15

77

BARD

FINAL REPORT

PROJECT NO. I-867-84

**Leafy Inflorescence Key to Improve Fruit Set
and Yield of the 'Shamouti' and 'Washington'
Navel Orange Varieties**

Y. Erner, C. J. Lovatt, C. W. Coggins, Jr.

1989

31

630.72

BAR/ERN

2nd copy

32070

000000133654

BARD

P.O. Box 6

Bet-Dagan, Israel.

Bard Project No. I-867-84

Title of Research Project: Leafy inflorescence: key to improve fruit set and yield of the 'Shamouti' and 'Washington' navel orange varieties.

Names of Investigators:

Principal Investigator: Yair Erner

cooperating Investigator(s): Carol J. Lovatt and C.W. Coggins, Jr.

Name and Address of Affiliated Institutions:

Principal Institution: ARO. The Volcani Center Bet-Dagan 50-250 Israel

Cooperating Institution(s): University of California Riverside CA 92521 U.S.A.

Project starting date: October 1985

Final Report

הספדיה המרכזית
למחקר ומקורות
בית-דגן

Signatures:

Principal Investigator

Institution's Authorizing Official

Yuval Eshdat

Dr. YUVAL ESHDAT
Head, Div. for Research Projects and Budgets
Agricultural Research Organization

630.72 ! 634.31 : 581.497 : 581.13
BAR/ERN
2nd copy

**LEAFY INFLORESCENCE: KEY TO IMPROVE FRUIT SET AND YIELD OF THE
'SHAMOUTI' AND 'WASHINGTON' NAVEL ORANGE VARIETIES.**

Y. Erner., C. J. Lovatt, and C. W. Coggins, Jr.

TABLE OF CONTENTS

	PAGE
TITLE	2
ABSTRACT	4
ORIGINAL OBJECTIVES	8
RESULTS	11
PUBLICATION	52
BENEFITS TO AGRICULTURE	53

FINAL REPORT TO BARD
FOR GRANT # I-867-84

Y. Erner, C.J. Lovatt, and C.W. Coggins, Jr.

ABSTRACT

Research was undertaken to improve fruit set and yield in the commercial production of the shy-bearing varieties, 'Shamouti' and 'Washington' navel orange. We proposed that an understanding of how leafy inflorescences were initiated on the tree, and why leafy inflorescences set more fruit that survive to harvest was the key to improving fruit set and yield in these varieties. The roles of hormones, carbohydrate availability, and nitrogen metabolism in shifting leafless (LS) to leafy (LY) inflorescences was examined. Transpirational flux, carbohydrate status, and hormone levels were compared in LS and LY inflorescences to determine which contributed to the preferential preformance of leafy inflorescences.

In the preferential set on (LY) over (LS) inflorescences was studied in order to evaluate the roles of carbohydrate, minerals and bioregulators. A $^{14}\text{CO}_2$ puls was used as a marker of sugar translocation from either a one-year-old leaf, or a young current leaf on 30 year-old 'Shamouti' orange trees. Total nonlabelled sugars and starch levels together with the labelled material, gave a better insight of the carbohydrate involvement in fruit set. It was found that fruit set was favored on LY while abortion of reproductive organs was nearly total on LS. Our data indicated that there was no deficiency of carbohydrate in fruitlets borne on LS. Moreover,irrespective of fruitlet

position on the branch, fruitlet borne on LS accumulated more ^{14}C -labelled photosynthate than fruitlets borne on LY. The total soluble sugars, and starch were found to be higher in LS than in LY fruitlets. Whether borne on LS or LY, the fruitlet was a strong metabolic sink and could compete with other plant parts. Presence vegetative growth increased fruit set on both LS and LY, although it is a competition for assimilates. We concluded that fruitlets borne either on LS or LY had the growth potential to survive but other factor(s) than carbohydrate might cause differential abscission of the fruitlets borne on LS.

Macroelement (N,P,K) measurements failed to correlate with the differential set on LY over LS. Although small, significant difference was found in phosphorus content in fruitlets one month after anthesis, it can hardly account for the better set.

Exogenous application of growth regulators in substitution for young leaves of the LY as well as in correlation with the endogenous content of the hormones, indicate that GA can enhance fruit set only when applied to the ovary. No differences were been found in the endogenous GA content of the ovaries and fruitlet until 63 days after flower opening. A significantly higher amount of abscissic acid (ABA) was found in fruitlets borne on LS. The higher ABA level might be associated with early metabolic events leading to fruit drop. The turnover of water in fruitlets adjacent to young leaves (LY) was found to be greater than that in LS adjacent to one-year-old leaf. It is suggested that a non-detectable, transient water stress during midday can cause ABA build up which could lead to abscission.

Exogenous application of GA+BA shifted the spring flush type towards the vegetative growth with a higher ratio of LY to LS. The increase of LY might increase fruit number per tree and thus contribute to the achieving of the overall goal of this project. Further experiments are needed in order to bring

these first important data to the level of a recommendation for commercial use by the farmer.

The clear cut data about carbohydrate translocation, the competition for reserves, the accumulation of ABA in LS and the possible manipulation of the inflorescence type bring the citrus researcher to a better understanding of the physiology of fruit set.

Rooted cuttings of navel orange were used in the study of flower induction as response to water and low temperature stress. Changes in photosynthesis and leaf concentrations of glucose, starch, NO_3 and ammonia (as combined pool of $\text{NH}_3\text{-NH}_4^+$), were monitored prior to, during and after the stress treatment.

The results provide strong evidence that floral intensity is depended on both starch and ammonia content, which are easily monitored in leaves. Leaves and floral buds undergo parallel changes in nitrogen metabolism in response to low temperature stress-induced flowering and provide evidence that flower $\text{NH}_3\text{-NH}_4^+$ content and putrescine synthesis via arginine are metabolically correlated during flower development in *C. sinensis*.

