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Proc. Fla. State Hort. Soc. 101:157-161. 1988.

METHODS FOR REDUCING ACIDITY IN CITRUS FRUIT

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Abstract. **PRO-GEN® (arsanilic acid or P-G) has been shown to reduce acidity of Florida grapefruit as effectively as lead arsenate. A series of experiments in the 1987-88 citrus fruit season reconfirmed that P-G was effective for this use with concentrations of 600-1500 ppm. P-G is water soluble. However, deliberate attempts to wash it off immediately following spray application reduced its effectiveness significantly, but did not completely eliminate the effect of P-G.**

Reduction of acidity of 'Hamlin' and 'Valencia' oranges occurred within 2-3 weeks following spraying with CITRUS 10 and triacontanol (TRIA). Both compounds effectively reduced acidity in oranges, but results were inconsistent. The advent of colder winter temperatures greatly reduced the effectiveness of both chemicals. Chemical application by standard airblast sprayers and fixed-wing aircraft was effective. Neither compound was effective in reducing acidity of 'Marsh' grapefruit. Both are biological materials with no known toxicology problems. Both compounds were effective at very low concentrations, but CITRUS TEN (20 ml/100 gal or 52.8 ppm) required 3-4 sprays 2-4 days apart, while TRIA (1-5 ppb) required a single spray.

Sales of grapefruit (*Citrus paradisi* MacFadyen) have long been aided by the use of the plant growth regulator (PGR) lead arsenate (LA) to reduce total fruit acids, thus improving fruit flavor as well as beneficially lengthening the shipping season. However, the safe use of LA, as well as the entire inorganic arsenic group, is under special review by the U.S. Environmental Protection Agency (EPA). LA is no longer being manufactured and sold for use on Florida grapefruit. A summary of the biological effects of arsenic on the environment was made by Fowler (3). A recent review on the use of arsenic on Florida grapefruit was made by Wilson (9).

Pro-Gen® (arsanilic acid or P-G) has been found to be an effective substitute for LA (9, 10). This compound is sold commercially as a growth stimulant in animal feed and a request has been made to the EPA for its use on Florida grapefruit. It has 0.5 ppm food tolerances in chicken and hog meat and 2.0 ppm in livers. These values are far in excess of any residues found on grapefruit (G. F. Fleming, Jr. Unpublished data). The compound is a synthetic organic arsenical, and this class of compounds is considered to have much lower toxicity than inorganic arsenics (7). Marine fish and invertebrates which contain arsenic in the organic form (2) may often have total arsenic contents 500-1000 times higher than that reported following P-G treatments on grapefruit (G. F. Fleming, Jr. Unpublished data).

Sales of certain orange (*C. sinensis* [L.] Osbeck) cultivars could also benefit from PGR's which would reduce fruit acidity in a manner similar to that which occurs for grapefruit. However, until recently, no PGR with an EPA clearance was available for this purpose. Although arsenicals reduce the acidity of oranges, they do so excessively and are, therefore, illegal for this purpose in Florida. Newly tested natural products are now available which show promise for acidity reduction for some citrus cultivars. The first of these, CITRUS 10 (C 10 or BRIX PLUS), is a product of Bio-Organics, Inc., Longwood, FL and has demonstrated the ability to increase the ratio, and to some extent, increase °Brix of some citrus cultivars (8). It is also reported to have beneficial PGR effects on other fruits, vegetables and grains (8). It is a safe natural product (8), is used at a very low concentration (20 ml of manufactured product/acre), but usually requires 3-4 sprays. Its active ingredient(s) are unknown. The product is sold commercially in Florida and in a number of agricultural areas.

Florida Agricultural Experiment Stations Journal Series No. 9412. The authors acknowledge the technical help of Mr. Charles Welch.
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The second chemical, triacontanol (TRIA) is a PGR well known to plant scientists. A thorough review of TRIA was made by Ries (6), and a recent review of its effects on the growth of 45 plant species was summarized by Biernbaum et al. (1). It is a 30-carbon, straight chain primary alcohol and is natural and safe and found in many plant and insect parts, particularly the wax or lipid portions, as well as in the environment generally. TRIA is active at 1 ppb or less and only 1 spray is usually required. Most formulations are dispersions of fine particles (6). The major drawback to wide spread use of TRIA has been erratic results (1, 8). It is subject to the adverse effects of cold temperature, soft plastics, water contaminants and other unknown deactivating agents (8). TRIA is soluble in a number of non-polar solvents, but is almost completely insoluble in water and other polar solvents (8). TRIA is not sold in Florida but is sold commercially for agricultural uses in the Far East to increase yields of rice and other grains.

