

INFLUENCE OF NITROGEN, CARBOHY-  
DRATE, AND PLANT GROWTH REGULATORS  
ON FLOWERING, FRUIT SET, AND YIELD OF  
*CITRUS*

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**Abstract:** Flower formation in Citrus species is promoted by drought and low temperature, followed by restoration of climatic conditions favorable for growth. Thus, stress applied in a quantitative manner provides a controlled system during which changes in nitrogen, carbohydrate and plant growth regulators can be monitored in relation to flower initiation. Data on the relationships between nitrogen, carbohydrate and plant growth regulators and floral intensity from experiments employing Citrus species induced to flower by low-temperature or water-deficit stress established a cause and effect relationship between tree ammonia status and floral intensity. During the development of Citrus flowers, putrescine content paralleled ammonia and arginine metabolism; and P-coumaroylputrescine, feruloylputrescine and caffeoylputrescine were present in flowers and leaves during flowering, but not in leaves from mature non-flowering trees. Further, we report results of experiments demonstrating that key nitrogen compounds influencing flower initiation are important in the formation, development, and subsequent set of the ovary. Tissue concentrations of ammonia and putrescine and activity of the de novo arginine biosynthetic pathway were greater in flowers borne in the terminal position of leafy inflorescences than on leafless inflorescences. During the second week after petal fall, fruit born in the terminal position of

leafy inflorescences had significantly greater total polyamine content, faster growth rates, and exhibited a greater percent fruit set than fruit born in the terminal position of leafless inflorescences. Winter applications of foliar urea to commercially-producing, nitrogen-sufficient 'Washington' navel orange trees just prior to or during flower initiation increased yield ( $P \geq 0.05$ ) in three successive years without a reduction in fruit size.

## INTRODUCTION

Citrus growers are not unique among producers of tree crops in desiring to increase profits by increasing yield per tree without reducing fruit size to one of little economic value. However, increasing fruit number while maintaining a marketable fruit size is not a trivial pursuit.

Fruit number can be increased indirectly by increasing floral intensity to augment total set, or directly by improving percent set. In *Citrus* species, only a fraction of the shoot apices flower; others continue the vegetative growth of the tree. Thus, to increase flower number, it is necessary to shift the apical meristem of a vegetative shoot within a resting bud to a floral apex or to prevent the redirection of the floral apex of an inflorescence resting bud to vegetative growth. The shoot apex determines the fate of the lateral meristems, which always show retarded expression with respect to the apex (Lord and Eckard, 1987). If the apex is floral, the laterals will form flowers; if the apex is vegetative, the laterals will be diverted to thorns.

In *Citrus sinensis*, flowers are borne in cymose inflorescences of one to many flowers with zero to many leaves (Lord and Eckard, 1985; Monselise, 1985). Typical inflorescences terminate with a flower and contain ca. 6 nodes with zero to five axillary flowers and zero to five developing leaves (Lovatt *et al.*, 1984; Lord and Eckard, 1987). Shoots of ten nodes terminating in a flower rarely have more than nine flowers or nine leaves under conditions occurring in Riverside, CA, USA (Lovatt *et al.*, 1984) and Mildura, Victoria, Australia (Sauer, 1951). Citrus inflorescences arise by elongation of axillary buds borne principally on 1-year-old wood, although substantial numbers arise on 2-year-old wood. Flowering on older wood is limited (Sauer, 1951).

In our laboratory, we are investigating the hypothesis that endogenous conditions optimal for the promotion of flower formation, which includes formation of the ovary, are also optimal for early ovary growth and, thus, are prerequisite for fruit set and maximum fruit size. Our investigation includes examination of the roles of nitrogen compounds, carbohydrates, and hormones in flower initiation, fruit set, and fruit growth.

In this communication, research on nitrogen, carbohydrate, and plant growth regulators and their role in flowering and fruit set are reviewed. Emphasis is given to the results of our research demonstrating that ammonia and/or its metabolites increase flowering, fruit set, and yield of the 'Washington' navel orange.

