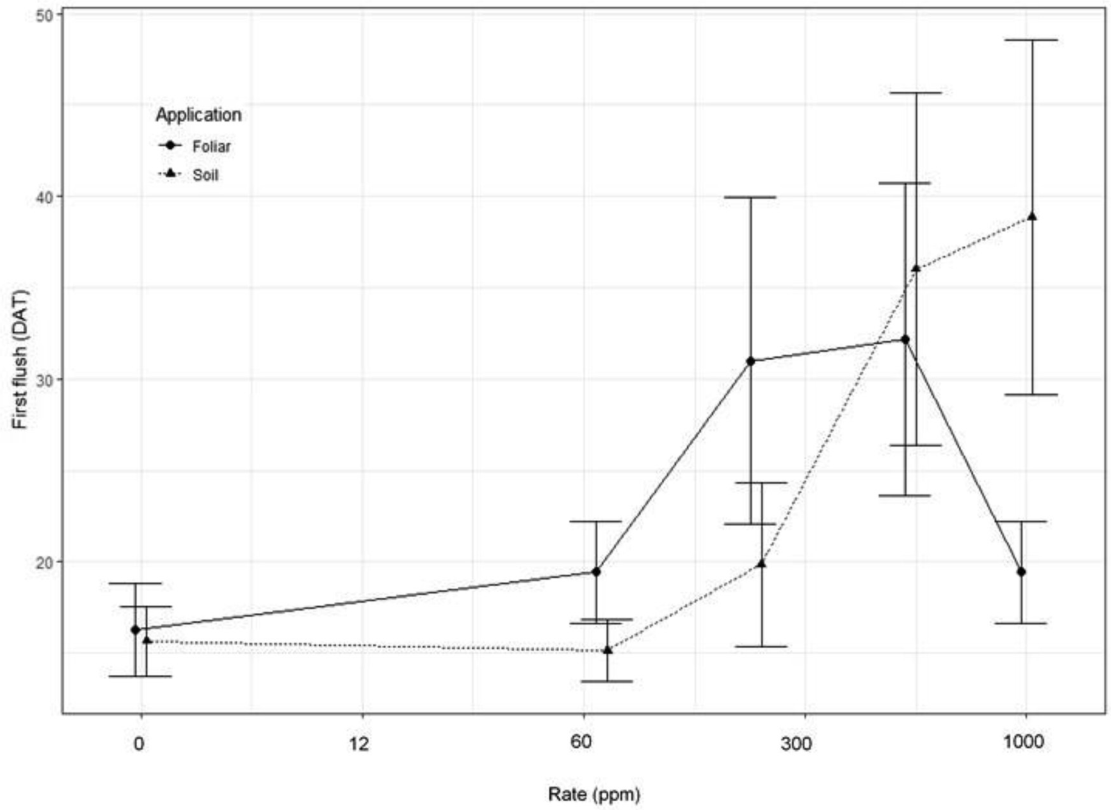

Fig. 1. Day of first flush of sweet orange, *Citrus sinensis*, after tipping (DAT) affected by different rates and application methods of naphthalene-acetic acid (NAA). The vertical bar represents standard error.

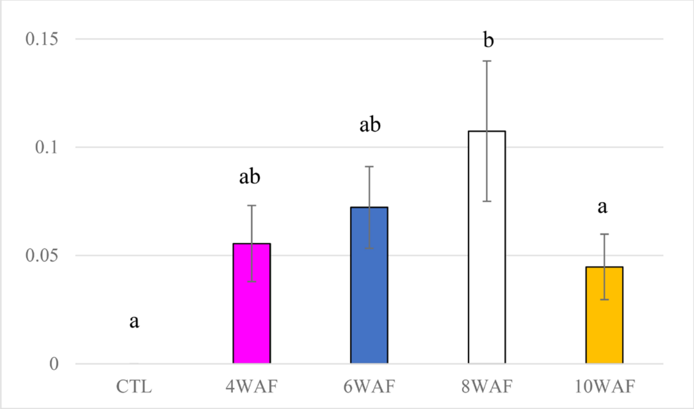

Fig. 2. The average flush shoot count of 20 labelled sweet orange (*Citrus sinensis*) shoots in the tree canopy in response to GA_3 application timing. GA_3 was applied at different weeks after flush initiation (WAF). The bars represent means and vertical bars represent standard error (n = 12). Different letters indicate the significant difference at $P \leq 0.05$ via Tukey test.

Discussion

In greenhouse experiments, the inhibitor role of NAA was shown to delay flush initiation. The efficacy of application method is different at comparable rates. Foliar application of NAA delays the flush up to 40 d at the lower rate but a similar delaying effect requires higher rate in soil application. Unlike the application in direct foliar contact, the efficiency of roots absorbing NAA may be lower, which would explain the difference.

Shoots with older nodes can have higher flush rates in the coming spring than recent flush shoots with younger nodes from the pruned stub (Krajewski and Rabe, 1995). No new flush on most current flush shoot in control plants in our study were shown as late as 12 weeks after flush initiation induced by pruning. In comparison, the plants with at least 4-week-old nodes showed the sprouting tendency in response to GA_3 application. The plants with 8-week-old nodes showed a significantly higher sprouting rate than the control plants. This fact indicates that GA_3 can induce new sprouting. The concept of GA_3 economy in citrus may give another hint to explain this field observation. Comparing to the antisense and sense overexpression of GA_{20}-oxidase gene, the shoot growth and endogenous GA_1 content are not different once they are grafted onto the non-transgenic ‘Carrizo’ citrange rootstock (Fagoaga et al., 2007). This study supports that the root plays the role in GA economy, which contributes to the bioactive GA formation or distribution. Then, the higher sprouting nodes on older branches and shoots can be attributed to the GA economy. Exogenous GA_3 may not immediately affect the sprouting on the applied young flush shoots but may travel to the root then distribute to the canopy in another bioactive GA form. If the root really plays a role in GA economy which affects flush phenology, the root should be considered as a factor in flush phenology.

Conclusions

NAA can effectively delay flush either applied to soil or canopy, but effective rates can vary with different application methods. Foliar application delays new flush at lower rates than soil application. GA_3 foliar application at 20 ppm can induce new flush but was most effective in the plants with 8-week-old flush shoots. Most sprouting nodes are the terminal nodes. Larger amounts of sprouting nodes were observed on the previous shoots and branches located in the inner canopy. Flush induction by GA_3 application likely results from whole plant signals, rather than individual bud maturity.

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