The purpose of this paper is to report results and draw conclusions on the effect on acidity of grapefruit treated with P-G, and oranges and grapefruit treated with certain biologicals.

Materials and Methods

A series of field experiments were conducted during 1987-88 in the principal citrus growing areas of Florida. The first group of experiments was principally for the purpose of generating residue data for eventual EPA clearance of P-G, but maturity comparisons were also conducted among these treatments. Two concentrations (1000 and 3000 ppm) levels were applied to 'Marsh' grapefruit, and 1/2 of the group of trees receiving the 3000 ppm spray were immediately washed (within 20-30 minutes) with 50 gal (189.3 l) of water each. The purpose was to simulate rains occurring immediately after spraying. Tests consisted of a randomized block design with 3 replications of 3 trees each. All trees were approximately 15 ft (4.6 m) in height. Sprays were applied using a modified FMC 200 gal handsprayer. Trees were sprayed to runoff or until 15 gal (56.8 l) of spray per tree were applied. Non-ionic surfactant ORTHO X-77 (0.1%) was added to all sprays. Random samples of 35-45 fruit were harvested from each replication. An FMC In-Line Extractor with automatic titration and computerized sample read-out was used to remove juice from the fruit and compute °Brix/acid ratio. In the second series of tests, 3 levels of P-G were applied to 'Ruby Red' grapefruit to measure the effect of concentration of chemical and its relation to acidity reduction in this cultivar. Tree size was approximately 12 ft (3.7 m) in height. Experimental design, tree gallonage, sampling procedures and °Brix/acid ratio tests (hereinafter referred to as ratio) were as previously described for 'Marsh' grapefruit.

A third group of tests compared the biological chemicals C 10 and TRIA. Three to 4 sprays of C 10 were applied to 'Hamlin' and 'Valencia' oranges and 'Marsh' grapefruit, usually with 3 replications of 3-4 trees each and with 2-5 day separations between sprays. Tree sizes ranged from 10-15 ft (3-4.6 m) in height. The chemical was furnished by Bio-Organics, Inc., Longwood, FL and was applied at 10-20 ml(26.4-52.8 ppm)/100 gal or 20 ml/acre. Non-ionic surfactant ORTHO X-77 (0.1%) was usually included, and spray tank pH was adjusted to between 6 and 7 by adding small amounts of sulfuric acid to the spray tank while simultaneously measuring the pH change with a portable meter. Tests were made using an airblast sprayer (500 gal), an FMC handsprayer, or fixed-wing aircraft as indicated. Random samples of fruit (60-100 oranges and 35-45 grapefruit) were made per replication. When large solid blocks of trees were treated (such as the fixed-wing air test), 10 trees were randomly sampled from the treated areas and 10 trees from an adjacent control block. Tests were harvested 2-1/2-4 weeks following the final spray treatments. Ratio was obtained as previously described for 'Marsh' grapefruit.

TRIA was applied usually as single treatment sprays to 'Hamlin' and 'Valencia' oranges and 'Marsh' grapefruit. Experimental design, sample sizes and ratio were obtained as previously described for 'Marsh' grapefruit. The chemical was furnished by either Dr. Stanley Ries, Michigan State University, E. Lansing, MI or Teijin Limited, Tokyo, Japan. Formulations furnished ranged from 10 to 1000 ppm, and field application rates ranged from 0.67 to 100 ppb. Non-ionic surfactant ORTHO X-77 at 0.1% was included in all sprays. Trees were sprayed to runoff with an FMC handsprayer or SOLO® Backpack sprayers. Fruit was harvested 2-3 weeks after spray application.

Two experiments were conducted in which 'Valencia' oranges were dipped in C 10 (70 ppm) and TRIA (10 ppb) solutions. Fruit treatments were immersed for 2 minutes, removed, air dried, and kept at room temperature (about 72°F or 22.2°C). Representative samples of 60-80 fruit each were taken from both treated and control fruit at various times over a 0-14 day period. °Brix/acid ratios were obtained as previously described for 'Marsh' grapefruit.

