Table 2. Analysis of variance for *Phytophthora palmivora* resistance in papaya measured by transformed percent mortality in a diallel crossing design.

Source	df	MS
Blocks	9	1,795**
Crosses	14	1,214**
GCA	4	3,666
SCA	10	233*
Error	126	102

*,**Significant at 5% (*) or 1% (**) level.

(Table 2). The GCA:SCA mean square ratio was 15.7 which confirms GCA is more important than SCA in the set of lines studied.

There was a significant correlation (r = 0.89) between the parental and hybrid array means, which indicates resistant parents give resistant offspring and susceptible parents give susceptible offspring and supports the high GCA:SCA ratio.

The hybrid mortality mean (24.3), in the transformed scale was slightly lower than the midparental mean, 27.2 (Table 3) but was not significant indicating there was no average heterotic effect for greater susceptibility. The largest negative GCA effects were shown by lines Waimanalo-23, Waimanalo-24 and line 40, indicating their progenies had significantly lower mortality regardless of the other parent. There were large negative SCA effects in 'Higgins' × Waimanalo-24, 'Higgins' × 40, 'Higgins' × 45-T_{22}, and 45-T × 40. These crosses probably contributed to the significant SCA mean square in the diallel analysis.

Inasmuch as these papaya inbred lines were selected for this study because they showed a range of susceptibility to phytophthora root rot, a fixed model was used for genetic interpretation. In spite of this, estimates of variance components were made (Table 4) as if a random model were used, following Griffing (3). This analysis suggested that genetic variation for phytophthora root rot in papaya is largely additive. Narrow sense heritability was 81% and broad sense heritability was 92%. These high heritability estimates indicate that selection should be effective in breeding for root rot resistance in papaya.

Table 4. Estimates of GCA, SCA, environmental, additive dominance, genotypic and phenotypic variances and heritability of *Phytophthora palmivora* root rot resistance in papaya.[z]

Variance component	Symbol	Estimated variance for percent mortality in the transformed scale
General combining ability	σ^2_{GCA}	491 ± 371
Specific combining ability	σ^2_{SCA}	131 ± 60
environmental	σ^2_e	102 ± 13
additive	σ^2_a	981
dominance	σ^2_d	131
genotypic	σ^2_G	1,112
phenotypic	σ^2_P	1,214
Narrow sense heritability		0.81
Broad sense heritability		0.92

[z]Griffing's relationship among variances (3): $\sigma^2_a = 2\sigma^2_{GCA}$; $\sigma^2_G = 2\sigma^2_{GCA} + \sigma^2_{SCA}$; $\sigma^2_d = \sigma^2_{SCA}$; σ^2_{SCA}; $\sigma^2_P = \sigma^2_G + \sigma^2_e$

Table 3. Transformed percent mortality of 5 parental papaya lines (underlined) and their progeny inoculated with *Phytophthora palmivora*, with GCA effects in the bottom line and SCA effects within the triangle below diagonal.[z]

Seed parent	Pollen parent: Waimanalo - 23	Waimanalo - 24	40	45-T_{22}	Higgins	Hybrid array mean
Waimanalo - 23	12.3	14.3	17.2	25.8	28.6	21.5
Waimanalo - 24	–0.4	14.8	17.4	31.3	26.3	22.3
40	2.5	1.4	19.3	20.7	25.2	20.1
45-T_{22}	1.8	6.1	–4.6	35.0	36.0	28.5
Higgins	–1.4	–4.8	–5.9	4.5	54.6	29.0
GCA	–5.9	–4.7	–4.6	4.6	10.5	

[z]K-ratio t (1%) = 10.5
Grand mean = 25.3; Parental mean = 27.2; F_1 mean = 24.3

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Chilling Injury in Leaves of Citrus Plants at 1.7°C[1]

George Yelenosky

U. S. Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, FL 32803

Additional index words. low temperature stress

Abstract. **Leaves of different citrus selections (*Citrus* sp.) developed visible bleaching (low chlorophyll content) within 14 days during constant 1.7°C and less than 500 μE/m² per sec (PAR) continuous light regimes in controlled temperature facilities. Leaves did not bleach at 21.1° and 10° in continuous light and/or in the dark at 1.7°. Larger amounts of amino acids leaked from bleached than nonbleached leaves and leaf disks from bleached leaves had lower rates of O_2 uptake during respirometry at 30°. Plants at 1.7° for 14 days in continuous light were injured more than plants conditioned at 10° during 4-hr freeze tests at −6.7°.**

In past work where carbohydrate accumulation was determined in leaves of 'Valencia' orange [*Citrus sinensis* (L.) Osbeck] at progressively colder temperatures, a metabolic disorder was indicated with carbohydrate concentrations decreasing sharply between 5° and 0°C (12). Subsequent observations on citrus plants, in a series of tests in controlled temperature facilities, support the contention that citrus leaves are chilling-sensitive. This characteristic is not readily apparent under natural conditions in Florida.

