

Characterization of Water Stress and Low Temperature Effects on Flower Induction in Citrus¹

Received for publication September 3, 1985 and in revised form December 20, 1985

STEPHEN M. SOUTHWICK AND THOMAS L. DAVENPORT*

Tropical Research and Education Center, University of Florida, IFAS, Homestead, Florida 33031

ABSTRACT

Experiments were conducted with containerized 'Tahiti' lime (*Citrus latifolia* Tan.) trees in order to define conditions needed to induce flowering. Cyclical or continuous water stress for 4 to 5 weeks induced flowering. Moderate (–2.25 megapascals, midday) or severe (–3.5 megapascals, midday) water stress as measured by leaf xylem pressure potential, for as little as 2 weeks induced flowering, but the response was more significant in severely stressed trees. Low temperature (18°C day/10°C night) induced a time dependent flowering response much like that of moderate water stress. Significantly negative leaf xylem pressure potentials as compared to controls were found only under water stress treatment, suggesting that a common stress-linked event, separate from low plant water potential is involved in floral induction. Leafless, immature cuttings from mature, field-grown trees were induced to flower by water stress treatment, suggesting that leaves are not essential for a flower inductive response.

'Tahiti' lime, *Citrus latifolia* Tan., is a sterile triploid that is vegetatively propagated thereby avoiding a juvenile period that is common to many tree species. Flowers are produced and fruit set and mature on leafy rooted cuttings as well as on plants propagated by air-layering. This unusual habit makes 'Tahiti' lime suitable as a test plant to study flowering in trees (20). Flowering in citrus can be induced by lower temperature (4, 10, 14–16, 20) or water stress (3, 11, 18, 20) and inhibited by applied gibberellin (5, 6, 9, 12, 13, 18). Regulation of flowering by water stress is not common in trees and generally is reported to be effective in tropical and subtropical species (1–3, 18). These studies, however, have been conducted under varying field conditions and are not descriptive (2) in the sense that the studies do not define the quantitative relationship between imposed stress and the flowering response. These same shortcomings are true for studies involving low temperature regulation of flower induction.

In our effort to understand the chemical control of flowering in 'Tahiti' lime we felt it important to manipulate flowering in small containerized trees and to have more than one method of flower induction available to us. By utilizing several floral-inductive treatments, we hope to ascertain whether a common regulatory event or signal controls flower induction. This report describes the quantitative relationship between low temperature and water stress in floral induction.

MATERIALS AND METHODS

Plant Material. In most experiments we used 1- to 2-year-old 'Tahiti' lime trees propagated by air-layering or by bud-grafting

on to *Citrus macrophylla* Wester. rootstock. The trees ranged in height from 0.5 to 1.0 m and were grown in the greenhouse under South Florida growing conditions in 16-cm black plastic pots in a mix of 1 peat:1 perlite:1 sand, and fertilized regularly with a 20:20:20 NPK soluble fertilizer plus micronutrients. In other experiments, cuttings were obtained from 18-year-old 'Tahiti' lime trees on rough lemon (*Citrus jambhiri* Lush.) rootstock in Rockdale limestone soil at the Homestead Tropical Research and Education Center. Cuttings were selected by clipping immature current season's growth, which bore mostly fully expanded, but nonhardened, immature leaves, and were reclipped near the base under water. Leaves were removed so that 5 to 8 nodes and 2 to 4 or no leaves remained, depending upon treatment. Cuttings with one end were stuck in vermiculite and were placed either in the greenhouse or on another bench under intermittent misting.