In this paper, whenever significance (or lack of it) was achieved between and among treatments in any individual experiment, it was at the 5% level ($P < 0.05$).

Results

Applications of 1000 and 3000 ppm P-G to 'Marsh' grapefruit resulted in a significant increase in ratios (Table 1). The higher concentration of P-G was the most effective in increasing ratio. Immediate washing of the 3000 ppm concentration of P-G following spraying significantly reduced its effectiveness, but the ratio of the "wash" treatment remained significantly higher than that of both the 1000 ppm treatment and control.

As with 'Marsh' grapefruit, ratio of 'Ruby Red' grapefruit was found to be concentration responsive to P-G (Table 2); the highest concentration (1500 ppm) resulted

Table 1. The effect of PRO-GEN® (P-G) application and immediate washing on the effectiveness of P-G to increase ratio of 'Marsh' grapefruit.[z]

Treatment	Concentration (ppm)	Ratio	Ratio increase (%)
Control	—	6.68 a	—
P-G	1000	7.59 b	13.6
P-G	3000	8.72 d	30 5
P-G	3000[y]	8.33 c	24.7

[z]Treatments were applied 21 May 1987. Fruit was harvested 2 November 1987. Location was Orange-Co., Inc., Ft. Pierce. Means separation in ratio column by Duncan's multiple range test, 5% level.
[y]At conclusion of spraying, each tree received a wash with 50 gal of water (elapsed time between spray and wash was approximately 20-30 minutes).

Table 2. The effect of various concentrations of PRO-GEN® (P-G) on the ratio of 'Ruby Red' grapefruit.[z]

Treatment	Concentration (ppm)	Ratio
Control	—	8.15 a
P-G	500	9.23 b
P-G	1000	9.94 c
P-G	1500	10.73 d

[z]Treatments were applied 1 June 1987 and fruit was harvested 23 November 1987. Location was A. Duda & Sons, Inc., LaBelle, FL. Mean separation in ratio column by Duncan's multiple range test, 5% level.

in a 2.5 ratio point increase above the control. Significant differences occurred between and among all treatments.

C 10 applications resulted in a decrease in percent acid and an increase in °Brix, and ratio for 'Valencia' orange at all concentrations tested, some of which were significant (Table 3). All treatments had similar ratios even though the C 10 concentration was doubled in each treatment.

Results of experiments comparing the acidity-reduction effectiveness of C 10 and TRIA to change ratio of oranges and grapefruit are shown in Table 4. In the series of tests near Lake Alfred, 4 applications of C 10 by airblast sprayer (initial spray 9/23/87) produced a non-significant (0.5 point) increase in ratio. However, the handspray treatment of C 10 (initial spray 9/29/87) and the higher concentration of TRIA (single spray treatment of 1.33 ppb) produced significantly higher ratios as compared with the control. The low TRIA concentration (single spray treatment of 0.67 ppb) resulted in a slightly higher ratio than the control, but not significantly so.

Table 3. Results of field tests with CITRUS 10 (C 10) applied to 'Valencia' oranges (1987).[z]

Treatment and conc.	°Brix	% Acid	Ratio
Control	13.44	1.01	13.31
C 10 (10 ml/100 gal)	13.80	0.93	14.84*
C 10 (20 ml/100 gal)	13.75	0.95*	14.47
C 10 (40 ml/100 gal)	13.67	0.94*	14.54*

[z]The test was applied in a grove near Ft. Pierce, FL belonging to Orange-Co. of Florida, Inc. C 10 sprays were applied 6, 8, and 9 April. Fruit were harvested 20 April and held at 40°F (4.4°C) until processed 23 April. No surfactant was used.
*Indicates significant difference from control, P > 0.05.

Table 5. Effect of sprays containing CITRUS 10 (C 10) and Triacontanol (TRIA) on °Brix, % acid, and ratio of 'Hamlin' oranges at LaBelle. FL.[z]

Treatment	Application method	°Brix[y]	% Acid[y]	Ratio[y]
C 10	Fixed-wing aircraft	10.08 c	0.79 c	12.76 b
C 10	Handspray	9.51 b	0.75 d	12.68 b
Control	—	8.97 a	0.97 a	9.25 a
TRIA (0.67 ppb)	Handspray	10.48 d	0.85 b	12.33 b
TRIA (1.33 ppb)	Handspray	10.39 d	0 75 d	13.85 c

[z]Additional information concerning concentration, spray dates, etc. is shown in Table 4 footnote.
[y]Means within a column followed by different letters are significantly different (Duncan's multiple range test, 5% level).