Observations were made in studies of 8-month-old plants of 'Valencia' orange; rough lemon (*C. limon* Burm. f.); and 'Cleopatra' mandarin (*C. reticulata* Blanco). Plants were grown from open-pollinated seed obtained from single-source trees. After germination in flats filled with vermiculite-peat mix, the seedlings were grown under natural daylight in a greenhouse with one transplant per 2.5 liter plastic pot containing equal parts of sphagnum peat moss; vermiculite; and perlite. Plants were watered as needed and fertilized bimonthly with commercial liquid fer-

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tilizer. Temperatures inside the greenhouse were 33°C maximum with 40% relative humidity during days and 18° minimum with 98% relative humidity during nights. Test plants were arbitrarily selected for uniform growth and appearance. Tests were done in four separate but adjacent walk-in temperature-controlled rooms described in previous reports (11). Three of the rooms were used simultaneously for different temperature regimes of constant 21.1°, 10°, and 1.7°, controlled to ± 0.5°. Light from fluorescent and incandescent overhead bulbs was continuous and passed through a mylar barrier 6 cm away. Light ranged from 420 to 480 $\mu E/m^2$ per sec (PAR) at the top of the plants with no appreciable difference among rooms during test periods. Relative humidity was maintained at 50% ± 5%. Freeze tests were done in the fourth room with similar relative humidity but dark. Freeze regimes were cam-controlled to start after 1 hr equilibration at 4.4° followed by a 1.1°/hr decrease to −6.7° for 4 hr and a return to 4.4° at 1.1°/hr. Frozen plants were rated as to amount of leaves and wood killed after 4 weeks of observations under greenhouse conditions. Tissue analyses were done on two leaves arbitrarily selected from the top one-half of each of 3 to 5 single plant replicates. Chlorophyll was extracted in the dark at about 4° with 80% acetone in a blender containing cut leaves (minus midvein). Extracts were made under vacuum filtration and chlorophyll concentrations determined according to Bruinsma (1). The sensitivity to leakage and apparent membrane damage were determined on three replications of single, whole leaves with blades immersed in 20 ml of glass-distilled water in test tubes for 24 hr at 6° in the dark. Leachates were decanted and spectrophotometrically analyzed using ninhydrin reagent and procedures of Moore and Stein (6) as modified by Rosen (9). Oxygen uptake was determined with a differential respirometer using Warburg procedures with 20 leaf disks per flask (replicated three times). Micro-Kjeldahl analyses were used to determine N in the leaf disks. Respirometry was done at 30° ± 0.01° with 20% KOH and a filter paper wick in the center well.

Results indicated that physiological disorders occurred in leaves of citrus plants subjected to constant 1.7°C and continuous light. Visible bleaching was noted after 8 days and was most pronounced in leaves of rough lemon. Only top surfaces of leaves were bleached. Such leaves had less chlorophyll, leaked more amino acids, and consumed less oxygen than leaves of plants maintained at higher temperatures (Table 1). Subsequent work indicated that bleaching of citrus leaves does not occur in the dark at 1.7° and that undersides of leaves become bleached if overturned to face the overhead lights. Also, leaf surfaces covered with nonphytotoxic white latex paint and whole leaves covered with aluminum foil remain green while noncovered matched areas become bleached (Fig. 1). These green areas were not checked for chlorophyll, leakage, or oxygen uptake.

Table 1. Chlorophyll content, leakage of amino acids, and O_2 uptake in leaves of potted citrus selections maintained in continuous light and at different constant temperatures for 14 consecutive days in controlled environment rooms.

Selection	Temp (°C)	Chlorophyll content (mg/g O.D. wt)	Amino acid leakage (μg/g O.D. wt)	O_2 uptake at 30°C (μl/mg N-hr)
Rough lemon	Greenhouse	3.5 a[z]	136 a	53 a
	21.1	3.7 a	133 a	52 a
	10	3.6 a	140 a	59 a
	1.7	1.8 b	923 b	10 b
Valencia Orange	Greenhouse	3.8 a	123 a	59 a
	21.1	3.7 a	110 a	60 a
	10	3.7 a	126 a	63 a
	1.7	2.3 b	878 b	18 b
Cleopatra mandarin	Greenhouse	3.6 a	140 a	56 a
	21.1	3.5 a	135 a	55 a
	10	3.5 a	140 a	62 a
	1.7	2.1 b	810 b	17 b

[z]Mean separation within columns per selection by Duncan's multiple range test, 5% level.

