Effects of drought stress and urea sprays on production of flower and vegetative buds of Tahiti lime

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Abstract — **Introduction**. Tahiti lime (*Citrus latifolia* Tan.) trees were subjected to different levels of water stress and urea sprays under a tropical climate in order to evaluate the effect on floral and vegetative growth. **Materials and methods**. The experiment compared four levels of irrigation intervals: frequent irrigation (every 3 or 4 d) starting at the end of the rainy season in order to maintain the soil moisture close to field capacity, and treatments with non-irrigation intervals of either 4, 8, or 12 weeks. These treatments were combined with foliar sprays of urea in a 4 × 2 factorial arrangement. There was a non-sprayed control and a 5% urea treatment sprayed 2 d before resuming irrigation. **Results and discussion**. The flowering response increased with the level of drought stress, but no response resulted from the single urea spray. Regression equations depicted curvilinear relationships between vegetative shoot growth and leaf water potential. Floral shoots and, especially, the number of flowers per branch exhibited a strong relationship to drought stress.

Venezuela / *Citrus latifolia* / irrigation / flowering / water potential / leaves / ammonia

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Effets du stress hydrique et de la pulvérisation d'urée sur la formation de bourgeons végétatifs et floraux chez la lime Tahiti.

Résumé — **Introduction**. Des limettiers de la variété Tahiti (*Citrus latifolia* Tan.), cultivés en climat tropical, ont été soumis à plusieurs types de stress hydrique et de pulvérisation d'urée afin d'évaluer l'effet de ces traitements sur la croissance végétative et la floraison. **Matériel et méthodes**. L'expérimentation a permis de tester quatre protocoles d'irrigation : irrigation fréquente (tous les 3 ou 4 d) débutant à la fin de la saison des pluies pour maintenir l'humidité du sol à une valeur proche de la capacité au champ, et traitements caractérisés par des périodes sans irrigation de 4, 8 ou 12 semaines. Ces traitements ont été combinés avec des pulvérisations foliaires d'urée selon un dispositif factoriel 4 × 2 : un lot de plants, constituant le témoin, n'a reçu aucune pulvérisation et un autre lot a reçu une pulvérisation à 5 % d'urée, 2 d avant le début de l'irrigation. **Résultats et discussion**. Le degré de sécheresse a influencé la floraison, mais la seule pulvérisation d'urée effectuée avant la reprise de l'irrigation n'a eu aucun effet. Les équations de régression ont montré une relation curvilinéaire entre la croissance végétative des tiges et le potentiel hydrique foliaire. Le taux de tiges florales et particulièrement le nombre de fleurs par branche se sont avérés très liés au stress hydrique.

Venezuela / *Citrus latifolia* / irrigation / floraison / potentiel hydrique / feuille / ammoniac

1. introduction

Flowering of citrus in tropical regions is enhanced by the alternating rainy and dry periods of the year. Flower differentiation in lemon occurs during the drought stress period and generative buds undergo flower development when plants are rewatered [1]. Regardless of the time of the year, Southwick and Davenport [2] induced flowering in Tahiti lime trees by subjecting them to prolonged periods of water stress. Drought stress in summer has induced flowering of lemon plants in Sicily [3] and California [4].

The number of flowers per tree has been related to the concentration of leaf ammonia during a drought stress period in a commercial lemon orchard [5]. Although leaf ammonia does not influence flower initiation directly, it may serve as a substrate for the synthesis of arginine and polyamines that could act to initiate the flowering process through stimulation of cell division and elongation [6].

The culture of Tahiti lime for exportation represents a growing industry in Venezuela. The enhancement of floral habits of trees could improve fruit yield in periods when international prices are the highest. This study, therefore, was conducted to evaluate the effect of different levels of drought stress and foliar sprays of urea on floral and vegetative response of Tahiti lime. Since several studies relating drought stress to flowering have not defined quantitative relationships [7] or have been conducted under controlled environments [2], the results may provide useful information about the tree response under its natural field growing conditions.