Tests with C 10 and TRIA on 'Hamlin' oranges in the LaBelle area (Table 4) showed significant differences between all treatments and the control. There were no significant differences between applications of C 10 applied as a handspray or by fixed-wing aircraft. TRIA significantly increased ratio compared to the control in both treatments, with the highest concentration (1.33 ppb) significantly higher than all other treatments. Application of C 10 to 'Marsh' grapefruit produced only a nonsignificant (0.2 point) ratio increase compared to the control. A comparison of the effects of C 10 and TRIA on °Brix and % acid, in addition to ratio, of 'Hamlin' oranges in LaBelle is shown in Table 5. Both compounds significantly increased °Brix compared to the control and significantly lowered percent acid.

Six of the 12 additional tests with TRIA conducted on 'Valencia' oranges between December 1987 and April 1988 showed significant differences in at least some juice quality measurement parameters (Table 6). Results of 5 experiments showed no significant differences among any of the measurement parameters. Although TRIA significantly increased ratio in these 6 'Valencia' orange experiments, the results were often inconsistent. Where significant ratio differences were observed, correspondingly significant decreases in % acid usually occurred. However, consistently

Table 4. Results of field tests with CITRUS 10 (C 10) and Triacontanol (TRIA) on 'Hamlin' orange (HA) and C 10 applied to 'Marsh' grapefruit (MA).[z]

Treatment and cultivar	Spray date (1987)	Location	Application method	Ratio[y]
C 10 (HA)	23, 25, 29, Sept., 12 Oct.	LA	Airblast sprayer (100 gal/acre)	8.72 a
Control (HA)	—	LA	—	8.22 a
C 10 (HA)	29 Sept., 2, 5, 8 Oct.	LA	Handspray (dilute)	8.46 a
Control (HA)	—	LA	—	7.78 a
Triacontanol (HA) (0.67 ppb)	9 Oct.	LA	Handspray (dilute)	8.19 a
Triacontanol (HA) (1.33 ppb)	9 Oct.	LA	Handspray (dilute)	9.81 b
C 10 (HA)	2, 5, 14, 19 Oct.	LB	Fixed-wing air	12.76 b
C 10 (HA)	1, 5, 13, 16 Oct.	LB	Handspray (dilute)	12.68 b
Control (HA)	—	LB	—	9.25 a
Triacontanol (HA) (0.67 ppb)	9 Oct.	LB	Handspray (dilute)	12.33 b
Triacontanol /HA) (1.33 ppb)	9 Oct.	LB	Handspray (dilute)	13.85 c
C 10 (MA)	15, 17, 22, 25 Sept.	FP	Handspray (dilute)	6.64 a
Control (MA)	—	FP	Handspray (dilute)	6.45 a

[z]LA—shinn grove, n. of Lake Alfred; LB—A. Duda & Sons grove, S. of LaBelle; FP—Orange Co., Inc. grove w. of Ft. Pierce; C 10 was applied at 10 ml/100 gal of water. ORTHO X-77 surfactant (0.1%) was added to all sprays and pH was adjusted to between 6 and 7. Fruit from the Shinn grove was harvested 29 Oct.; Duda grove 5 Nov.; MA were harvested 27 Oct. Airblast sprayer application was 100 gal/acre containing 20 ml (56.9 ppm) of C 10; fixed-wing air application was 20 ml of C 10/acre; hand-spray applications were 10 ml (25.4 ppm) per 100 gal of C 10 sprayed to tree runoff.
[y]For each experiment, means within a column followed by different letters are significantly different (Duncan's multiple range test, 5% level).

significant increases in °Brix were not observed. TRIA caused no significant changes in maturity parameters of 'Marsh' grapefruit (Table 6).

TRIA applied at 100 ppb by backpack sprayer in April (Table 6) was significantly higher than all other treatments except for the other backpack TRIA application (4 ppb). This latter concentration was substantially higher (though not significantly) than all other hand-spray applications.

The 2 experiments conducted, in which 'Valencia' oranges were dipped in C 10 (70 ppm) or TRIA (10 ppb) solutions, showed no significant differences between and among treatments and controls.