## FLOWERING

Under tropical and subtropical conditions, flowering is recurrent due to the tropical phylogenetic background of *Citrus* species (Monselise, 1985), unless synchronized into a well-defined period of concentrated bloom by external environmental conditions (Monselise and Goren, 1969; Goldschmidt and Monselise, 1972; Monselise, 1978). Flower formation in *Citrus* species is promoted by drought or low temperature, followed by restoration of climatic conditions favorable to growth (Monselise and Halevy, 1964; Monselise and Goren, 1969; Monselise, 1978, 1985; Southwick and Davenport, 1986; Lovatt *et al.*, 1988a,b). The action of these abiotic factors, together with that of cultural practices that promote flowering in *Citrus* species, i.e., girdling (Monselise, 1985), graft incompatibilities resulting in weak rootstocks (Mosse, 1962), confining of root systems in small pots (Furr *et al.*, 1947), and root pruning (Monselise and Halevy, 1964; Monselise, 1985), support the idea that the cessation of root growth is an essential prerequisite to flowering in *Citrus* as first proposed by Monselise (1947). Subsequent research employing growth regulators and antimetabolites has established the inhibiting effects of gibberellins (GA) on

flowering in *Citrus* species (Monselise and Halevy, 1964; Moss, 1970; Nir *et al.*, 1972; Goldschmidt and Monselise, 1972; Monselise, 1978; Guardiola *et al.*, 1977, 1982; Garcia-Luis *et al.*, 1986; Lord and Eckard, 1987). On this ground, Goldschmidt and Monselise (1972) suggested that from their inception in the adult phase, all *Citrus* buds are determined to flower, but the presence of GA may inhibit their flowering. This hypothesis is supported by the fact that compounds inhibiting plant growth and those reducing GA biosynthesis promote flowering (Monselise *et al.*, 1966; Nir *et al.*, 1972). In addition, a link between the abiotic factors promoting flowering in *Citrus* species and reduced GA content was established by work of Nir *et al.* (1972) demonstrating that GA<sub>3</sub> inhibits drought-induced flowering in 'Eureka' lemons, while the growth retardant Cycocel (trimethylammonium chloride) can replace the water-stress treatment and promote flowering in lemons. Extrapolation led to the idea that inhibition of root activity results in a reduction of the synthesis or transport of GA (Monselise, 1978, 1985). However, since antimetabolites of nucleic acid and protein synthesis such as chloramphenicol succinate, 5-fluorodeoxyuridine, and bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) enhance flowering in *Citrus sinensis* (Goren and Monselise, 1969; Jona *et al.*, 1971), the role of drought, low temperatures, and inhibitors of GA biosynthesis in promoting flowering may be due to their more general effect on overall growth inhibition (Iwasaki *et al.*, 1959). This would cause a temporary inhibition of mitosis followed by rapid division conducive to flower bud formation (Bernier *et al.*, 1970; Stebbins, 1965) once the inhibiting factor has been removed, as suggested by Goren in 1978 (Monselise, 1985). Thus, stress applied in a quantitative manner provides a controlled system during which changes in tree carbohydrate status, nitrogen metabolism, or plant growth regulator levels can be monitored in relation to flower initiation.

Our research employed: (1) low-temperature stress to induce flowering in 5-year-old rooted cuttings of the 'Washington' navel orange (*Citrus sinensis* L. Osbeck); (2) water-deficit stress to induce flowering in commercially grown 16-year-old 'Frost Lisbon' lemon (*Citrus limon* Burm. f.) trees on Troyer citrange rootstock (*Citrus sinensis* x *Poncirus trifoliata*); and (3) 30-year-old 'Washington' navel orange trees on Troyer citrange rootstock which flowered in spring in response to low temperatures experienced during the preceding winter.

Five-year-old rooted cuttings of the 'Washington' navel, orange induced to flower by low-temperature stress, were subjected to an 8-h day ( $500 \mu\text{m}^2/\text{sec}$ ) at 15 to 18°C, 16-h night at 10 to 13°C. After 4, 6, or 8 weeks of low-temperature treatment, the trees were transferred to 12-h day ( $500 \mu\text{m}^2/\text{sec}$ ) at 24°C, 12-h night at 19°C. Maximum flower number, or peak bloom, occurred 4 weeks after the trees were transferred to the warm temperature (Moss, 1969). Control trees maintained at the warm temperature throughout the experiment did not flower. Low-temperature stress did not cause water-deficit stress. The average predawn water potential of the trees during the 8 weeks of low-temperature treatment was the same as for the warm temperature control trees:  $-0.7 \pm 0.2$  MPa (Lovatt *et al.*, 1988a).