The research also investigated the influence of flower position and inflorescence type [leafy (inflorescences with more than one leaf per flower) versus leafless (inflorescences with no leaves or less than one leaf per flower)] on the metabolism of these key nitrogen compounds. During early flower development, terminal flowers had higher levels of $\text{NH}_3\text{-NH}_4^+$ than subterminal flowers ($p < 0.05$). For each flower type, the flower borne on the leafy inflorescence had the higher $\text{NH}_3\text{-NH}_4^+$ content ($p < 0.05$). One week after petal fall, these differences were insignificant at the 5% level. De novo arginine biosynthesis followed this same pattern ($p < 0.05$), except one week after petal fall, there was an increase in the novo arginine biosynthesis in the fruit,

which resulted in a marked increase in the putrescine content of the fruit, with the highest level of putrescine found in flowers borne on leafy inflorescences.

Fruit on leafy inflorescences exhibited the greater concentration of total polyamines. This was consistent with the fact that fruit borne on leafy inflorescences exhibit faster growth rates and a greater potential to set and survive to harvest and with the fact that polyamines promote cell division and growth of ovaries.

The results suggest that foliar applied low biuret urea would increase the $\text{NH}_3\text{-NH}_4^+$ content of developing ovaries, increase their polyamine content, and improve fruit set in the 'Washington' navel orange.

Last, low biuret urea applied foliary was demonstrated to increase the number of leaves per inflorescence and the proportion of leafy inflorescences per tree.

Transpirational flux was investigated in containerized *Citrus sinensis* (Osbeck) cv. 'Washington' navel and 'Valencia' trees exposed to Hydrogen-3 and/or Sulfur-35 via the irrigation solution. The containers were weighed at regular intervals to determine water loss. Samples from various aerial organs were collected at the same intervals and their isotopic content determined.

The accumulation of both ^3H and ^{35}S in aerial organs was highly correlated to weight loss from the containers. The rate of ^3H accumulation in petals of leafy inflorescences was greater than in the petals of leafless inflorescences. The content of both ^3H and ^{35}S was greater in ovaries of leafy inflorescences when compared to those borne on leafless inflorescences after 48 hours from introduction of the isotope. There was no difference in isotope flux to inflorescence leaves when compared to leaves developing in the absence of flowers.

Flowers from field grown 'Valencia' orange trees were collected at petal fall. The ovaries were removed, dried and weighed. The dried ovaries were then assayed for their content of N,P,K,Ca,Mg,Zn,Mn,Fe,B and S. The dry weight of ovaries from leafy inflorescences was greater than those from leafless inflorescences. Thus, the concentration of N,K and Fe was higher in ovaries from leafless when compared to leafy inflorescences. However, the total content of N,P,Mg and Zn was higher in ovaries from leafy inflorescences when compared to leafless inflorescences.

ORIGINAL OBJECTIVES

With fruit crops, the loss in potential yield due to the abscission of flowers and immature fruit that fail to set and are abscised during their early developments is well documented. This situation represents a considerable loss of energy and biomass to the tree and is of great concern to citrus growers, especially those involved in the commercial production of shy-bearers, i.e., the 'Shamouti' and 'Washington' navel orange.

Our overall objective was to improve fruit set and yield of the 'Shamouti' and 'Washington' navel orange varieties. "Leafy" inflorescences (shoot bearing leaves and flowers) have been shown to set more fruit than their "leafless" counterparts (bearing only flowers) in a number of citrus varieties, including the 'Shamouti' and 'Washington' navel orange. We believe that an understanding of how leafy inflorescences are initiated on the tree and why leafy inflorescences set more fruit that survive to harvest is the key to improving fruit set and yield.

Thus, our specific research objectives were to (1) determine why leafy inflorescences of shy bearing cultivars of *Citrus sinensis* L. Osbeck, 'Shamouti', and 'Washington' navel oranges set more fruit that survive to harvest than their leafless counterparts, and (2) to identify the physiological event(s) that increase the proportion of leafy inflorescences that develop during bloom.

The results obtained from the investigation of these two objectives will provide the basis for cultural practices to increase the number of leafy inflorescences on a tree and to cause leafless inflorescences to mimic leafy ones in order to improve fruit set and yield in these citrus varieties and others.

Objective 1: Determination of the Physiological Reason that Leafy Inflorescences Set More Fruit That Survive to Harvest Than Their Leafless Counterparts.

(1) We investigated the hypothesis that leafy inflorescences set more fruit that survive to harvest because they have a greater transpirational flux which improves not only their water status, but also their mineral nutrition and hormone balance.

This research was conducted in the laboratory of Dr. Charles W. Coggins, Jr., Department of Botany and Plant Sciences, Citrus Research Center and Agricultural Experiment Station, University of California, Riverside, CA 92521, USA.

(2) We investigated the hypothesis that intra-inflorescence differences contribute to the high yield potential of leafy inflorescences. Intra-inflorescence differences in endogenous growth regulator, and capacities to fix $^{14}\text{CO}_2$ and import ^{14}C -photosynthate were quantified. In addition,

competition between vegetative shoots, leafy and leafless inflorescences was studied by selective removal of competing sinks.

This research was conducted in the laboratory of Dr. Yair Erner, Department of Citriculture, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.

Objective 2: Identify the Physiological Event(s) That Increase the Proportion of Leafy Inflorescences That Develop During Bloom.

Flowering was induced by low temperature and water-deficit stress applied in a quantitative manner to provide a controlled system during which changes in carbohydrate and nitrogen metabolism and hormone content were monitored in relation to floral shoot initiation, leaf to flower ratio, and floral intensity.

This research was conducted in the laboratory of Dr. Carol J. Lovatt, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521, USA.

RESULTS

Objective 1: Determination of the Physiological Reason That Leafy Inflorescences Set More Fruit That Survive to Harvest Than Their Leafless Counterparts.

Hypothesis 1: Leafy inflorescences set more fruit that survive to harvest because they have a greater transpiration flux which improves not only their water status, but also their mineral nutrition and hormone status.

