RESPONSES OF HEALTHY AND VIRUS-INFECTED CITRUS TO GROWTH REGULATORS[1]

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Abstract. **Spray applications of gibberellic acid (GA) hastened the symptom expression of exocortis-infected plants of Arizona 861 selection of 'Etrog' citron (Citrus medica L.) and of tristeza-infected plants of 'Key' lime (C. aurantifolia (Christm.) Swing.) but not of xyloporosis-infected plants of 'Orlando' tangelo (C. paradisi Macf. x C. reticulata Blanco). Spray applications of 2,4-dichlorophenoxyacetic acid (2,4-D) and N⁶-benzyladenine (BA), caused morphological changes in both healthy and virus-infected plants of the 'Etrog' citron, 'Key' lime and 'Orlando' tangelo but neither compound hastened symptom expression of exocortis, tristeza or xyloporosis.**

Virus and virus-like diseases of citrus have caused major damage to citrus throughout the world. One problem of major concern is that viruses may be present in a tree without showing symptoms for many years or be present in scion cultivars or rootstocks without causing identifiable symptoms. Thus, many new trees have been produced from seemingly good budwood containing viruses that caused damage when placed on certain rootstocks or which expressed damaging symptoms after several years of productivity. Much attention, therefore, has been given to identifying viruses in trees without visible symptoms and the maintenance of budwood sources free of viruses through state-controlled budwood registration programs.

Attempts to identify viruses by chemical indexing, electron microscopy, color tests and serology have met with limited success. The most reliable method developed is the use of citrus indicator plants. This procedure generally consists of budding or grafting material from a tree in which the presence or absence of the virus is unknown onto a citrus cultivar which will express symptoms relatively soon after infection and which is free of the virus. This procedure is not entirely satisfactory, indexing still requiring several weeks to many months, depending on the virus.

Some growth regulators cause pronounced morphological changes in plants when applied at appropriate concentrations. Applications of growth regulators have modified symptom expression of virus-infected indicator plants in a few cases (1, 2); however, such work is sparse. Thus, research was initiated to determine the responses of several citrus indicator plants, both with and without viruses, to applications of several growth regulators known to induce morphological changes in plants in an effort to improve indexing techniques.

Materials and Methods

A series of experiments were performed to determine the influence of an auxin (2, 4-D), a cytokinin (BA) and gibberellin (GA) on the morphology of both healthy plants and those infected with tristeza, exocortis and xyloporosis viruses respectively.

Tristeza-infected and exocortis-infected plants received treatments of individual growth regulators. Xyloporosis-infected plants were treated with both individual growth regulators and combinations of them, Table 1. All treatments were applied as aqueous sprays. Plants were sprayed with a growth regulator, allowed to dry and then sprayed with another growth regulator when combination treatments were used.

Results and Discussion

Healthy Plants. Spray applications of 20 ppm of 2,4-D) are frequently applied to some cultivars of mature sweet orange (*C. sinensis* (L). Osbeck.) trees, without damage, to prevent preharvest fruit drop; however, this material is also used as a brush killer and a herbicide and can be very damaging. Concentrations of both 10 and 20 ppm of 2,4-D caused leaf distortion typical of 2,4-D damage on the immature leaves and leaves of the first growth flush developing after application of all 3 indicator plants, Table 2. No permanent damage or dieback occurred but the results suggested the upper limits of plant tolerance to 2,4-D were reached.

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Table 1. Concentrations and combinations of growth regulators used.

Growth regulators	Conc. (ppm)		
2,4-D	5	10	20
BA	25	50	100
GA	250	500	1000
2,4-D-BA+GA	5+25+250	10+50+500	20+100+1000
2,4-D+BA	5+25	10+50	20+100
2,4-D+GA	5+250	10+500	20+1000
BA+GA	25+250	50+500	100+1000

BA sprays did not result in damage to any of the plant material used in this research, suggesting higher concentrations could be safely applied. All concentrations of BA caused early bud growth, delayed leaf abscission and slightly increased thorniness, Table 1. Early bud growth and delayed leaf abscission resulted in plants with a dense canopy of foliage.