Discussion

P-G may not be as easily dislodged as was previously believed (Table 1). Therefore, if the chemical is eventually cleared by EPA, a drying period of 3 or more hours between spraying and precipitation (the waiting period proposed by the manufacturer) would appear to be adequate for complete chemical effectiveness. However, as P-G is more water soluble than LA, growers should apply it with caution during abnormally wet periods. Field experience has also shown that 5 or more minutes of agitation of the chemical in the spray tank should be allowed so that the chemical can dissolve. P-G is considered water soluble, but it dissolves slowly.

Previous data for 'Ruby Red' grapefruit had shown some inconsistencies, principally because of rainy conditions at the time of spraying. However, P-G application under "normal" Florida conditions (when at least several hours had elapsed between spray application and precipitation) produced almost ideal ratio increases (Table 2). Data showed that red grapefruit were more sensitive to P-G than white grapefruit, which confirms previously published data (9). Therefore, if EPA clearance for P-G is obtained, a lower concentration for red cultivars vs. white cultivars should be recommended as has historically been done for LA (5).

The initially successful test with C 10 in April 1987 (Table 3) showed that biological materials could be used to affect citrus maturity. It was also reasoned, because of its reported properties for increasing plant growth (6), that TRIA might prove useful for this purpose. Subsequent tests confirmed this (Tables 4, 5, and 6) and a single spray was sufficient. The chief drawback to both chemicals has proven to be inconsistency, a problem which has plagued TRIA since research on the PGR began (6). Although C 10 has proven more consistent than TRIA in these experiments, results such as observed in the airblast sprayer test

Table 6. Results of field tests with various concentrations and formulations of TRIA applied to Florida citrus 1987-88 fruit season.[z]

Conc. (PPB), Cutivar[y]		Spray date	Harvest date	Location	°Brix	% Acid	Ratio
2	VA OR	23 Dec.	12 Jan.	FP	12.27a	1.58 ab	7.77 ab
4	VA OR	23 Dec.	12 Jan.	FP	12.11 a	1.50 b	8.07 a
Control	VA OR	—	12 Jan.	FP	12.26 a	1.63 a	7.52 b
2	VS OR	23 Dec.	4 Feb.	FP	12.48 ab	1.39 ab	8.98 ab
4	VA OR	23 Dec.	4 Feb.	FP	12.32 b	1,32 a	9.33 a
Control	VA OR	—	4 Feb.	FP	12.69 a	1.46 b	8.69 b
1	VA OR	28 Dec.	26 Jan.	LB	11.91 a	1.08 a	11.03 a
2	VA OR	28 Dec.	26 Jan.	LB	11.66 ab	1.09 a	10.70 ab
4	VA OR	28 Dec.	26 Jan.	LB	11.70 ab	1.14 b	10.26 c
Control	VA OR	—	26 Jan.	LB	11.60 b	1.11 ab	10.45 bc
4	VA OR	19 Jan.	10 Feb.	BT	11.38 a	1.17 a	9.73 b
4	VA OR	19 Jan.	10 Feb.	BT	11.22 a	1.06 b	10.58 a
4	VA OR	19 Jan.	10 Feb.	BT	11.47 a	1.16 a	9.89 b
Control	VA OR	—	10 Feb.	BT	11.47 a	1.16 a	9.89 b
5	VA OR	11 Mar.	4 Apr.	LB	10.54 a	0.70 a	15.06 a
5	VA OR	11 Mar.	4 Apr.	LB	10.53 a	0.71 ab	14.83 a
Control	VA OR	—	4 Apr.	LB	10.71 a	0.75 b	14.28 b
4	VA OR	6 Apr.	21 Apr.	LB	12.38 a	0.88 a	14.07 a
4	VA OR	6 Apr.	21 Apr.	LB	12.15 a	0.85 a	14.29 a
4	VA OR[x]	6 Apr.	21 Apr.	LB	12.36 a	0.84 a	14.71 ab
100	VA OR[x]	6 Apr.	21 Apr.	LB	11.98 a	0.77 b	15.56 b
Control	VA OR	—	21 Apr.	LB	12.34 a	0.88 a	14.02 a
4	Marsh Gpft	23 Dec.	12 Jan.	FP	11.18 a	1.45 a	7.71 a
8	Marsh Gpft	23 Dec.	12 Jan.	FP	11.31 a	1.53 a	7.39 a
Control	Marsh Gpft	—	12 Jan.	FP	10.71 a	1.39 a	7.71 a