Containerized *Citrus sinensis* (Osbeck) cv. 'Washington' navel and 'Valencia' orange trees were exposed to tritiated hydrogen-3 and/or Sulfur-35 via the irrigation solution. The containers were weighed at regular intervals to determine water loss. Samples from various aerial organs were collected at the same intervals and their isotopic content determined. The accumulation of both ^3H and ^{35}S in aerial organs was highly correlated to weight loss from the containers. The rate of ^3H accumulation in petals of leafy inflorescences was greater than leafless inflorescences. The content of both ^3H and ^{35}S was greater in ovaries of leafy when compared to leafless inflorescences after 48 hours from introduction of isotope. There was no difference in isotope flux to LY inflorescence when compared to leaves developing in the absence of flowers.

Flowers from field grown valencia trees were collected at petal fall. The ovaries were removed, dried and weighed. The dried ovaries were then assayed for their content of N,P,K,Ca,Mg,Zn,Mn,Fe,B and S. The dry weight of ovaries from leafy inflorescences was greater than those from leafless inflorescences. The concentration of N,K and Fe was higher in ovaries from leafless when compared to leafy inflorescences. The total content of N,P,Mg and Zn was higher in ovaries from leafy inflorescences when compared to leafless inflorescences.

Hypothesis 2: Intra-inflorescence differences contribute to the higher yield potential of leafy inflorescence.

The preferential set on leafy inflorescence (LY) over leafless inflorescence (LS) has been positively correlated with carbohydrates, growth regulators and mineral elements in different varieties other than 'Shamouti'. Preliminary work done by us (Erner & Bravdo 1983) indicates that carbohydrate might not be a limiting factor for fruit set and therefore, LS has the same potential for fruit set as LY if carbohydrates are considered as the predominant factor for set. In order to prove our initial argumentation, which contradicts other data on carbohydrate translocation (Moss & Kriedemann), we conducted experiments on mature (30 years old) 'Shamouti' orange trees grafted onto 'Sweet lime' stock and inarched with 'Sour orange' on medium light soil. A branch model was chosen to serve for the feeding $^{14}\text{CO}_2$ and source-sink relationship (Fig 1).

Air containing 1000 dpm ml^{-1} $^{14}\text{CO}_2$ was generated from a 500 liter container (330 ppm of Hot/cold CO_2) through a humidifier at a flow rate of 50 ml min^{-1} to the entire lamina of selected source leaves. A 120 ml transparent plastic bag was used in exposing the source leaf for a 2 hr feeding period in full sunlight. Excess CO_2 was absorbed on Ascarite (Thomas Company, U.S.), packed in a column 20 cm long X 2 cm in diameter. Four replications were made in each experiment. Following the 2-hour feeding period, the plastic bag was removed and the component parts were separated, freeze-dried and either oxidized by using Tri-Carb Sample Oxidizer (Packard, Model 305) or extracted for soluble sugars and starch.

Previous experiments have not taken into account the fruitlet position and the xylem:phloem connection between source leaf and fruitlet sink. Moreover, competition between the fruitlets themselves and vegetative growth has been avoided.

Fig.1. Schematic model of Citrus spring inflorescence type used for $^{14}\text{CO}_2$ pulse experiments. A,B,C - Mature leaf, one-year-old.
a,b,c,d - Young leaf, current growth.
f - Fruitlet

Therefore, present experiments with both inflorescence types (LY and LS) on the same branch, have been examined for ^{14}C translocation. The feeding pulse of ^{14}C was made as above and soluble sugars were extracted with methanol:chloroform:water 12:5:3. The pellet was digested by amyloglucosidase to measure the starch content. A 100 mg of dry matter was used for each sample and the soluble sugars were measured with an anthrone reagent after discarding the chloroform. An aliquot was tested for radioactivity amount of sugars.

Our previous work (Erner & Bravdo 1983) showed that LS is a strong sink and no clear-cut observation has been made at that time for the change-over of the young leaves from sink to source status. In this work we could show that young leaves of the LY became a source about one month after anthesis (Fig. 2). The young leaves a,b,c and d attract as much ^{14}C as they need from one-year-old leaf (B) until the demand is smaller than the production. Similar results, which supported the previous experiment, showed that young leaf (a) can allocate ^{14}C to the fruitlet (f) while it was not attracting ^{14}C from the old leaf (B). Feeding the 4 fully expanded (50 days after anthesis) young leaves (a,b,c,d) together was not found to be more efficient for carbohydrate translocation to the fruitlet than feeding one-year-old leaf (B) on LS (Table 1).

Fig.2. Relative distribution of ^{14}C (%) in reproductive shoots, 96 h after treatment.

Table 1. Relative distribution of ^{14}C (%) in reproductive shoots 96h after treatment. Leaf B (LS) or leaves a,b,c,d (LY) were exposed to $^{14}\text{CO}_2$ 50 days after anthesis.

Plant	% ^{14}C	
Organ *	Leafy Infl.	Leafless Infl.
A	0.24	0.11
B	0.08	71.37
C	0.03	1.58
a	12.15	--
b	22.06	--
c	25.03	--
d	12.83	--
f	25.48	26.83

* See Fig. 1

All the experiments with the pulse $^{14}\text{CO}_2$ feeding showed that only small amounts of ^{14}C have been translocated to one-year old leaves (A,B,C), even with girdling applied to avoid ^{14}C basipetal translocation (data not shown).

Carbohydrate allocation from source leaf (A or B) to the fruitlets have examined when both fruitlets were at different locations -LS, LY- on the same branch (Fig. 3).

Fig.3. Relative distribution of ^{14}C (%) in reproductive Shoots 96 h after treatment.

Leaf A or B was exposed to $^{14}\text{CO}_2$ 25 days after anthesis

Feeder leaf A, Leafless infl. in axil C.

Leafy infl. in axil B.

Feeder leaf B, Leafless infl. in axil B.

Leafy infl. in axil C.