2. materials and methods

The study was carried out in an orchard of Tahiti lime (*Citrus latifolia* Tan.) on Volkamer lemon (*C. volkameriana* Pasq.). The trees were 28 months old (plus 10 months in nursery) and spaced 4 m in rows and 7 m between them. The orchard was located near Camatagua, Aragua state, Venezuela, at 9° 43' N and 180 m above sea level. The soil was a clay loam with a slope less than 5%.

The experiment compared four different levels of irrigation intervals. The frequent irrigation treatment maintained the soil moisture close to field capacity (0.03 MPa tension, assessed by tensiometer readings at a depth of 30 cm) by applying water every 3–4 d, starting at the end of the rainy season in October. Drought stress treatments consisted of non-irrigation intervals of either 4, 8, or 12 weeks. Irrigation was controlled by plugging the emitter of the existing undertree microsprinkler system of the trees subjected to drought stress. Irrigation was resumed January for all treatments.

The four irrigation treatments were combined with foliar sprays of urea in a 4×2 factorial arrangement. There were a non-sprayed control and a foliar spray treatment of 5% urea, in aqueous solution which completely covered the foliage, 2 d before resuming irrigation in an attempt to modify the ammonia levels in the leaves. Each treatment was replicated six times in a completely randomized design. There were two plants per plot, for a total of 96 trees.

To avoid the interfering effect of rains that could occur during the dry season, a 3×3 m plastic sheet was placed under each tree at the beginning of the period of no irrigation. To keep the microsprinkler system functional until the day when each treatment was to be imposed, the sheets were placed at different periods of time for each treatment; however, the plastic sheet, acting as an evaporation barrier, did not allow the trees to reach very high stress levels, even after 12 weeks without irrigation. For this reason, 12 similar additional trees, lacking plastic sheets and showing moderate to severe levels of drought, were selected from the close vicinity. The 108 trees (96 + 12) were used to generate a regression analysis between leaf water potential (LWP) and either floral or vegetative activity in the trees.

Just prior to resuming irrigation, soil moisture and leaf water potentials (LWP) were determined. One soil sample per tree was taken at the top 30 cm, midway from the dripping edge, and water content obtained gravimetrically. LWP was measured at predawn with a Scholander pressure bomb using the terminal portion of a nonbearing, nonflushing twig (carrying 3–4 leaves) coming from each of the 108 trees. Midday LWP was measured the same day between noon and 1:00 pm (solar time) on four well-exposed, sunlight twigs per treatment, coming from the terminal flush of growth.

Five weeks after the resumption of irrigation, the floral and vegetative response was assessed in 32 tagged terminal branches (mature resting twigs coming from the last flush of growth) per plot. The proportion of terminal branches producing new flowering shoots, expressed as a percentage, represented the floral activity. A shoot carrying at least one flower was considered a flowering shoot. On the other hand, the percentage of terminal branches producing new vegetative shoots (carrying no flowers at all) represented the vegetative activity.

Additionally, the total number of flowers and shoots per branch (flushing density) were counted. Shoots were defined as: vegetative, generative (carrying only flowers), mixed (carrying both flowers and leaves) and floral shoots (generative plus mixed).

The data were analyzed using factorial ANOVA, and polynomial regressions were generated from the statistical program Costat (version 4.21, 1990, Berkeley, Calif., USA). Overall goodness of fit was described by the model r^2.

3. results and discussion

3.1. drought effects

Different irrigation intervals caused different levels of water stress in the four treatments (*table I*). As soil water content dropped from 19.7 to 10.8%, the predawn LWP fell from –0.49 to –0.81 MPa, and the midday LWP from –1.05 to values as low as –2.10 MPa. Unexpectedly, the average soil water content and plant water potential in the treatment with 4 weeks of no irrigation were lower than those with 8 or even 12 weeks of drought. This may be due to the fact that the plastic covers were first placed on the treatments with 12 and 8 weeks of no irrigation. Soil water may have been depleted by direct evaporation by the time the plastic covers were placed on the 4-week drought treatment.