## RESULTS

### CARBOHYDRATE

- 1) Leaf levels of carbohydrate (starch and glucose) did not change during low-temperature stress.
- 2) Tree starch content had a significant effect on the number of floral shoots the tree produced.
- 3) Interestingly, tree starch content did not have an effect on the number of vegetative shoots the tree produced. This is a significant finding. It says that starch is not simply serving as an energy source to increase budbreak, but is actually influencing floral shoot development.

**Table 1.** Linear regression analyses of stress, starch, and flowering in *Citrus*.

	Independent variable	Dependent variable	Probability	Coefficient of linear correlation
	x	y	p	r
Stress				
Low temperature*	Duration	Starch of stress	NS**	-0.186
	Starch	Floral shoots	$P \leq 0.0001$	0.750
	Starch	Vegetative shoots per tree	NS**	0.212

\* Starch (mg/g fr wt) content of leaf tissue collected at the end of the first week in the warmer temperature after completion of the low-temperature treatment.

\*\* Not significant at  $P \leq 0.10$ .

From: Lovatt *et al.*, 1988b.

## NITROGEN

- 1) Neither leaf total nitrogen nor nitrate content changed during or after the low-temperature stress treatment.
- 2) There was a significant increase in leaf  $\text{NH}_3\text{-NH}_4^+$  content during low-temperature stress.
- 3)  $\text{NH}_3\text{-NH}_4^+$  accumulated in a manner that paralleled the duration of the stress.
- 4) The amount of  $\text{NH}_3\text{-NH}_4^+$  that accumulated was directly proportional to the number of flowers produced by the tree.
- 5) Tree  $\text{NH}_3\text{-NH}_4^+$  status had no effect on the number of vegetative shoots the tree produced.

Table 2. Linear regression analyses of stress,  $\text{NH}_3\text{-NH}_4^+$ , and flowering in *Citrus*.

	Independent variable	Dependent variable	Probability	Coefficient of linear correlation
Stress	x	y	p	r
Low temperature*	Duration of stress	NH - NH	$P < 0.001$	0.605
	NH - NH	Flowers per tree	$P < 0.0001$	0.803
	NH - NH	Floral shoots per tree	$P < 0.01$	0.413
	NH - NH	Vegetative shoots per tree	NS**	0.077

\*  $\text{NH}_3\text{-NH}_4^+$  ( $\mu\text{g/g}$  dry wt) content of leaf tissue collected at the end of the first week in the warmer temperature after completion of the low-temperature treatment.

\*\* Not significant at  $P \leq 0.10$ .

From: Lovatt *et al.*, 1988b.

## CAUSE AND EFFECT: THE USE OF FOLIAR UREA

To convince ourselves that there was a cause and effect relationship between leaf  $\text{NH}_3\text{-NH}_4^+$  content and flower initiation and floral intensity, we subjected trees to only 4 or 6 weeks of low-temperature stress and artificially raised  $\text{NH}_3\text{-NH}_4^+$  status of the trees by foliar application of low-biuret urea at the rate of 1.5 g/tree. For trees receiving only 4 or 6 weeks of low-temperature stress, foliar application of low-biuret urea increased the leaf  $\text{NH}_3\text{-NH}_4^+$  content 166 and 215% of the values obtained for trees not receiving foliar urea and resulted in a concomitant and equal increase in flower number per tree of 194 and 230% of the value obtained for trees not receiving foliar urea, respectively.

**Table 3.** Effect of foliar urea (1.5 g per tree) applied at the end of the low-temperature treatment on leaf  $\text{NH}_3\text{-NH}_4^+$  content and flowering of the 'Washington' navel orange.

Duration of low temperature stress (weeks)	Increase in leaf $\text{NH}_3\text{-NH}_4^+$ content during the first week after transfer to the warm temperature as a percent of the control without urea for each treatment	Increase in flower number as a percent of the control without urea for each treatment
4	166 %	194 %
6	215 %	230 %

From: Lovatt et al., 1988a.