Irrespective of fruitlet position on the branch, all experiments showed that fruitlets borne on LS accumulated more ^{14}C labelled photosynthate than fruitlets borne on LY. The experiments precluded the possibility that the pattern of ^{14}C translocation was influenced primarily by alignment of the source leaf with fruit. At that stage of the experiments, the young leaves of the LY were capable of exporting carbohydrates (Fig. 2).

Measuring the total soluble sugars, starch and the radioactivity after a $^{14}\text{CO}_2$ pulse at leaf (a) showed that fruitlets borne on LS contained more ^{14}C which has been transported from leaf (a) (Table 2). No differences were found in starch content, although the specific activity of the labelled starch was higher in LS than in LY. Fruit weight had no correlation with allocation of carbohydrate.

Table 2. Sugar and starch content in fruits borne on leafy or leafless inflorescences, 50 days after anthesis.

Fruit sample	Fresh Wt. mg	S u g a r		S t a r c h	
		mg g ⁻¹ D Wt	dpm* mg ⁻¹	mg g ⁻¹ D Wt	dpm* mg ⁻¹
Leafy	399± 94	46.5±2.5	1503±351	29.7±8	4815±810
Leafless	415±107	59.7±3.6	4393±404	29.6±7	8680±903

Feeder Leaf a.

Leafless Infl. in Axil. A

Leafy Infl. in Axil B.

* Disintegrations per minute.

The fruitlet, whether borne on LS or LY, was a strong metabolic sink and could be expected to compete with other plant parts. Therefore, experiments were conducted to determine the interrelationship between fruitlet and vegetative growth in the spring flush on 10 year-old branches (Table 3).

Table 3. Relationship between shoot type and fruit set.

(Averages of 2 years).

Treatment*			Fruit set** (%)		
V	LY	LS	All infl.	Leafy	Leafless
+	+	+	14.5±3	28.3±4	
-	+	+	6.8±2	17.2±4	
+	+	-		33.7±7	
-	+	-		28.5±6	
+	-	+			18.7±4
-	-	+			11.5±4

* Vegetative (V), leafy (LY) and leafless (LS) inflorescence were left on (+), or removed from (-) 10 years old branches.

** First count at petal drop stage, final count after 'June drop'.

Leafless inflorescence could set fruit in significant amounts when LY and vegetative flush were removed. Moreover, a higher percent set in LS was observed when vegetative flush was left on (Only LY removed).

Removal of vegetative growth reduced fruit set both in the LY and LS. It is likely that developing fruitlets influence the survival of other fruits, possibly by direct interactions rather than a lack of photosynthates. The highest percent set was found on LY together with vegetative branches, while the set on LY by itself was significantly higher than on normal branches (V+LY+LS, Table 3). Removal of vegetative growth (V) should increase fruitset if lack of carbohydrate exist.

We could not able to show any differences in the fresh weight and percent dry matter of ovaries from LY and LS (Table 4). The ovaries sampled were taken at a precise stage in their physiological development, 100 flowers being tagged for this purpose at first petal opening (see drawing in table 4). These were sampled 0, 14, 28 days. The similar weights of the ovaries and fruitlets growth, borne on LY or LS, indicate that the potential growth of the fruitlets is exists in both and factor(s) other than carbohydrate might cause differential abscission of the fruitlets borne on leafless inflorescences.

Plant growth regulators were applied to the petiole or onto the ovary at flower opening in order to examine the possible involvement of these hormones in fruit set and the possible substitution for the presence of young leaves (LY) (Table 5). GA and BA were found to be effective in increasing fruit set only if applied directly to the ovary. Hormone treatment on to the petiole of the LY did not increase fruit set. 2,4-D could delay fruitlet abscission but did not increase set, and in some cases even enhanced abscission. Different auxins and concentrations were tried over 3 years of experiments but have never been able to show any promotion.

Table 4. The relationship between inflorescence type and weight of ovary and fruitlet.

Days From Anthesis	<u>Leafless Inflorescence*</u>		<u>Leafy Inflorescence**</u>	
	Fresh Wt. mg	% Dry Wt.	Fresh Wt. mg	% Dry Wt.
0	27.6±4.4	35.5±0.5	29.2±5.8	36.0±0.6
14	97.1±30	34.4±0.5	88.9±20	35.9±0.3
28	338.2±169	31.7±0.9	341.0±214	33.9±1.0

* One flower

** One terminal flower with 4 leaves

Table 5. Influence on fruit set by lamina removal and application of growth regulators either on the ovary or on the petiole.

Treatment Application	PERCENT FRUIT SET			
	Leafless Ovary	Leaves Intact Ovary	Leaves Removed Ovary	Leaves Removed Petiole
Control	2	16	8	8
GA ₃ 200 ppm	10	28	32	10
BA 50 ppm	4	28	28	6
2,4-D 50 ppm	-	10	8	6

The influence of exogenous application of growth regulators on sugar and starch content were measured in fruitlets 50 days after anthesis (Tables 6 and 7). The hormones were applied 3 days before $^{14}\text{CO}_2$ treatment to leaf B (Fig.1) and had affected the translocation and metabolism of LS and LY in different ways.

Table 6. The influence of growth regulators on sugar and starch content in the fruitlet from leafless Inflorescence, 50 days after anthesis.

Treatment*	SUGAR		STARCH	
	mg g	% ^{14}C	mg g	% ^{14}C
Control	101±11	49±5	45±7	36±3
GA ₃	35±4	59±7	41±1	51±6
BA	53±3	31±6	33±2	18±2
2,4-D	61±13	46±2	33±2	32±2

* GA₃ 5μl of 10 ppm; BA 50 ppm; 2,4-D 10 ppm - all Applied 3 days before $^{14}\text{CO}_2$ treatment to leaf B.

The sugar content was highest in LS control fruitlets, and considerably lower in the hormone treated ones, while almost no difference was found in starch contents. In the LY fruitlets, sugar contents in the control was lowest, and highest in GA₃. The same relations were seen in starch content of LY. The amounts of ^{14}C in the sugar and starch fraction in LS fruitlets were significantly higher than in LY.