Vegetative shoot growth in the treatment with continuous irrigation (predawn LWP of –0.49 MPa), either expressed as a percentage or number of shoots per branch, was less than the rest of treatments (*table II*). Floral activity consistently increased with increased drought stress, and the differences became greater as the LWP decreased.

The regression analysis showed a close relationship between the predawn LWP, and the floral or vegetative response of the tree (*figure 1*). The floral activity increased linearly with increased stress (r^2 = 0.67). The vegetative activity increased until a

Table I.
Effect of withholding irrigation cut off on soil water content and leaf water potential (LWP) in Tahiti lime immediately before rewatering.

Weeks without irrigation	Soil water content (% weight)	Leaf water potential (-MPa)	
		Predawn	Midday
0	19.7^{a}	0.49^{a}	1.05^{a}
4	10.8^{c}	0.81^{c}	2.10^{b}
8	13.3^{b}	0.67^{b}	1.40^{a}
12	12.6^{bc}	0.74^{bc}	1.90^{b}

$^{a, b, c}$: within the same columns, groups differ according to Duncan's multiple range test, 5% level.

Table II.
Vegetative and floral activity of Tahiti lime, 5 weeks after rewatering, in relation to the minimum predawn leaf water potentials (LWP) reached (LWP values were rearranged in sequential order from *table I*).

Leaf water potential (-MPa)	Shoot %		Number of shoots per branch			Number of flowers per branch
			Vegetative	Floral		
	Vegetative	Floral		Generative	Mixed	
0.49	44.11^{a}	7.54^{a}	0.67^{a}	0.02^{a}	0.09^{a}	0.44^{a}
0.67	72.67^{b}	10.89^{ab}	1.62^{b}	0.03^{a}	0.18^{a}	0.64^{a}
0.74	64.43^{b}	21.35^{bc}	1.37^{b}	0.06^{a}	0.34^{a}	1.12^{a}
0.81	67.45^{b}	34.81^{c}	1.92^{b}	0.16^{b}	0.71^{b}	2.75^{b}

Generative shoot: shoot carrying only flowers; mixed shoot: shoot carrying both flowers and leaves; floral shoots: generative plus mixed shoots. $^{a, b, c}$: within the same columns, groups differ according to Duncan's multiple range test, 5% level.

threshold LWP of about –1.2 MPa, and then decreased at lower potentials as the number of vegetative shoots decreased in favor of floral shoots, thus showing a curvilinear relationship (r^2 = 0.42). The total number of shoots per branch increased until a similar LWP threshold, and then tended to stabilize. Since the threshold LWP was similar to that for the maximum total vegetative plus floral shoots (*figure 1*), it may represent that at this LWP the branch would have produced the maximum number of total shoots according to its genetic capacity and growing conditions.