### $\text{NH}_3\text{-NH}_4^+$ THRESHOLD FOR FLOWERING

For the more than 100 trees used in the many experiments conducted, including trees subjected to 2, 4, 6, or 8 weeks of low-temperature stress and trees treated with a foliar application of low-biuret urea, leaf  $\text{NH}_3\text{-NH}_4^+$  content ranged from 389 to 2639  $\mu\text{g/g}$  dry wt leaf tissue. The corresponding number of flowers for each tree was 4 to 3065. These results suggest that there is a threshold level of  $\text{NH}_3\text{-NH}_4^+$  below which flowering will not take place in citrus. That value is approximately 400  $\mu\text{g/g}$  dry wt leaf tissue (Lovatt et al., 1988b).

### WATER-DEFICIT STRESS-INDUCED FLOWERING IN LEMON

Water-deficit stress was also used to induce flowering in 16-year-old 'Frost Lisbon' lemon trees on Troyer citrange rootstock under commercial production in the San Joaquin Valley of California.

Trees were subjected to water-deficit stress during July and August and rewatered at the end of August to give maximum bloom by the end of September to produce a summer crop of *verdelli* lemons in addition to the winter crop harvested from the spring bloom. The summer lemon crop has 5 to 10 times the market value of the winter lemon crop.



## RESULTS

- 1) Leaf carbohydrate content (glucose and starch) did not change during water-deficit stress.
- 2) Leaf carbohydrate content was not related to either floral or vegetative shoot development.
- 3)  $\text{NH}_3\text{-NH}_4^+$  accumulated during water-deficit stress.
- 4) The amount of  $\text{NH}_3\text{-NH}_4^+$  that accumulated increased with the severity and duration of the water-deficit stress.

Table 4. Effect of water-deficit stress and foliar urea on leaf  $\text{NH}_3\text{-NH}_4^+$  content and flowering of lemon.\*

Treatment	Number of flowers per tree	Average leaf $\text{NH}_3\text{-NH}_4^+$ content during stress (g/g dry wt)
Control-no water stress (>-1MPa)**	14 b	519 c
Severe water stress of short duration (-3 MPa in 30 days)	53 b	646 b
Severe water stress of short duration (-3 MPa in 20 days) followed by moderate water stress for 40 days (-2 MPa)		
Moderate water stress (-2MPa for 50 days)	165 b	683 b
Moderate water stress (-2 MPa for 50 days) with foliar urea (0.1 kg of nitrogen per tree)	426 a	863 a

\* Values within a column followed by a different letter are significantly different at  $P \leq 0.05$  by Duncan's Multiple Range Test.

\*\* 1 MPa is equal to 10 bars.

From: Lovatt et al., 1988a.

## CAUSE AND EFFECT: THE USE OF FOLIAR UREA

One set of lemon trees was subjected to moderate water stress. The trees were maintained at  $-2$  MPa for 50 days by deficit irrigation. Half of the trees received a foliar application of low biuret at the rate of 0.1 kg N as urea per tree. Foliar application of low-biuret urea raised the leaf  $\text{NH}_3\text{-NH}_4^+$  content of the trees to  $863 \mu\text{g/g}$  dry wt and increased the number of flowers per tree 2.6-fold over trees not receiving a foliar application of urea. This degree of floral intensity was not significantly different at the 5% level from the number of flowers produced by trees receiving the maximum stress. For lemon trees induced to flower by water-deficit stress, the average  $\text{NH}_3\text{-NH}_4^+$  content of the leaves during the stress was significantly correlated with the number of flowers per tree ( $P_{.05}$ ;  $r = 0.56$ ).

## IS IT TIME TO REVISE THE KRAUS-KRAYBILL HYPOTHESIS?

The potential regulation of flower initiation by the balance of carbohydrate to nitrogen or by a precise mathematical ratio of carbohydrate/nitrogen is recognized internationally as the Kraus-Kraybill hypothesis.

Consistent with the broadest interpretation of the Kraus-Kraybill Hypothesis, the results of our research provide evidence that carbohydrate, specifically starch, and nitrogen, specifically  $\text{NH}_3\text{-NH}_4^+$ , do influence the number of floral shoots and total number of flowers initiated. It has been suggested previously that carbohydrate levels are a factor limiting flower formation in *Citrus* (Ogaki *et al.*, 1963; Goldschmidt and Golomb, 1982; Goldschmidt *et al.*, 1985), but a role for tree carbohydrate status in *Citrus* flowering remains equivocal (Davenport, 1990). We obtained equivocal results in our experiments: starch level was related to low temperature-induced flowering in the navel orange, but not to water-deficit-stress-induced flowering in the lemon. Our data do not support a role for a carbohydrate-to-nitrogen ratio in flower initiation and demonstrate that only  $\text{NH}_3\text{-NH}_4^+$  consistently influenced flower number, while other metabolic species did not. Glucose, total N, and  $\text{NO}_3^-$  did not influence flower initiation.