Water Stress Treatments. Water stress of trees was either continuous or cyclical. Continuous water stress was established by sealing the pot, soil, and roots in a plastic bag with only stem and leaves exposed to the environment and withholding water. Transpirational water loss from each containerized tree was determined by monitoring the daily weight decrease. Lime trees were transpiring approximately 140 ml water/d. From these measurements 67% of the amount of water lost per d was added back to the tree daily so that stress could be gradually imposed and leaf drop minimized. When all leaves became wilted and the mature leaves had a xylem pressure potential of at least –3.5 MPa (severe stress as defined by Syvertsen, [21]), 100 ml of water per d was added to each in order to approximately replace transpirational water lost and to keep these trees under constant stress. Addition of 100 ml of water per d to soil briefly saturated the soil until it drained to field capacity. In absolute terms, continuous water stress may not precisely define this sequence of events, but continuous water stress best describes our observations with regard to the water status of these trees. Cyclical water stress was achieved by stressing each tree to the point of wilting as above and then refilling the container to the full capacity, which set the soil at field capacity. The dry (wilting), wet (container soil at field capacity) cycle or continual stress was continued for the duration of each experiment. Control and treatment trees were preconditioned for at least 1 month through maintenance of container soil at field capacity by applying water twice daily through automatic drip irrigation. Leaf xylem pressure potentials were measured at predawn and midday by the pressure bomb technique (19). Leaves were removed at the petiole-blade abscission zone of each leaf, and measurements were made within 30 s of leaf removal. Leaf xylem pressure potential measurements were made at weekly intervals in the time course experiments, and two leaves per tree (10 leaves/treatment) were measured at predawn and midday. Only two leaves were used from each tree to maximize the number of leaves remaining on stressed trees and maintain uniformity of treatment.

Low Temperature Treatments. Growth chamber experiments

¹ Florida Agricultural Experiment Stations Journal Series No. 6738.

were conducted at 18/10°C (day/night) temperatures with 12-h photoperiods at a photon flux ranging from 350 to 850 $\mu\text{E}/\text{m}^2\cdot\text{s}$. Prior to placing each tree in the growth chamber, approximately one-half of all branch apices including 2 to 3 leaves and nodes were clipped off (4). Controls were treated likewise and were grown in the greenhouse under South Florida conditions (17) and 29/24°C (day/night) temperatures. In these experiments, "total shoots produced" represents the sum of vegetative, mixed, and generative shoots. Those three shoot types are defined here as they have been previously (6, 12, 14). Briefly, vegetative shoots carry leaves only, mixed shoots carry both leaves and flowers, and generative shoots carry flowers only. Tables have been obtained from at least two replicate experiments in all cases.

RESULTS AND DISCUSSION

Continuous or cyclical water stress for 4 to 5 weeks (from the initiation of reduced water application to restoration of daily irrigation to container soil field capacity) resulted in flower induction of 'Tahiti' lime (Table I). Continuous and cyclical water stress resulted in more total shoot production as well as a significantly greater number of flowers than controls. After these trees had completed flowering, the flowers and fruitlets were removed. The trees were allowed to resume vegetative growth for a period of 2 months under controlled greenhouse growing conditions. The same trees were induced to flower a second time by the above procedure. The rationale for using the same trees in experiment 2 was that flower reinduction in the same population of trees by the same treatment should indicate that our treatments were truly effective since heavy flowering in subsequent flushes does not occur in greenhouse-grown citrus. The results of the second experiment were similar to those of the first. Continuous and cyclical water stress resulted in trees producing more total shoots and flowers than controls, which produced only random and insignificant numbers of flowering shoots and flowers. There were more flowers/plant produced in continuous than cyclical stress of experiment 1, but not in experiment 2. No significant differences were found between continuous or cyclical stress with regard to shoots per plant, shoot type, or flowers per plant, but continuous stress generally resulted in greater numbers of total shoots per plant and flowers per plant. In fact, as long as the severity of stress resulted in prolonged wilting or a leaf xylem pressure potential of -3.5 MPa for 4 to 5 weeks, the flower inductive response was similar and significantly different from controls. Therefore, the continuous stress condition was used because it was easy to produce and allowed for uniformity of treatment.