[z]Five additional experiments were applied to 'Valencia' oranges on the following dates: 20 Jan.; 3 Feb.; 8 Mar.; 11 Mar.; and 19 Apr. However, none of these showed significant differences ($P < 0.05$) among treatments or controls. Abbreviations: BT—Bok Tower; FP—Ft. Pierce; LB—LaBelle; Gpft—grapefruit; VA OR—Valencia orange. For each experiment, mean separation in each column is by Duncan's Multiple Range Test, 5% level.
[y]All sprays contained 0.1% ORTHO X-77 surfactant or equivalent.
[x]These sprays were applied by backpack sprayers.

(Table 4) are not uncommon. While some growers have reported excellent results from C 10 usage, many growers have reported mediocre activity or complete failure. It is probable that colder winter temperatures reduced the effectiveness of both chemicals as has been reported for other crops (1, 6); and of the 5 experiments which showed no TRIA activity, most were applied during the Florida winter period (Table 6). In addition, TRIA has also been found to be adversely affected by certain plastics (6). Florida spray waters can also vary greatly, with most being pH 8 or above. The manufacturers of C 10 recommend that spray tank pH be adjusted to 6-7 by appropriate additives. Water sources are important for both C 10 and TRIA but more research will be necessary to further understand this problem.

Attempts to harvest 'Valencia' oranges followed by dipping in a solution of C 10 or TRIA proved ineffective as a substitute for field application. Although tests with C 10 and TRIA have not effectively reduced white grapefruit acidity (Tables 4 and 6), researchers in Texas (4) report that in some tests, C 10 significantly increased °Brix and ratio, and significantly reduced percent acid with 2 red grapefruit cultivars.

Future research will be aimed at trying to achieve consistent results in controlling acidity in citrus fruit. To this end, the FMC handsprayer used by the Florida Department of Citrus has been modified by removing all plastic materials where possible, and with the addition of a 100% rubber spray hose and a 200 gal (757 l) stainless steel tank. Similar modifications have also been adapted by A. Duda and Sons, Inc. Relatively pure water sources will be utilized as much as possible.

The extreme high cost of registering new chemicals for use on food crops has greatly reduced chemical development for agricultural purposes, including PGR materials. The situation will probably not improve as environmental protection is an important consideration for everyone and the necessary testing is very expensive, perhaps as much as $40 million per chemical (E. H. Beyer, personal communication). Therefore, research with environmentally safe biological materials should be pursued when available, even though at times inconsistent results may prove frustrating to the researcher.

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Proc. Fla. State Hort. Soc. 101:161-165. 1988.

JUICE VESICLE DISORDERS AND IN-FRUIT SEED GERMINAION IN GRAPEFRUIT

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Abstract. **Juice vesicle disorders (vesicle collapse [VC] or vesicle granulation [G]) and in-fruit seed germination were studied in fruit of 2 grapefruit cultivars (*Citrus paradisi* Macf. cv. Marsh Seedless and Ruby Red) harvested from the same trees in each of 8 groves (4 per cultivar) during the late season (March-May) in 3 successive years. Fruit were examined at harvest and after 40 days storage at 21°C. At harvest, section drying (SD) was found primarily at the stem-end whereas during storage they also appeared in the stylar-end and central fruit portions. The occurrence of VC was slightly higher than G at harvest. Both disorders increased during storage but VC much more than G. Moderate to severe VC occurred in fruit from the last harvest date (May). Grove to grove and year to year variability in VC and G was observed. In-fruit seed germination appeared to be associated with SD. SD and in-fruit seed germination were significantly positively correlated each year with 50% of the variation in seed germination accounted for by SD.**

Florida Agricultural Experiment Station Journal Series No. 9530.

[1]This manuscript was prepared from the dissertation of the senior author as partial fulfillment of the requirements for a PhD degree in Horticultural Science at the University of Florida.

Grapefruit production is a major part of the citrus industry in Florida. Grapefruit harvest usually extends through May when physiological disorders often occur. These disorders result in quality reduction of fruit at harvest or postharvest. In previous studies, at least 2 distinct vesicle disorder types (granulation [G] and dehydration disorders [dry juice sac and core dryness]) have been re-