Table 7. The influence of growth regulators on sugar and starch content in the fruitlet from leafy Inflorescence, 50 days after anthesis.

Treatment	S U G A R		S T A R C H	
	mg g	% ^{14}C	mg g	% ^{14}C
Control	27±4	3±2	23±3	4±1
GA ₃	97±23	9±4	52±8	10±2
BA	59±3	5±3	37±5	5±4
2,4-D	46±3	18±1	32±3	10±4

* GA₃ 5μl of 10 ppm; BA 50 ppm; 2,4-D 10 ppm all applied 3 days before $^{14}\text{CO}_2$ treatment to leaf B.

At this point, we have no viable explanation for these differences. Much more work needs to be done in elucidating these phenomena.

Our results of the translocation of unlabelled growth regulators from the petiole to the fruitlet (Table 5) needed further proof by experiments using labelled hormones, since we could not show that the applied hormones reached the fruitlet. Application of the labelled hormones on the petiole resulted in a significant amount of the label being found in the fruit, but this might be part of the wound effect. Therefore, experiments in which the labelled hormone was applied to the basal end of the lamina. This gave penetration to the leaf and allocation to other plant parts without wound effect (Table 8).

Table 8. Translocation of labelled GA, Kinetin and IAA from one-year-old leaf to fruitlet 10mm in Diameter, 24h after application on the lamina base (20.000 dpm leaf⁻¹).

Plant part		GA	Kinetin	IAA
		% of Total dpm		
Leafless	Mature leaf*	91.6	90.3	91.9
	Fruitlet	8.4	9.7	8.1
Leafy	Mature leaf*	39.1	29.7	94.4
	Young leaves	57.3	69.1	5.1
	Fruitlet	3.6	1.2	0.5

* Application leaf

Ready translocation of the hormones were found, although high levels of ¹⁴C were found in the application site. IAA moved acropetally which was unexpected for such movement. The relative movement of the various hormones to LS fruit were similar while the movement to LY was highest with GA, medium with Kinetin and least with IAA. IAA is known to conjugate immediately with amino acids or sugars. Therefore, it might not move out of the application leaf (especially in the LY case). The results prove that unlabelled hormones did reach the fruitlets and affected the set according to the specific response.

Our data again confirm the better fruit set on LY as reported previously, but do not support the concept that adjacent young leaves provide a better supply of photosynthate. If limited metabolite accumulation was due to low sink activity,

or low source availability, and/or intersink competition, fruitlets borne on LS should have had a lower carbohydrate concentration than those borne on LY. However, fruitlets borne on LS or LY were both strong sinks for assimilates during their early growth. It has been proposed that fruitlets borne on LS have to obtain their assimilates from one-year-old leaves, the photosynthetic capacity of which is lower than that of young leaves. It is also known that fruits can draw their assimilates from distant leaves and that young vegetative growth can support both types of inflorescence. The greater amount of carbohydrates found in fruitlets borne on LS may be due to lack of competition with young leaves. Girdling, which has been shown to increase the carbohydrate supply above the girdle, and to improve fruit set, does not substitute for the young leaves (previous work) since the increased fruit set associated with girdling is supported by the presence of the young leaves.

Young leaves go through a sink-to-source transition about 25 days after anthesis (Fig.2) but by that time the leaves are no longer essential to fruit set (previous work).

Macro-elements were considered to affect fruit set. The demand for N,P,K was studied during fruitlet development until end of "June drop". Nitrogen concentrations of leaves and fruitlets from anthesis until the end of "June drop" are presented in table 9.

The young flushes in the spring drew nitrogen from reserves of old leaves. The LS and the LY drew nitrogen from one-year-old leaves for a longer time than did adjacent vegetative growth. Old leaves which had no adjacent flush, transported N to the new flush and started to reaccumulate N before the completion of "June drop".

Table 9. Nitrogen concentration in leaves and fruitlets from anthesis until end of "June Drop".

	<u>April 7</u>	<u>May 5</u>	<u>May 28</u>	<u>June 25</u>
Sample	N-(NO ₃) ppm in Dry Matter			
Old leaf*				
no flush	40.8bc**	21.5c	42.5c	230.0b
adjacent LS	33.5bc	27.3bc	40.5c	82.0cd
adjacent LY	24.3c	18.8c	33.5c	81.3cd
adjacent V	22.5c	27.0bc	51.3bc	191.8bc
Young leaves				
of leafy	68.3ab	38.5abc	69.0b	132.3bcd
of vegetative	80.3a	32.8abc	102.5a	390.0a
Fruitlet				
of leafless	30.8c	52.3ab	59.5bc	81.3cd
of leafy	41.0bc	59.3a	53.5bc	63.5d

* No flush - node did not flush

Adjacent LS - node flushed with leafless inflorescence

Adjacent LY - node flushed with leafy inflorescence

Adjacent V - node flushed vegetatively

** Mean separation in columns by Duncan's Multiple Range Test, 5% level.

Fruitlets borne on LY competed with the young leaves for nitrogen since the recovery of nitrogen in the young leaves of the LY was slower than on the young

הספדיה המדעית
למחקר ולתאור
ב"ח - 111

vegetative shoot. However, the concentration of N in the fruitlets borne either on LS or LY were not significantly different from each other (Table 9).

Potassium concentration in the different organs are presented in Table 10.

Table 10. Potassium concentration in leaves and fruitlets from anthesis until end of "June Drop".