The above experiment, which had been performed at two different times of the year including that when 'Tahiti' lime typically does not flower, indicated that flowering could be induced in containerized lime trees by a period of water stress lasting for a 4 to 5 week period. To more clearly define the duration and severity of water stress needed to induce flowering, leaf xylem pressure potentials were measured at weekly intervals over a 4 to 5 week period in a population of trees that were stressed. Table II shows the level of moderate stress maintained above controls at predawn and midday for each time interval measured. We defined moderate levels of stress (-2.1 to -3.0 MPa) as those levels intermediate between control and severe stress (-3.5 MPa, [21]). Midday leaf xylem pressure potentials were generally lower and measurements less variable than those at predawn, except at week 5 where predawn stress was as great as that at midday. Inter-tree variability and daily climatic changes were presumably responsible for the variability in the pressure potential measurements. Control trees produced the least number of shoots/plant and those shoots were vegetative. More shoots/plant were produced as a result of water stress and the numbers generally increased in trees exposed to greater durations

of water stress. Flowering was induced after 2 weeks of stress. The percent flowering shoots and number of flowers per plant increased with time under stress. The highest percentage of flowering shoots and flowers per plant were found after 5 weeks of water stress. Apparently, moderate levels of stress can induce flowering in a relatively short period of time (2 weeks), but the inductive response is much greater after an extended time period (5 weeks).

In a similar experiment, 'Tahiti' lime trees were severely stressed as indicated by leaf xylem pressure potentials ranging from -3.25 to -3.67 MPa (Table III). Predawn and midday leaf xylem pressure potentials were significantly different from controls at each measured time. Predawn and midday stress measurements were significantly different from one another at 2 weeks, but thereafter, pressure potentials were not different from one another, and a constant level of water stress prevailed in these trees throughout the experiment. At these severe stress levels, a less variable leaf xylem pressure potential was maintained than those measured for moderate stress (Table II) indicating that control of stress (water potential) was obtained under severe water stress conditions. As in the preceding experiment, control trees showed very little shooting, and those shoots produced were vegetative. On the other hand, severe water stress when compared to moderate water stress, resulted in much greater shooting and flowering per plant as well as increased percentages of flowering shoots at all measured time intervals. Flowering trends between moderate and severely water-stressed trees were dissimilar over time, with severely water stressed trees producing the same number of flowers and flowering shoots at each measured time interval. Under moderately water stressed conditions, water stress duration was a factor regulating the flower inductive response. The flowering response appears to be time dependent when regulated by moderate levels of water stress, but at some point floral induction is more immediately reached under conditions of more severe water stress.

Similar to water stress, low temperature stress 18/10°C (day/night) time course experiments were conducted with containerized 'Tahiti' lime trees growing in the growth chamber as previously reported (4, 10, 14). Leaf xylem pressure potentials did not significantly differ from one another at predawn except at the 4-week time interval (Table IV). At midday, however, control trees growing in the greenhouse had significantly more negative pressure potentials at all intervals measured. Control trees, as in the previous water stress experiments, produced very few shoots per plant, and those produced were vegetative (Tables III and V). Flowering was induced in trees after having been in the growth chamber for as little as 2 weeks, but the response was not as great as that of severely water stressed trees within the same time period (Table V). The low temperature stress of the growth chamber resulted in a flower inductive response like that of moderate water stress, but apparently not through a common reduction in leaf xylem pressure potential. A different signal, mediated through a common mechanism, may be regulating floral induction. Severe water stress rather than low temperature stress consistently produced the greatest number of flowers and flowering shoots. Floral induction best describes the floral response observed after both water and low temperature stress. This belief is based upon results obtained from other experiments which will be presented elsewhere (SM Southwick, TL Davenport, unpublished data) indicating that lime trees forced to produce shoots by branch pruning produced a greater percentage of flowering shoots after imposing the above stress treatments.

Table I. Effect of Continuous or Cyclical Water Stress on Flower Induction in Containerized 'Tahiti' Lime Trees

Water Stress Treatment	Shoots/Plant	Shoot Type			Flowers/Plant
		Vegetative	Mixed	Generative	
%					
Experiment 1 ^a					
Control	5.7 ^b ± 0.5	13.0	0	87.0	5.0 ± 1.4
Continuous	41.7 ± 8.0	23.3	17.4	59.3	145.7 ± 48.5
Cyclical	29.5 ± 4.4	44.1	16.1	39.8	44.2 ± 14.3
Experiment 2 (Repeat)					
Control	0.3 ± 0.5	0	0	100.0	0.3 ± 0.5
Continuous	37.7 ± 5.0	11.3	53.6	35.1	75.7 ± 18.1
Cyclical	29.0 ± 5.0	19.0	48.3	32.7	78.5 ± 51.9

^a Experiment No. 1, February 2, 1984 to March 17, 1984. Experiment No. 2, May 11, 1984 to June 12, 1984. These data represent one of two replicate experiments. In this experiment, the same four tree replicates/treatment were used. ^b Values represent means ± SD.