Table 2. Freeze injury to citrus selections at −6.7°C for 4 hr after 14 days of conditioning in continuous light at different constant temperatures in controlled environment rooms.

Selection	Temp (°C)	Kill (%)	
		Leaf	Wood
Rough lemon	Greenhouse	100 a[z]	100 a
	21.1	100 a	94 a
	10	90 a	62 b
	1.7	100 a	99 a
Valencia orange	Greenhouse	100 a	100 a
	21.1	100 a	90 a
	10	74 b	38 b
	1.7	99 a	86 a
Cleopatra mandarin	Greenhouse	100 a	98 a
	21.1	100 a	94 a
	10	62 b	12 b
	1.7	96 a	88 a

[z]Mean separation within columns per selection, by Duncan's multiple range test, 5% level.

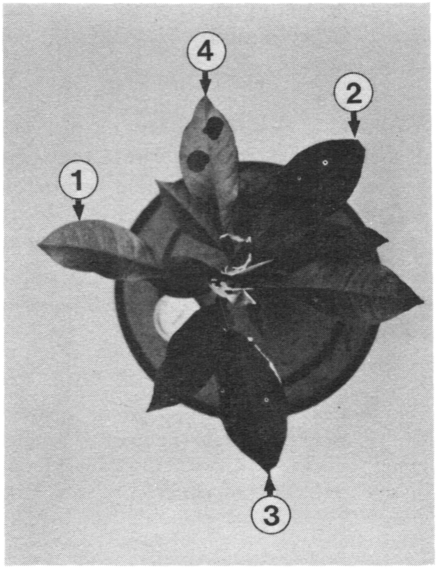

Fig. 1. Chilling injury (bleaching) in leaves of a rough lemon seedling (*Citrus limon* Burm. f.) exposed to continuous light during constant 1.7°C for 2 weeks; 1) = uncovered, bleached leaf, 2) = aluminum foil covered, nonbleached leaf, 3) = latex paint surface covered nonbleached leaf, and 4) = uncovered, bleached leaf with two paint-covered, nonbleached disk-shaped areas.

The bleaching of leaves observed in this study along with the loss in chlorophyll, increased leakage, and low oxygen uptake are considered classical symptoms of chilling injury to plants (4, 5, 8). Chilling injury to leaves of citrus is not known to occur in the field in Florida either because of nonrecognition or because the necessary chilling conditions are of insufficient duration to induce visible symptoms in the leaves. Such may not be the case in colder citrus growing areas. In the Georgian region of the USSR, shade seemingly protected young lemon trees from apparent chilling injury as a result of low but nonfreezing temperature in combination with exposure to direct sunlight (3). Exposure to direct sunlight may also play a role in increasing the susceptibility of grapefruit to chilling injury (7).

The bleaching of leaves observed in these controlled temperature studies is considered typical of chilling injury to other plants, especially as described by researchers for *Cucumis* leaves where damage is largely attributed to photooxidation (2). In other tests, we found that citrus plants do not cold harden satisfactorily in continuous light and constant 1.7°C (Table 2). This was also found but not recognized as such in 1971 (10). Unusually prolonged low temperatures between 5° and

0° in combination with direct exposure to light are suspect in predisposing citrus tissues to greater freeze injury.

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Growth Regulator Effects on Ethylene Production from Calamondin Flowers[1]

Stephen M. Southwick[2] and Frederick S. Davies

Fruit Crops Department, University of Florida, Gainesville, FL 32611

Additional index words. calcium, cytokinins, gibberellic acid, *Citrus madurensis*

Abstract. **Ethylene production by senescing flowers of calamondin (*Citrus madurensis* Lour.), at rates as high as 15 nl/g fresh weight-hour did not necessarily induce absicission. Moreover, combinations of gibberellic acid (GA), calcium dihydrophosphate and 6-benzylamino purine (BA), which are known to increase fruit set in citrus, did not significantly decrease ethylene production. Abscission of calamondin fruitlets and increases in fruit set appear to be independent of ethylene production.**