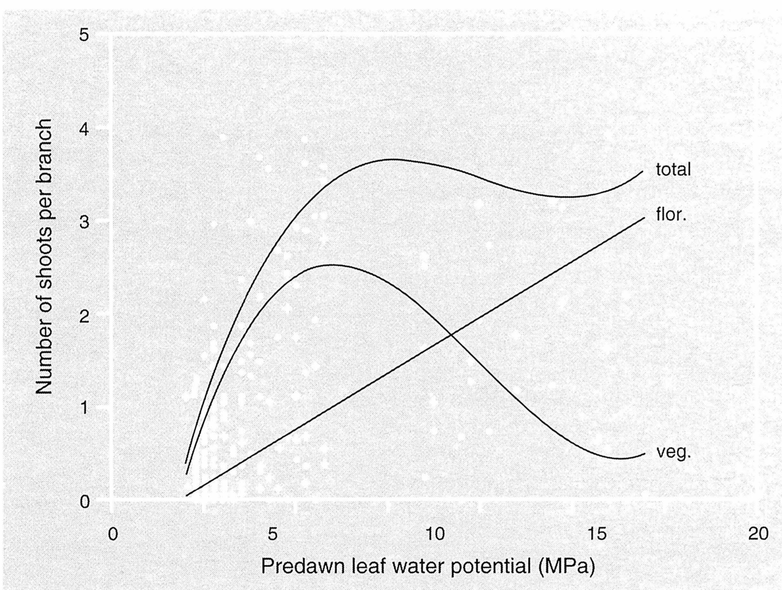

Figure 1.
Vegetative (veg.), floral (flor.) and total activity of Tahiti lime, 5 weeks after rewatering, in relation to the minimum predawn leaf water potential reached. Y(veg.) = –3.16 – 11.01 X – 6.53 X^2 – 1.09 X^3; r^2 = 0.42*** and Y(flor.) = –0.45 – 1.21 X; r^2 = 0.67*** (each equation *n* = 108). (o) and (+) represent vegetative and floral activity, respectively. Total activity line was drawn from direct summation of vegetative and floral lines.

The number of flowers per branch increased in a curvilinear trend (r^2 = 0.76) as drought stress increased (*figure 2*). The drought stress favored the development of both flowering shoots and the number of individual flowers per shoot, and this compounding effect remained even at very low water potentials. Drought stress may reduce the growth of roots that are implicated in hormonal synthesis, thus altering the hormonal balance to favor floral initiation [3, 8], or a limited transport of cytokinins from the roots may occur when transpiration rate is decreased [9].

Vegetative and reproductive growth began about 3 weeks after rewatering and was the highest at the fourth or fifth week, when measurements were completed. Similarly, Cassin et al. [10] examined the flowering response to extended periods of water stress in citrus orchards located in eight tropical countries and observed that the new growth occurred 20 to 28 d following the first effective rain or irrigation. Limes are well adapted to tropical climates and tend to respond better to drought stress periods than oranges, which appear to require an additional condition of rest [9]. Pire et al. [11] found that drought stress in an orange orchard favored reproductive activity in the growth flushes following rewatering but the effect was reversed in the next two flushes of the year, thus rendering no important differences in total yield between drought stressed and well watered trees.

3.2. urea effects

Foliar application of urea failed to improve the floral or vegetative response of the trees as compared to the nonsprayed controls. In contrast to the finding of Lovatt et al. [5], who used urea to increase the ammonia status and flowering of lemon trees, the urea spray in our assay had no significant effect on drought stressed or unstressed trees (*table III*).

In the present study, no analysis was performed to determine whether the single spray of 5% urea actually increased the foliar concentration of ammonia (no leaf burn was observed). Davenport [9] stated that urea sprays at concentrations of 1 to 4% on nonstressed Tahiti lime trees also failed to stimulate production of additional new shoots or flowering. The author suggested that a possible explanation for the correlation observed by Lovatt et al. [5] is that the water stress condition was responsible for floral induction, but the nitrogenous compounds stimulated initiation of the new shoots by providing substrate for protein synthesis. In this experiment, the maximum level of drought stress of -2.1 MPa midday LWP (*table I*) might not be high enough to produce similar results.

Our data indicate that single sprays of 5% urea did not promote floral induction in Tahiti lime, at least, at moderate levels of drought. However, drought stress strongly enhanced the production of both vegetative and floral buds, and as drought stress increased, the number of vegetative shoots decreased in favor of floral shoots.