## PLANT GROWTH REGULATORS INCLUDING POLYAMINES AND PHENOLIC AMIDES

While Southwick and Davenport (1986) demonstrated that the degree of flowering in *Citrus latifolia* Tan. also was directly proportional to the severity or duration of water-deficit or low-temperature stress. They found no correlation between the endogenous level of any of several plant growth regulators and flower number (Davenport, 1990). Davenport (1990) concluded in his thorough review that at present there are no specific candidates for a chemical inducer of flowering in *Citrus*.

The aliphatic free di- and polyamines putrescine, spermidine and spermine have been designated as plant growth regulator substances (Bagni *et al.*, 1982) and phenolic amides have been correlated with the flowering process in a number of annual plant species (Martin-Tanguy *et al.*, 1982; Martin-Tanguy, 1985). Consistent with the potential role of these compounds in the regulation of flowering in *Citrus*, we demonstrated that p-coumaroylputrescine, feruloylputrescine and caffeoylputrescine were present in flowers and leaves during flowering of the 'Washington' navel orange and 'Frost' Lisbon lemon, but not in leaves of mature non-flowering trees or either cultivar. The three phenolic amides were highest in flower buds and decreased during development through petal fall, a developmental pattern identical with that we (Sagee and Lovatt, 1991) previously reported for flower  $\text{NH}_3\text{-NH}_4^+$  content and putrescine synthesis via arginine. p-Coumaroylputrescine was the dominant phenolic amide in flowers and leaves from flowering trees. In contrast, putrescine, spermidine and spermine and the catecholamine dopamine and its precursors, phenylethylamine and tyramine, were present in leaves of both non-flowering and flowering trees.

## FRUIT SET

In *Citrus*, it is well known that leafy inflorescences (shoots bearing leaves and flowers) set more fruit than leafless inflorescences (for a recent review see Erner, 1989). In addition, young fruit that are faster-growing have a greater potential to set and survive to harvest than slower-growing fruit (Zucconi *et al.*, 1978). This provides populations of fruit with very distinct capacities for fruit set, which can be readily identified and collected in the field for biochemical studies.

Most studies on fruit set have emphasized changes in content of growth-promoting versus growth-inhibiting plant growth regulators and carbohydrate allocation.

Erner (1989) in his thorough review of fruit set in *Citrus* provided evidence negating the popular concept that fruit borne on leafy inflorescences import more photosynthate and have higher levels of sugar and/or starch.

No differences in concentrations of gibberellic acid (GA) or abscisic acid (ABA) (ng/g dry wt) were observed for 'Valencia' fruit borne on leafy versus leafless inflorescences but because fruit borne on leafy inflorescences were larger in mass than fruit of the same age borne on leafless inflorescences, fruit on leafy inflorescences had more GA and ABA per fruit. High temperatures 40 to 55 days after full bloom of 'Valencia' were associated with a large decrease in GA concentration in the fruit, but there was a lesser effect on ABA (Hofman, 1986). Thus, a shift towards a greater ratio of growth inhibitor to growth promoter occurred during the stress. The increased ratio of growth inhibitor to growth promoter may be the cause of increased abscission. Abscission would be greater for fruit borne on leafless inflorescences because these fruit had less GA than fruit of leafy inflorescences even before the stress.

Erner (1989) proposed that fruit borne in leafy inflorescences have a greater ability to withdraw water from the transpiration stream than those borne on leafless inflorescences. Since the transpiration stream (xylem sap) of citrus contains high levels of cytokinins, fruit borne on leafy inflorescences would be expected to have a higher ratio of growth-promoting PGR to growth-inhibiting PGR than fruit borne on leafless inflorescences. In addition, it would be expected that fruit borne on leafless inflorescences would be more susceptible to water-deficit stress. Thus, fruit borne on leafless inflorescences would be expected to accumulate more ABA than fruit borne on leafy inflorescences. Since fruit of leafless inflorescences have lower levels of growth-promoting cytokinins, the ratio would be shifted significantly toward growth inhibition and fruit abscission.