The 3 cultivars used in this research responded similarly to GA sprays in some cases but differed markedly in others, Table 2. 'Orlando' tangelo did not suffer twig dieback even though typical internode elongation, strap-shaped leaves, increased thorn length, and early abscission of old leaves were evident. Leaves of immature growth became broader but leaves on the first new flush following spraying were smaller than normal. No morphological changes were noted on later growth flushes. 'Key' lime and 'Etrog' citron, on the other hand, suffered extensive twig dieback following pronounced internode elongation of immature growth. Early leaf fall was severe on 'Key' lime but slight on citron. Trunk girth of citron increased greatly and new shoots that were much thicker than normal arose from near the base of GA treated plants following dieback. Multiple buds, which formed on all cultivars, were particularly prominent on citron.

Combinations of growth regulators were applied to 'Orlando' plants only. Plant response varied greatly with the combinations used, Table 3. Combinations containing GA resulted in more varied and intense responses than did combinations of BA and 2,4-D. Combinations of GA and 2,4-D produced the most bizarre responses. The 2 lowest concentrations of GA and 2,4-D combined resulted in tendril-like growth of new leaves, as a result of severe leaf rolling and elongation, and the new branches were slender and vine-like. On the other hand the highest concentration induced a pronounced dormancy and no visible damage for at least 4 months. GA plus BA increased internode elongation, length of thorns and number of multiple buds, as compared with plants sprayed with GA only, but leaf abscission was less severe than when GA alone was applied. Combinations of BA and 2,4-D intensified some of the responses of each applied singly but neither growth regulator dominated the response. Multiple buds were pronounced and leaves were tendril-like at the highest concentration, Combinations of GA, BA and 2,4-D produced morphological changes somewhat similar to those in which combinations of GA with 2,4-D were used.

None of the growth regulators alone or in combinations caused morphological changes that were similar to those caused by tristeza, exocortis or xyloporosis viruses.

Virus-Infected Plants. The mild strain (T_1) of tristeza is generally determined by leaf cupping and vein clearing symptoms. Identification is sometimes difficult because vein clearing symptoms may not develop under conditions of high temperature and sunlight (3), a situation true in this research. Neither BA nor 2,4-D hastened symptom expression. GA hastened the time of appearance of the leaf cupping symptoms (Table 4, Expt. 1) but produced fewer cupped leaves before leaf abscission and dieback due to GA

Table 2. Generalized responses of healthy virus indicator plants to growth regulators.

Response	Indicator Plant		
	Key lime	Etrog citron	Orlando tangelo
2,4-D			
Abscission of matured leaves	xx	x	x
Distortion of expanding and new-flush leaves	xxx	xxx	xxx
Reduced new-flush leaf size	xx	x	xx
BA			
Termination of bud dormancy	xx	xx	xx
Multiple bud formation	x	xx	xx
Increased thorn length	x	x	xx
Delayed senescence	xxx	xxx	xxx
GA			
Termination of bud dormancy	xxx	xxx	xxx
Multiple bud formation	x	xxx	xxx
Chlorotic new-flush leaves	xxx	xxx	xxx
Strap-shaped new-flush leaves	xxx	xxx	xxx
Broadening of expanding leaves	---	---	xx
Abscission of matured leaves	xxx	x	x
Drooping of terminal shoot	xxx	xxx	xxx
Internode of elongation	xxx	xxx	xxx
Increase in thorn length	xxx	xxx	xxx
Increased girth of stem	x	xxx	x
Tip necrosis and die back	xxx	xxx	x

Evaluations of intensity categories were arbitrary and not precise, but the relative differences were so pronounced and consistent there was no question of their existence. x - slight; xx - moderate; xxx - severe.

developed. Plants were not evaluated for stem pitting until 89 days after inoculation at which time all except the uninoulated controls were pitted. Symptom expression was not influenced by concentration. Additional plants were inoculated with T_1 tristeza and part of them sprayed with 1000 ppm of GA. These plants were evaluated approximately every 2 weeks for stem-pitting. No plants were treated with 2,4-D or BA because all evidence in this and other experiments indicated these materials were not influencing symptom expression. GA treated plants developed stem pitting within 45 days but these symptoms were not found on the inoculated controls until the evaluation made 84 days after inoculation, Table 4, Expt. 3. Thus, GA treatments reduced the time required for identification of the T_1 tristeza virus by several weeks based on leaf cupping symptoms and identification was made more positive by the development of stem pitting within 45 days.