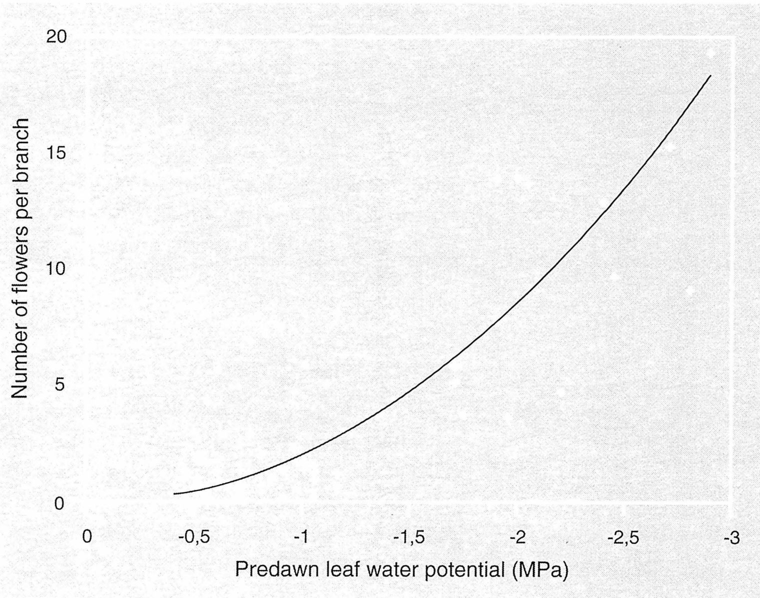

Figure 2.
Flower density of Tahiti lime, 5 weeks after rewatering, in relation to the minimum predawn leaf water potential reached. $Y = 0.14 + 0.24\ X + 2.26\ X^2$; $r^2 = 0.76^{***}$ ($n = 108$).

Table III.
Vegetative and floral activity of Tahiti lime in relation to urea sprays, 5 weeks after rewatering.

Urea spray	Shoot %		Number of shoots per branch			Number of flowers per branch
			Vegetative	Floral		
	Vegetative	Floral		Generative	Mixed	
Control	62.39	16.91	1.49	0.06	0.31	1.32
Urea 5%	62.09	20.28	1.30	0.08	0.35	1.16
Test significance	ns	ns	ns	ns	ns	ns

Generative shoot: shoot carrying only flowers; mixed shoot: shoot carrying both flowers and leaves; floral shoots: generative plus mixed shoots; ns: not significant for main effects or interactions with irrigation treatments, 5% level.

This study also provided useful information on the use of plastic covers to protect from rain and induce drought stress in the field. If covering is placed too soon following rains or irrigations, it will take a long time to get the stress and the beneficial effect may be lost. Growers should be aware that a waiting time with sunny days is needed for the surface soil evaporation to take place.

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Efecto del estrés hídrico y la aspersión de úrea sobre la formación de yemas vegetativas y florales en lima 'Tahiti'.

Resumen – Introducción. Se sometieron plantas de lima 'Tahiti' (*Citrus latifolia* Tan.) cultivadas en clima tropical a diferentes niveles de estrés hídrico y aspersiones foliares de úrea para evaluar su efecto sobre el crecimiento vegetativo y floral. **Material y métodos.** El ensayo comparó cuatro intervalos de riego: riego frecuente (cada 3-4 d) comenzando al final de la época de lluvias con el propósito de mantener el suelo cercano a su capacidad de campo, y riegos aplicados a intervalos de 4, 8, y 12 semanas. Estos tratamientos fueron combinados con las aspersiones de úrea de acuerdo a un arreglo factorial de 4 × 2: un lote de plantas, que constituyó el testigo, no recibió ninguna aspersión, mientras que el otro lote recibió una aspersión de úrea al 5% dos días antes de reiniciar los riegos. **Resultados y discusión**. La producción de brotes florales aumentó a medida que se incrementaba el nivel de sequía pero no fue afectada por el tratamiento de úrea. Se generaron ecuaciones de regresión que mostraron una relación curvilínea entre el crecimiento vegetativo y el potencial hídrico foliar. La formación de brotes florarles y, especialmente, el número de flores por rama mostraron una relación muy estrecha con el estrés hídrico.

Venezuela / *Citrus latifolia* / riego / floración / potencial hídrico / hojas / amoníaco