The hypotheses of both Hofman (1988) and Erner (1989) are consistent with previous research reported in earlier literature. Exogenous application of ABA to citrus fruit demonstrated that this PGR causes fruit abscission (Cooper and Henry, 1973). In addition, exogenous application of GA alone or with calcium dihydrophosphate or 6-benzylamino purine as whole-tree sprays after full bloom or at petal fall increased fruit set. The effect on yield was variable from year to year. Calcium was effective even though trees had "sufficient" calcium for normal tree growth (Southwick and Davies, 1982).

Since we demonstrated that flower initiation is influenced by  $\text{NH}_3\text{-NH}_4^+$ , our research examined the metabolism of key nitrogen compounds synthesized from  $\text{NH}_3\text{-NH}_4^+$  as possible factors influencing ovary development of the 'Washington' navel orange.

## POLYAMINE CONTENT OF CITRUS FLOWERS

Early in their development, apical flowers borne on leafy inflorescences had higher tissue levels of polyamines (Table 5) and  $\text{NH}_3\text{-NH}_4^+$  and higher rates of *de novo* arginine biosynthesis (data not shown) than apical flowers borne on leafless inflorescences.

**Table 5.** Polyamine content of apical flowers of leafy versus leafless inflorescences\*.

	Leafy	Leafless
mg/flower		
Flower fresh weight	99 a	74 b
nmol/g fresh weight		
Putrescine	472	231
Spermidine	170	149
Spermine	31	19
	673 a	401 b
mg/g fresh weight		
Flower protein content	19 a	12 a
nmol/mg protein		
Putrescine	26	14
Spermidine	9	8
Spermine	1	1
	36 a	23 b

\*Data are the mean of four replicates from two separate experiments inducing flowering by low-temperature stress in 5-year-old rooted cuttings of the 'Washington' navel orange and for four replicates from spring bloom of 30-year-old 'Washington' navel orange trees under commercial production. Mean separation was by Duncan's Multiple Range Test. Values within a horizontal row followed by different letters are significantly different at  $P \leq 0.05$ .

From: Lovatt *et al.*, 1992

### POLYAMINE CONTENT OF FRUIT WITH DIFFERENT POTENTIALS TO SET

Navel orange fruit exhibiting faster growth rates during the first weeks after petal fall were borne on leafy inflorescences and had higher levels of polyamines than slower-growing fruit borne on leafless inflorescences (Table 6).

**Table 6.** Characteristics of fruit borne on leafy versus leafless inflorescences during thesecond week after petal fall.<sup>z</sup>

	Leafy	Leafless
mm/day		
Fruit growth rate	0.130 a	0.067 b
mg/fruit		
Fruit fresh weight	382 a	259 b
mg/g fresh weight		
Fruit protein content	6.4 a	6.8 a
nmol/g fresh weight		
Putrescine	388	186
Spermidine	132	81
Spermine	61	40
	581 a	307 b
nmol/g/protein		
Putrescine	62	32
Spermidine	21	15
Spermine	10	6
	93 a	53 b
nmol/organ		
Putrescine	144	55
Spermidine	49	22
Spermine	23	12
	216 a	89 b

\* Data are the mean of four replicates from two separate experiments inducing flowering by low-temperature stress in 5-year-old rooted cuttings of the 'Washington' navel orange and of four replicates from spring bloom of 30-year-old 'Washington' navel orange trees under commercial production. Mean separation was by Duncan's Multiple Range Test. Values within a horizontal row followed by different letters are significantly different at P<0.05.

From: Lovatt *et al.*, 1992

## YIELD

We tested our hypothesis that key nitrogen compounds that influence flower initiation may be important in the formation, development, and subsequent set of the ovary in a field experiment employing 30-year-old 'Washington' navel orange trees on Troyer citrange rootstock to determine whether a winter foliar-application of low biuret urea at the rate of 0.17 kg per tree prior to or during the normal period of floral initiation preceding spring bloom of *Citrus* in California would increase floral intensity and result in increased fruit set and yield.