	<u>April 7</u>	<u>May 5</u>	<u>May 28</u>	<u>June 25</u>
Sample	K % in Dry Matter			
<hr/>				
Old leaf*				
no flush	0.81de**	0.90cd	0.86b	1.05b
adjacent LS	0.96cd	0.80d	0.69d	0.88cd
adjacent LY	0.73e	0.80d	0.72cd	0.85d
adjacent V	0.70e	0.78d	0.80bcd	1.04bcd
Young leaves				
of leafy	1.19ab	1.17abc	0.88b	1.03bc
of vegetative	1.27a	1.25a	1.20a	1.45a
Fruitlet				
of leafless	1.08bc	1.12ab	0.82bc	0.98bcd
of leafy	1.08bc	1.02bc	0.78bcd	0.89cd

* No flush - node did not flush

Adjacent LS - node flushed with leafless inflorescence

Adjacent LY - node flushed with leafy inflorescence

Adjacent V - node flushed vegetatively

** Mean separation in columns by Duncan's Multiple Range Test, 5% level.

The general pattern of potassium levels was found to be very similar to that of nitrogen. One-year-old leaf can serve as a reserve organ for the young flush in the spring. The concentration of K in the old leaf increased at the time when the young vegetative growth (25 June) became mature. On the other hand, the same old leaf was still in competition for the current K with LS and LY growing fruitlets. The young leaf of the vegetative flush, tended to contain more K than the leaves of the LY, indicating some competition with the fruitlet. No differences were found in K concentration of either fruitlets.

Phosphorus concentration was found to be higher in the fruitlets than in young and old leaves (Table 11). Fruitlets borne on LY had more P than LS in the first month after anthesis. In spite of the overall small differences in the P concentration of the fruitlets, the 0.01 % concentration difference between LY and LS was significant.

High nitrogen fertigation levels (used in a different project), combined with partial wetting of the root zone, significantly increased yield through the increase of leaves and flower number per tree and not by altering the inflorescence type towards the LY. Potassium and phosphorus have been known to increase yield only when applied to nutrient deficient trees. Competition for nutrients among the developing organs was observed but could not be definitively associated with abscission.

Table 11. Phosphorus concentration in leaves and fruitlets from anthesis until end of "June Drop".

	<u>April 7</u>	<u>May 5</u>	<u>May 28</u>	<u>June 25</u>
Sample	P % in Dry Matter			
<hr/>				
Old leaf*				
no flush	0.05d**	0.04d	0.04d	0.04c
adjacent LS	0.05d	0.04d	0.04d	0.04c
adjacent LY	0.05d	0.04d	0.04d	0.04c
adjacent V	0.04d	0.03d	0.04d	0.04c
Young leaves				
of leafy	0.08c	0.07c	0.05c	0.04c
of vegetative	0.09c	0.07c	0.06b	0.06b
Fruitlet				
of leafless	0.15b	0.14b	0.11a	0.11a
of leafy	0.16a	0.15a	0.12a	0.11a

* No flush - node did not flush

Adjacent LS - node flushed with leafless inflorescence

Adjacent LY - node flushed with leafy inflorescence

Adjacent V - node flushed vegetatively

** Mean separation in columns by Duncan's Multiple Range Test, 5% level.

Endogenous growth regulators - IAA, GA, Cytokinin and ABA - were extracted simultaneously from 'Shamouti' orange ovaries, fruitlets and leaves (according to the branch model in Fig.1) sampled for 63 days after anthesis. Since, we need to check four different plant growth regulators, it was important to develop the best technique and procedure for determining all the plant hormones in the same plant extracts. Plant material of LY and LS inflorescences were collected, frozen immediately and kept at -20°C until processed. Extraction of 1 g F Wt was made with 5ml 80% methanol (MeOH) containing 0.1 M ammonium acetate and 10 mg ml^{-1} B.H.T.

^{14}C -labelled IAA, GA_3 , ABA and Kinetin at 1000 dpm were added to the macerated material for recovery, calculations and marker determinations. After 30 min. of incubation at 4°C the material was centrifuged at 5000 rpm for 10 min. The pellet was resuspended in 3 ml of extraction solution, incubated and centrifuged as above. The 2 supernatants were combined and diluted 1:1 with H_2O to bring the concentration of the ammonium acetate to 0.01 M. This was done to eliminate the problems of separation by anion exchange resin. The extract was loaded on to a PVPP column (5 ml bed volume) following passage through DEAE-Sephadex column (3ml bed volume) and washed with 30 ml of H_2O . The cytokinin was not absorbed by either of the columns. The solution was acidified to pH 3 after passing both columns and was loaded on to SP-Sephadex. Elution was made with 1M NH_4OH and absorbed on Sepack c-18. This was eluted with methanol (MeOH). The acidic hormones (IAA, ABA, and GA) were eluted from DEAE-Sephadex with 1M acetic acid. GA was eluted first, ABA soon after and last IAA. GA and ABA were not separated from each other, both were absorbed on sepac C-18 and eluted with MeOH.

The sample was dried and redissolved in 20% MeOH for separation with HPLC. Fractions containing ABA and GA were collected from HPLC with fast column LC-18 DB (Supelco) of C-18 reverse phase. The appropriate fractions of GA and ABA were tested by the lettuce hypocotyl elongation bioassay for GA and methylated ABA with GC-EC. The lettuce hypocotyl test was improved by us for small volume, and quantities. Methylated IAA was quantified by ELISA.

Exogenous GA was found (Table 5) to increase set when applied to the ovary but we could not find any significant differences in endogenous GA content between fruitlets born on LY or LS on fresh weight basis (Table 12). A peak of GA₃-like activity was found 7 days after anthesis and thereafter a decrease in its content. At this time, the GA content in the fruitlets borne on LS was higher than the content in the adjacent one-year-old leaves. These results show that fruitlets borne on LS are a strong sink, considering their GA content at each stage of development. Later in the season the amount of GA's decreased ca-10-fold of that at 7 days after flower opening (FO). Adjacent leafy mature leaves (till the 63 days) contained more GA than adjacent LS leaves. GA in fruitlets may be synthesized locally or transported from other plant parts. If the young leaves of the LY are the source of GA, the amount of GA in them should be higher than in one-year-old leaves. Our data do not show this (Table 12).

Table 12. Changes in GA-like activity in young and mature leaves and fruitlets borne on leafy inflorescence (LY) or leafless inflorescence (LS) during 2 months after flower opening.