Table II. Effect of Moderate Water Stress over Time on Leaf Xylem Pressure Potential and Flower Induction in 'Tahiti' Lime

Duration of Water Stress	Leaf Xylem Pressure Potential		Shoots/Plant	Shoot Type			Flowers/Plant	Flowering Shoots ^a
	Predawn	Midday		Vegetative	Mixed	Generative		
<i>weeks</i>	<i>MPa</i>			<i>%</i>				<i>%</i>
Control	-0.34 ± 0.08 ^b	-1.48 ± 0.15	4.50 ^c ± 1.9	100.0	0	0	0	0
2	-0.90 ± 0.42	-2.25 ± 0.08	6.25 ± 2.2	68.0	16.0	16.0	3.0 ± 0.82	32.0
3	-1.62 ± 0.82	-2.21 ± 0.25	8.00 ± 2.6	46.9	21.9	31.2	5.0 ± 2.16	53.1
4	-0.87 ± 0.09	-2.89 ± 0.23	9.75 ± 3.0	43.6	20.5	35.9	9.0 ± 2.16	56.4
5	-2.89 ± 0.62	-2.83 ± 0.19	9.75 ± 1.5	10.3	56.4	33.3	21.0 ± 8.04	89.7

^a % Flowering shoots = sum of mixed and generative shoot percentages. ^b Values represent the means of 10 leaf replicates/treatment ± SD. ^c Values represent the means of 5 tree replicates/treatment ± SD.

Table III. Effect of Severe Water Stress over Time on Leaf Xylem Pressure Potential and Flower Induction in 'Tahiti' Lime

Duration of Water Stress	Leaf Xylem Pressure Potential		Shoots/Plant	Shoot Type			Flowers/Plant	Flowering Shoots ^a
	Predawn	Midday		Vegetative	Mixed	Generative		
<i>weeks</i>	<i>MPa</i>			<i>%</i>				<i>%</i>
Control	-0.24 ± 0.05 ^b	-1.38 ± 0.29	3.25 ^c ± 2.0	100.0	0	0	0	0
2	-2.00 ± 0.35	-3.25 ± 0.07	70.50 ± 18.0	16.0	23.7	60.3	246.75 ± 15.8	84.0
3	-3.41 ± 0.83	-3.67 ± 0.24	45.00 ± 12.0	8.9	36.7	54.4	97.2 ± 33.2	91.1
4	-3.56 ± 0.25	-3.66 ± 0.21	49.00 ± 25.0	6.9	26.6	66.5	144.6 ± 41.6	93.1
5	-3.54 ± 0.27	-3.58 ± 0.23	49.80 ± 10.2	6.4	34.1	59.4	168.8 ± 22.8	93.5

^a % Flowering shoots = sum of mixed and generative shoot percentages. ^b Values represent the means of 10 leaf replicates/treatment ± SD. ^c Values represent the means of 5 tree replicates/treatment ± SD.

Table IV. Effect of Low Temperature over Time on Leaf Xylem Pressure Potential in 'Tahiti' Lime

Time of Measurement	Duration of Continual Water Stress (weeks)								
	0	2	C ^a	4	C	6	C	8	C
<i>leaf xylem pressure potential (MPa)</i>									
Predawn	-0.360 ± 0.098 ^b	-0.400	-0.166	-0.479	-0.373	-0.413	-0.426	-0.340	-0.326
Midday	-1.446 ± 0.203	-0.613	-1.306	-0.623	-1.926	-0.633	-1.840	-0.500	-1.94
		0.105	0.041	0.055	0.101	0.069	0.043	0.092	0.064
		0.109	0.118	0.072	0.086	0.052	0.149	0.066	0.08

^a C, control treatments at each time measurement. ^b Values represent means of 10 leaf replicates/treatment ± SD.