The trees employed in this study have a history of average fruit yields ranging from 500 to 700 fruit per tree in an "off" year to 1,300 fruit per tree in an "on" year. These yields are equal to the state average. During the first year of our study, the trees yielded close to the average for Southern California. The 1989-90 season shipments of navel oranges from Southern California averaged 655 cartons per tree (Source: Navel Orange Administrative Committee). The control trees in our experiment, i.e. those under standard commercial management and receiving soil-applied urea, averaged 689 cartons per acre for the 1989-90 season. All trees were nitrogen-sufficient according to September leaf analyses conducted at the start of the experiment.

In year one, all trees receiving a winter foliar application of low biuret urea had significantly higher yield per tree than trees receiving soil-applied urea ( $P \leq 0.05$ ) (Table 7). The increase in yield was just over one packing carton (17 kg) per tree.

The results of the second year of our research were compromised by the freeze which occurred in December, 1990. Yield of the control trees, i.e. those under standard commercial management and receiving only soil-applied urea, was reduced 50%: compare 333 cartons per acre in 1990-91 to 689 cartons for 1989-90. In addition, this level of production was 25% lower than the average for Southern California for 1990-91 (Source: Navel Orange Administrative Committee).



For 1990-91, there was a statistically significant effect due to the time of foliar urea application ( $P \leq 0.10$ ). Trees receiving foliar applications of urea in January or February had greater yield than the control trees ( $P \leq 0.05$ ) (Table 7). Trees receiving a foliar application of urea in January or February averaged 29 and 26 kg more fruit per tree, respectively, than trees receiving soil-applied urea.

For the year following the freeze, 1991-92, all trees receiving a winter foliar application of low biuret urea had significantly higher yield per tree than trees receiving soil-applied urea ( $P \leq 0.002$ ), approximately three packing cartons per tree (Table 7).

**Table 7.** Effect of a January or February foliar application of low biuret urea on yield of the 'Washington' navel orange.

Kg fruit/tree				
date urea applied to the foliage				
Year	Control (none)	January	February	Significance level*
1	109 a	134 b	131 b	$P < 0.05$
2	49 a	78 b	75 b	$P < 0.05$
3	131 a	189 b	182 b	$P < 0.002$

\*Data are the mean of 12 individual tree replicates per treatment. Mean separation was by Duncan's Multiple Range Test.  
From: Lovatt *et al.*, 1992

For all three years, the increase in yield resulting from the winter foliar application of low biuret urea did not change the number of large or medium-sized fruit that were harvested, i.e. fruit with diameters from 7.0 to 8.0 cm (box sizes 88 and 72) or from 6.1 to 6.9 cm (box sizes 138 and 113), respectively ( $P \leq 0.05$ ).

The increase in yield resulting from the winter foliar application of low biuret urea was not a result of improved nitrogen status of the trees. At the end of the three-year experiment, leaf total nitrogen content of the 1991 spring flush leaves collected in September was not significantly different at the 5% level for control trees receiving soil-applied urea versus trees receiving a foliar application of low biuret urea. Leaf total nitrogen content was between 2.5 to 2.6 percent.

## CONCLUSION

Results of our basic research demonstrated (i) that stress-induced flowering was causally related to tree ammonia status; (ii) that accumulated ammonia was metabolically linked to the synthesis of polyamines via arginine in developing flowers; and (iii) that fruit with a greater potential to set had significantly higher levels of putrescine + spermidine + spermine. Results of our field experiment demonstrated that a winter application of low biuret urea in January or February significantly increased yield by more than one packing carton (17 kg of fruit) per tree for three consecutive years, without reducing fruit size or significantly increasing tree total nitrogen status compared to control trees receiving a winter soil application of urea.

The results are consistent with and strongly support the interpretation that application of urea to the foliage of the 'Washington' navel orange provides sufficient ammonia to accelerate *de novo* arginine biosynthesis and lead to an increase in one or more species of polyamine that promotes flower initiation and ovary growth by cell division resulting in increased fruit set and yield. Results of experiments with apple provide additional support for this proposed sequence of metabolic events. Ammonia, arginine, and putrescine have all been shown to increase flowering in apple (Edwards, 1986), and exogenous application of putrescine to apple trees during early bloom increased fruit growth during the cell division phase with a concomitant increase in fruit set and yield (Costa *et al.*, 1986). Taken together, these results suggest practical approaches for improving yield that are economically viable and environmentally safe in the absence of clear roles for carbohydrate and plant growth regulators in enhancing flowering and fruit set in *Citrus*.

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