The severe tristeza strain (T_3) is characterized by leaf cupping, vein clearing, corky vein and stem pitting. All of these symptoms developed in control plants inoculated with T_3 tristeza and in plants inoculated with T_3 tristeza and treated with GA, but not in uninoculated control plants. All GA treatments, but none of

Table 3. Generalized response of 'Orlando tangelo to combinations of growth regulators.

Response	Growth Regulators			
	2,4-D+BA	2,4-D-GA	GA+BA	2,4-D+BA+GA
Termination of bud dormancy	xx	xx	xxx	xx
Multiple bud formation	x	x	xxx	xx
Chlorotic new-flush leaves	---	xxx	x	xx
Strap-shaped new-flush leaves	---	x	xxx	x
Broadening of expanding leaves	---	---	xxx	x
Distortion of expanding and new-flush leaves	x	xxx	---	xx
Abscission of matured leaves	x	xxx	x	x
Cessation of bud growth	---	xxx	---	---
Tendril-like leaf formation	x	xxx	---	xxx
Internode elongation	x	xxx	xxx	xxx
Increased thorn length	x	xxx	xxx	xxx
Vine-like stem formation	x	xxx	---	xxx

Evaluation of intensity categories were arbitrary and not precise, but relative differences were so pronounced and consistent there was no question of their existence. x - slight; xx - moderate; xxx - severe.

the other growth regulators hastened the occurence of leaf cupping, vein clearing and corky vein symptoms of T_3 tristeza (Table 4, Expt. 2). Leaf cupping occurred about 2 weeks earlier, vein clearing about 10 days earlier and corky vein about 10 days earlier when plants were sprayed with GA as compared to virus-infected, untreated plants, and the corky vein symptom occurred more commonly on plants sprayed with GA. Stem pitting symptoms were present in all inoculated plants, regardless of treatment, when first evaluated 53 days after inoculation but pitting was more intense in the GA treated plants. Additional plants inoculated with T_3 tristeza were treated with 1000 ppm and evaluated approximately every 2 weeks to determine the time required to develop stem pitting more precisely. GA treated plants all showed stem pitting within 32 days while the inoculated controls required 49 days.

All exocortis-infected control plants developed characteristic leaf epinasty symptoms within 35 to 44 days. Small, vertical stem splits developed on some plants within 51-50 days, generally preceded slightly by the occurrence of yellow stem blotches (Table 5, Expt. 1). Applications of GA greatly modified the pattern of symptom expression in exocortis-infected plants, Table 5. The immature terminals of the young plants developed elongated internodes and strap-shaped leaves, typical of healthy GA-treated plants. Small, vertical stem splits, which opened widely and became very corky, developed on the green, vigorously growing internodes of exocortis-infected plants treated with GA within 20 to 25 days when treated 10 days after inoculation and within 13 to 16 days when treated at the time of inoculation. Splitting and yellow blotch symptoms usually appeared simultaneously just below a leaf petiole. The terminals with elongated internodes and strap-shaped leaves died back later and thick shoots with severe leaf epinasty arose from the bases of the plants. Many small bark splits or cracks occurred at the bases of the new shoots. Thus, even though GA delayed development of leaf epinasty, the time required for identification was shortened appreciably as a result of the earlier, consistent appearance of pronounced bark splits on the central axis of the plant. All concentrations of GA were equally effective in hastening and modifying symptom expression. The results obtained with GA treatments were verified several times with additional plants. Both 2,4-D and BA were ineffective in

Table 4. Influence of growth regulators on the time for symptom expression of mild (T_1) and severe (T_3) strains of tristeza in 'Key' lime.