A final experiment was conducted to determine if water stress would induce flowering on cuttings that had been obtained from trees growing in the field. Cuttings ("Materials and Methods") were separated into two populations: those with leaves and those with leaves removed. From each population, one set was placed

in the greenhouse and allowed to desiccate as the result of being irrigated only occasionally (water stress), and another set was put on a different bench under intermittent mist (nonstressed) to prevent desiccation (8). During the period in the greenhouse prior to flower production, all leaves, except for the most im-

Table V. *Effect of Low Temperature over Time on Flower Induction in 'Tahiti' Lime*

Duration of Reduced Temperature	Shoots/Plant ^a	Shoot Type			Flowers/Plant	Flowering Shoots ^b
		Vegetative	Mixed	Generative		
<i>weeks</i>			%			%
Control	5.20 ± 0.4	100.00	0	0	0	0
2	6.20 ± 3.8	54.84	12.90	32.26	5.60 ± 4.03 ^a	45.16
4	9.80 ± 3.8	44.90	18.37	36.73	14.20 ± 6.76	55.10
6	13.20 ± 3.7	33.33	36.37	30.30	25.40 ± 10.02	66.67
8	15.20 ± 5.8	22.37	14.47	63.16	30.00 ± 9.97	77.63

^a Values represent means from 5 tree replicates/treatment ± SD. ^b Flowering shoots = sum of mixed and generative shoot percentages.

Table VI. *Effect of Leaves and Misting on Flowering of Immature 'Tahiti' Lime Cuttings*

Treatment	Total Shoots	Shoot Type (mean)			Flowers	Flowering Shoots
		Vegetative	Mixed	Generative		
	<i>no</i>				<i>no</i>	%
Greenhouse						
No leaves	8.3 ^a ± 1.5	5.6 ± 2.1	0	2.7 ± 0.6	3.0 ± 1.0	32.0
Leaves	16.7 ± 1.1	5.0 ± 3.6	2.7 ± 1.1	9.0 ± 1.7	22.7 ± 11.7	70.0
Mist bed						
No leaves						
Leaves	14.7 ^b ± 6.5	8.0 ± 4.0	2.3 ± 1.5	4.3 ± 2.1	10.3 ± 1.5	45.4

^a Each value represents the mean of 3 experiments where at least 10 replicates/treatment were used. ± SD. ^b Cuttings in the mist bed did not have shoots or flowers after 33 d; therefore, those cuttings were removed from the mist bed, placed on another bench in the greenhouse, and data recorded 5 weeks later.

mature ones, wilted and abscised. After 33 d had elapsed, those cuttings that had been placed in the greenhouse (desiccated), both leafy and those with leaves removed, produced flowers (Table VI). The greatest number of shoots, flowers, and flowering shoots were produced on cuttings that had initially borne leaves. Nevertheless, those cuttings that had their leaves manually removed produced both vegetative and flowering shoots. Since immature, leafy cuttings were selected for these experiments, it is improbable that a previously stored floral message was present. Furthermore, flowers produced on leafless cuttings suggest that it is not essential for leaves to be present for floral induction and that perception of flowering cues occurs within the shoot, or as most likely in the bud itself. Although it has been speculated that citrus roots may produce a substance(s) which can be transported to shoots and exert control over bud break and flowering (7), these immature cuttings never flower in the field while attached to the tree until they go through a period of maturation or possibly a freezing event (FS Davies, personal communication).

Cuttings that were placed in the mist bed did not produce shoots or flowers. Therefore, after the same 33 d period, these cuttings were removed from the mist bed and placed on an open bench in the greenhouse, and after another 5 weeks those cuttings bearing leaves produced vegetative and flowering shoots as a result of water stress (Table VI). Cuttings without leaves which had been placed in the mist bed did not produce any new shoots and eventually died.