Days after flower opening	GA ₃ equivalent ng g ⁻¹ F Wt.				
	Mature leaves		Young leaves	Fruitlets	
	LY	LS		LY	LS
2	103.2ab*	85.0ab	73.3b	152.7a	129.4a
7	190.0bc	75.0c	126.5bc	244.0ab	327.5a
33	174.5a	98.5b	92.0b	101.9b	80.0b
63	123.0a	110.0a	110.0a	30.5b	29.6b

* Mean separation in rows by Duncan's Multiple Range Test, 5% level.

The total amounts of GAs per fruitlet increased continuously during the first month after FO with a dramatic 10-fold increase after 63 days (Fig.4). These changes correlated with the rate of fruit growth (Table 13).

Fig.4. Total amount of GA-like activity in fruitlet during 2 months after flower opening.

Table 13. Changes in fruit fresh weight during first two months after flower opening in fruitlets borne on LY and LS (Average of 6 replicates).

Days after Flower opening	LY	LS
mg Fresh Weight per Fruitlet		
2	34±2	33±3
4	36±4	34±6
7	36±6	36±8
20	122±19	112±32
33	464±59	393±47
63	3356±297	3124±211

IAA was found to reach maximum concentration 7 days after flower opening (Table 14), similarly to GA. Although, no significant differences were found between LY and LS fruitlets 7 days after FO, the amount in the LS was more than 50% of that in the LY fruitlets.

Absciscic acid (ABA) content was found to be over 10-fold in fruitlets, 2 and 7 days after FO, than in fruitlets at 33 and 63 days (Table 15). Fruitlets borne on LS had significantly larger amounts of ABA than the LY till 7 days after flower opening. The ABA content dropped sharply in the fruitlet after 7 days from FO but no such dramatic decrease found in young or mature leaves.

Table 14. Changes in IAA activity in young and mature leaves and fruitlets borne on leafy inflorescence (LY) or leafless inflorescence (LS) during 2 months after flower opening.

Days after flower opening	IAA ng g ⁻¹ F Wt.				
	Mature leaves		Young leaves	Fruitlets	
	LY	LS		LY	LS
2	88.7b*	122.0ab	80.3b	163.1a	187.8a
7	252.5b	437.0ab	133.5b	402.0ab	669.0a
33	33.0b	63.0a	27.5b	52.9a	33.7b
63	39.5ab	51.0a	46.5a	13.4b	10.1b

* Mean separation in rows by Duncan's Multiple Range Test, 5% level.

Since the contents of GA, IAA and ABA fluctuated during the experimental period, the ratio between promoters (GA+IAA) to inhibitors (ABA) emphasizes the regulatory role of these hormones (Table 16). The promoter-inhibitor ratio in fruitlets borne on LY was higher than in fruitlets borne on LS, especially 33 days after FO while opposite results were found in mature leaves. This might indicate the preferential fruit set on LY as compared with LS. The ratio in all organs increased till 33 days after FO and decreased thereafter. The shift in the ratio is correlated with the transition of the leaves from sink to source.

Table 15. Changes in ABA concentration in young and in mature leaves and fruitlets borne on leafy inflorescence (LY) or leafless inflorescence (LS) during 2 months after flower opening.

Days after flower opening	ABA ng g ⁻¹ F Wt.				
	Mature leaves		Young leaves	Fruitlets	
	LY	LS		LY	LS
2	183.3b*	193.7b	355.3b	454.3b	812.7a
7	312.0b	242.0c	324.0b	316.5b	512.5a
33	74.5ab	41.0ab	114.0a	29.8b	57.7ab
63	134.0ab	264.0a	117.0ab	52.2b	70.4b

* Mean separation in rows by Duncan's Multiple Range Test, 5% level.

Table 16. Changes in the ratio of growth promoter (IAA+GAs) to growth inhibitor (ABA) content in young and in mature leaves and fruitlets borne on leafy (LY) or leafless inflorescence (LS) during 2 months after flower opening (FO).

Days after flower opening	GAs+IAA ABA ⁻¹						
	Mature leaves			Young leaves	Fruitlets		
	LY	LS	LY/LS		LY	LS	LY/LS
2	1.04	1.07	0.97	0.43	0.69	0.39	1.77
7	1.42	2.12	0.67	0.80	2.04	1.94	1.05
33	2.80b	3.93	0.71	1.04	5.20	1.96	2.65
63	1.21b	0.61	1.98	1.33	0.84	0.56	1.50

In previous work we were able to show that fruitlets can be set initially on LS but 98% of the harvest fruit were from LY. Furthermore, 70-80% of the flowers are from the LS types while the small proportion borne on LY are responsible for the yield. Increasing the number of LY might increase the yield through a better set. Gibberellin spray in winter can prevent flowering, depending on the concentration. We found that the application of 10-20 ppm GA in late December can alter the inflorescence type by reducing the LS and increasing the vegetative (V) growth (Table 17), but the percent of LY was smaller than in the control. Promalin, which is a commercial formula of GA₄₊₇ and BA, increased the number of leaves per flower in the LY, which, in earlier work had been found to have a better set at ratio of 3-4 leaves to one flower. The percent of vegetative flush increased dramatically and a decrease in LS was found.

Table 17. Effect of promalin and GA₃ on spring flush growth of 'Shamouti' orange.

Treatment	Leaves	Leaves	No.sprout LY branch	No.sprout LY branch	Flush Type (%)		
	Flower ⁻¹	Flower ⁻¹ LY			branch	LY branch	V
Exp. I							
Control	0.77b ^{**}	1.01b	4.3a	0.98a	8.3b	23.7a	68.0a
Promalin [*]	6.96a	2.15a	2.8b	0.86a	31.7a	29.3a	39.0b
Exp. II							
Control	1.24b	1.34a	3.6a	1.27a	14.0b	38.0a	48.0a
GA ₃ [*]	4.54a	1.80a	2.9a	0.80a	52.2a	28.8b	20.0b

* 20 ppm spray on December 20

** Mean separation in columns by Duncan's Multiple Range Test, 5% level.

The addition of BA was expected to increase bud break (sprouts per branch) but failed to do this in this experiment. Sprout number per branch in LY was less inhibited with promalin than with GA alone. Treatment of BA did not alter the proportion of the spring flush types (Table 18) while the combination of GA+BA in this experiment increased the ratio of LY to V branches.