Low concentrations of GA and 2,4-dichlorophenoxyacetic acid (2, 4-D) increase fruit set or delay abscission of navel orange (20) and other citrus cultivars (10, 11, 18, 21). Combinations of GA with BA and calcium dihydrophosphate increased fruit set of navel orange in some years (20), and GA_{4+7}+BA has shown promise as a fruit setting agent (19). In addition, GA and 2, 4-D stimulate ethylene production in plant tissues (3, 8, 13, 15). Greater amounts of ethylene (g/fresh wt·hr) were produced from younger fruit than from fruit at later stages of development in citrus (7, 9). Ethylene induced or accelerated abscission of mature citrus fruit (5, 6) but failed to cause similar responses in young citrus fruit (9). Ethylene has been associated, however, with premature flower abscission of apple and cherry (2). The objective of this study was to determine whether growth regulators that increase citrus fruit set do so by affecting ethylene production.

Calamondin is uniquely suited to flowering and fruit-set studies because it can be induced to flower at any time of the year by witholding water and nitrogen. Flowers also respond to growth regulators in a fashion similar to other citrus species (12), thus providing a convenient test plant for fruit set studies.

Three-year-old calamondin plants were transferred from the nursery to 20 cm plastic pots containing sand: pine bark: peat (1:1:1:v/v/v) plus macro- and micronutrients. Plants were fertilized biweekly with approximately 12 g per 3.8 liters of 20N:8.6P:16.6K water-soluble fertilizer and micronutrients. Plants were induced to flower after about a month-long period of acclimation to greenhouse growing conditions.

Ethylene determinations were made at various flower developmental stages. Flowers were collected and placed into one of 4 groups: 1) closed flowers that would open in 24 to 48 hr; 2) opening flowers with a visible stigma and free but not recurved petals; 3) senescing flowers with brownish, recurved petals, water-soaked flaccid stamens, and a yellow-green ovary; and 4) fruitlets with no flower parts persisting. Three flowers were collected from each of 3 trees for each developmental stage. Flowers and fruitlets were clipped with 1.0 to 2.0 mm of the pedicel attached and put into 19.8 ml glass shell vials. A serum rubber stopper was used to seal the open end of the vial. Ethylene levels were determined following 2- and 6-hr incubation periods at 25°C. One ml gas samples were withdrawn from each container into a disposable plastic syringe and injected into a Hewlett-Packard 5710A gas chromatograph equipped with a flame ionization detector and a stainless steel, 1 m x 6.35 mm ID column packed with activated alumina. Flow rate of nitrogen carrier gas was 75 ml/min and oven temperature was 100°C.

Five terminal leafy inflorescences per treatment, each borne on a different plant, were randomly selected and dipped for 10 sec into growth regulator solutions (see Table 1 for materials and concentrations). Growth regulators were dissolved in distilled water and 0.1% X-77 adjuvant. Flowers were allowed to dry before they were clipped from the plant and put into 19.8 ml shell-vials. Excised flowers were incubated for an hour in the dark at 25°C. Ethylene was collected at hourly intervals up to 8 hr after sealing the vial with a serum rubber stopper for an hour. A 1 ml gas sample was withdrawn from each vial as described previously; thereafter, vials were opened, flushed with air and reincubated in the dark at 25°C until the next hourly

Table 1. Effect of growth regulator dips on ethylene production from excised calamondin flowers.[z]

Growth regulator treatment (ppm)	Ethylene production (nl/g fresh wt·hr)[z] Incubation time[y] 1–2 hr	3–4 hr	7–8 hr
Control (water)	16.6[x]	11.9	8.9
GA (25)	20.5	14.9	15.4
BA (20)	20.9	20.6	17.3
GA (25) + BA (20)	11.7	17.0	18.3
$Ca(H_2PO_4)_2$ (1000)	7.0	9.0	9.5
GA (25) + $Ca(H_2PO_4)_2$ (1000)	13.0	21.5	22.7
GA (25) + BA (20) + $(Ca(H_2PO_4)_2$ (1000)	11.8	16.0	18.5
GA (25) + BA (20) + 2, 4-D (20)	13.6	19.3	23.2
Promalin[w] (500)	14.5	10.0	9.8

[z]Means are from 6 replicates.
[y]Effect of incubation time on ethylene production nonsignificant by Duncan's multiple range test, 5% level.
[x]Effect of treatment on ethylene producton nonsignificant by Duncan's multiple range test, 5% level.
[w]Concentration of Promalin (equal parts GA + BA) was 500 ppm of formulated material [GA_{4+7} (25 ppm) + BA (25 ppm)].

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[2]Present address: Agricultural Resource Management, P.O. Box 993, Vero Beach, FL 32960.