In conclusion, 'Tahiti' lime trees preconditioned at container soil field capacity for about 1 month can be severely water stressed for a period of as little as 2 weeks and consistent flower inductive responses obtained. The floral response seems to be time dependent under conditions of moderate water stress and low temperature. However, floral induction from low temperature when compared to water stress is not mediated through a common decrease in leaf xylem pressure potential. Immature, leafless cuttings can produce flowering shoots under water-stress conditions, indicating that leaves are not essential for flower induction in 'Tahiti' lime.

LITERATURE CITED

- ALVIM P DE T 1960 Moisture stress as a requirement for flowering in coffee. *Science* 132: 354
- BERNIER G, JM KINET, RM SACHS 1981 *The Physiology of Flowering*, Vol I. CRC Press, Boca Raton, FL
- CASSELLA D 1935 L'agrumicoltura siciliana. *Ann R Staz Sper Fruit Agrum Acireale* NS 2: 147
- DAVENPORT TL 1979 Effect of pruning and chilling temperature on flower induction in 'Tahiti' lime. *Plant Physiol* 63: S-70
- DAVENPORT TL 1983 Daminozide and gibberellin effects on floral induction of (*Citrus latifolia*). *HortScience* 18: 947-949
- GOLDSCHMIDT EE, SP MONSELISE 1972 Hormonal control of flowering in citrus and other woody perennials. In DJ Carr, ed, *Plant Growth Substances* 1970. Springer Verlag, New York, pp 758-766
- HALL AE, MMA KHAIRI, CW ASBELL 1977 Air and soil temperature effects of citrus. *J Am Soc Hortic Sci* 102: 261-263
- HARTMANN HT, DE KESTER 1975 *Plant Propagation Principles and Practices*. Prentice-Hall, Inc., Englewood Cliffs, NJ, pp 252-254, 297-300
- IWAMASA M, Y OBA 1973 Precocious flowering of citrus seedling. *Congresso Mundial de Citricultura*, Vol II. Muria-Valencia, Spain, pp 205-213
- LENZ F 1969 Effect of daylength and temperature on the vegetative and reproductive growth of Washington Navel orange. In HD Chapman, ed, *Proceedings, 1st International Citrus Symposium*, Vol I. University of California, Riverside, pp 333-338
- MARANTO J, KD HAKE 1985 Verdelli summer lemons: a new option for California growers. *Calif Agric* 39: 4
- MONSELISE SP 1973 Recent advances in the understanding of flower formation in fruit trees and its hormonal control. *Acta Hortic* 34: 157-166
- MONSELISE SP, R GOREN 1978 The role of internal factors and exogenous control in flowering, peel growth, and abscission in Citrus. *HortScience* 13: 134-139
- MOSS GI 1969 Influence of temperature and photoperiod on flower induction and inflorescence development in sweet orange (*Citrus sinensis* L. Osbeck). *J Hortic Sci* 44: 311-320
- MOSS GI 1971 Promoting flowering in sweet orange. *Aust J Agric Res* 22: 625-629
- MOSS GI 1976 Temperature effects of flower initiation in sweet orange. *Aust J Agric Res* 27: 399-407
- National Oceanic and Atmospheric Administration 1976 *Climate of Homestead, Florida*. Climatology of the United States No. 20
- NIR I, R GOREN, B LESHAM 1972 Effects of water stress, gibberellic acid and 2-chloroethyl trimethylammonium chloride (CCC) on flower differentiation in 'Eureka' lemon trees. *J Am Soc Hortic Sci* 97: 774-778
- SCHOLANDER PF, HT HAMMEL, ED BRADSTREET, EA HEMMINGSEN 1965 Sap pressure in vascular plants. *Science* 148: 339-346
- SOUTHWICK SM, TL DAVENPORT, AL CASTRO 1985 Development of radioimmunoassays for GA₃ and (±)ABA for use in the study of flower induction in 'Tahiti' lime (*Citrus latifolia* Tan.). *Proc 11th Annu Meet Plant Growth Reg Soc Am*, p 59
- SYVERTSEN JP 1982 Minimum leaf water potential and stomatal closure in citrus leaves of different ages. *Ann Bot* 49: 827-834