Symptom	Uninoculated control	Inoculated control	Growth Regulator[1] 2,4-D	BA	GA
			(days after inoculation)		
Expt. 1 (T_1)					
Leaf cupping	none	54-66	54-63	50-61	20-25
Vein clearing[2]	none	none	none	none	none
Stem pitting	none	89	89	89	89
Expt. 2 (T_3)					
Leaf cupping	none	30-36	28-33	28-31	19-23
Vein clearing	none	35-31	30-36	34-39	22-26
Corky vein	none	75-83	71-80	69-76	39-45
Stem pitting	none	53	53	53	53
Expt. 3 (T_1)					
Stem pitting	none	84	--	--	45
Expt. 4 (T_3)					
Stem pitting	none	49	--	--	32

[1] Time required not concentration-dependent.

[2] Vein clearing apparently masked by temperature and sunlight.

Table 5. Influence of growth regulators on time for symptom expression of exocortis in Arizona 861 'Etrog' citron.

Symptom	Inoculated control	Growth Regulator[1] 2,4-D	BA	GA
		(days after inoculation)		
Leaf epinasty	35-44	35-41	38-53	56-67
Stem yellow blotch	40-48	38-44	42-56	20-25
Stem vertical splits	51-50	50-53	46-49	20-25

[1] Applied 10 days after inoculation. Blotches and splits occured in 10-15 days when GA was applied at time of inoculation in a subsequent experiment. Essentially the same results were repeatedly obtained in additional experiments with GA.

hastening or intensifying the expression of exocortis symptoms.

Xyloporosis symptoms did not develop on any of the inoculated plants within the time limits of the experiment (6 months), regardless of treatment. Expression of xyloporosis symptoms in 'Orlando' seedlings may take up to 3 years, although they may occasionally develop within 6 months. Thus, treated, xyloporosis-infected plants might yet develop symptoms in less than the average time required in untreated, inoculated plants or symptoms might be modified in some other manner.

The 'Orlando' seedlings were very responsive to growth regulators, based on the morphological changes they caused but there was no evidence of symptom modification.

Conclusions

The results indicating GA sprays reduced the time for symptom expression of tristeza and exocortis viruses could be used immediately to improve routine indexing for these viruses. More importantly, however, the results clearly demonstrated that citrus is highly responsive to a representative compound of auxins (2,4-D), kinins (BA) and gibberellins (GA_3) respectively and that GA, at least, modified the *morphological* symptoms of 2 citrus virus diseases. These results suggest further research with growth regulators which could result in greatly improved virus indexing techniques and possibly even reduce or delay the damaging effects of virus diseases. Plants were not checked until 89 days after inoculation, at which time stem pitting was present in the inoculated control and in inoculated plants treated with GA, 2,4-D and BA respectively. Thus, another experiment (Table 1, Expt. 3) was conducted in which the plants were examined for stem pitting at approximately 2 week intervals. All inoculated plants treated with GA showed stem pitting within 45 days, as compared with 84 days for the uninoculated controls and the other growth regulator treatments. Moreover, only a few of the plants of inoculated controls and those treated with BA and 2.4-D were stem pitted at that time.

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AN EVALUATION OF NEW HERBICIDES FOR MILKWEED VINE CONTROL

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Abstract. **Herbicides presently available for use in Florida citrus groves have offered only limited control of established milkweed vines on a short-term basis. However, satisfactory control has been achieved over a period of 2 to 3 years when a program approach has been followed. A number of new herbicides tested over the past 2 years are showing considerable promise for improved control in an integrated program. Most of these materials are systemic in mode of action with some growth regulating type of activity. Those known to have little or no soil residual activity were evaluated alone and in combination with Krovar II and Princep. Four formulations of 2,4-D were tested at 2 and 4 lb/A rates with good topkill but rapid vine recovery. Silvex at 2 lb/A was more effective than 2,4-D with greater vine kill and slower recovery. Roundup and DPX 1108 (Krenite) at 4 and 6 lb/A, respectively, were especially effective, although the activity of the latter is very slow. R-24191 also worked slowly and was more effective than the 2,4-D products. Most of these herbicides varied in activity from**

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