Table 18. Effect of BA and GA on spring flush growth of 'Shamouti' orange.

Treatment	LY/V branch	Flush Type (%)		
		V	LY	LS
Control	1.6	14bc	24b	61a
BA	1.3	17ab	24b	58ab
GA	1.2	23a	28ab	48b
BA+GA	1.7	18ab	30a	52b

* Mean separation in columns by Duncan's Multiple Range Test, 5% level.

The percent LY in the BA+GA treatment was higher than in the control. GA decreased the percent of LS and increase the percent of vegetative (V).

General discussion.

It has been recognized that fruit set is favoured on leafy inflorescences, while abortion of reproductive organs is nearly complete on leafless inflorescences, during the first two months after anthesis - except in grapefruit and lemons. It has been suggested that this phenomena is controlled by carbohydrate translocation, a deficiency in certain elements and changes in growth regulator content.

Our first data indicate that carbohydrate might not be a limiting factor for fruit set, contradict earlier other scientists data. Therefore, research was done in different ways and techniques to evaluate this basic argument which has been quoted in the literature for many years. All the experiments failed to show

any lack of carbohydrate in fruitlets borne on LS compared with fruitlets borne on LY. Moreover, it was thought that vegetative growth would support the young fruitlet as a source of carbohydrates. We found that during the first month after anthesis, the vegetative branches, while still strong sinks compete for the carbohydrate with the LS and LY fruitlets. Our previous work showed that 4-6 weeks after anthesis, the young leaves of LY cease to be important for fruitlet survival. Removal of the growth, at the time of flowering, reduced fruit set whether on LS or LY. It is likely that developing fruitlets influence the survival mutually, possibly by direct interaction rather than a lack of photosynthate. The message translocated between the fruitlets is not yet known and believed (by other scientists also) that it might also be the same compound which is involved in the inhibition of the flower induction process.

Orchard management might affect fruit set by the increase of the numbers of leafy inflorescences through increasing tree size and leaf numbers. The small significant difference in P found in fruitlets at the first month after anthesis should be checked. Exogenous application of GA to seedy varieties or to the ovary of seedless ones can increase set. Exogenous application of auxin (normally 2,4-D) might decrease preharvest drop. Therefore, endogenous growth regulators, especially IAA and GA, were expected to be correlated with fruit set.

Abscissic acid has been found to be involved in accelerating abscission in citrus tissues. Fruitlet abscission occurs after an increase in ABA either as a cause or the effect. The higher concentration of ABA in fruitlets borne on LS might be associated with early metabolic events leading to fruit drop. Another work showed that increase in ABA concentration was observed after water stress in leaves of 'Shamouti' orange seedlings as well as in 'Washington' navel orange trees. The turnover of water in fruitlets adjacent to young leaves (LY) was

found to be greater than that in LS fruitlets adjacent to one-year-old leaves (has reported in this work by prof. Coggins). Additionally, non-detectable transient water stress during midday can cause ABA build up which lead to abscission.

Experiments done years ago showed that a shifting in spring flush type was possible by using GA or antigeberellin in the autumn. The increase of LY compared with LS is a desirable phenomenon since LY are responsible for the yield. Our data indicate that it is possible to shift the inflorescence type by the application of GA+BA towards the LY and vegetative, and to decrease the percent of LS. The equation of this manipulation can be as follow:

Comercial evaluation of the possible shift in the inflorescence types by GA + BA and the reasons for ABA accumulation in fruitlet borne on LS more than on LY should be subjects for further study.

Objective 2: Identify the Physiological Event(s) That Increase the Proportion of Leafy Inflorescences That Develop During Bloom.

Regulation of flowering has long been a goal of growers, horticulturalists, and plant physiologists. There are many practical benefits to be derived from the ability to regulate flowering. It would be of benefit to be able to increase floral intensity to achieve maximum yield or to decrease flower number to improve fruit set, fruit size, or quality. In the specific case of the 'Shamouti' and 'Washington' navel orange, it would be of benefit to decrease the number of flowers per inflorescence to increase the leaf to flower ratio of the inflorescences, and thus increase the proportion of leafy inflorescence per tree. An ability to decrease flower number would make it possible to correct alternate bearing. An ability to regulate flowering would make it possible to change the time of flowering - earlier, later, or to a completely different time of the year to capture a more lucrative marketing window. Finally, regulation of flowering would permit crop production in marginal areas because we would be able to insure a good bloom.

Flowering in Citrus is recurrent under tropical and subtropical conditions unless synchronized into a well-defined period of concentrated bloom by external environment conditions. Flower formation in Citrus is promoted by drought and low temperature stress followed by restoration of climatic conditions favorable for growth. Thus, stress applied in a quantitative manner provided a controlled system during which changes in the ratio of carbohydrate to nitrogen and hormone levels were monitored in relation to flower initiation.

Five-year-old rooted cuttings of the 'Washington' navel orange induced to flower by low temperature stress were subjected to an 8h-day ($500\mu\text{E}/\text{m}^2\cdot\text{sec}$) 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 ($500\mu\text{E}/\text{m}^2\cdot\text{sec}$) at 24°C, 12-h

night at 19°C. Maximum flower number, or peak bloom, occurred four weeks after the trees were transferred to the warm temperature. 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 ± 0.2 MPa.

There was no significant change in the glucose or starch content of the youngest, fully expanded leaves of the 'Washington' navel orange during 8 weeks of low temperature stress. The correlation between the length of the cold treatment and starch content was negative but not significant at the 10% level ($r=-0.186$). Previously, it been assumed that photosynthesis continues during low temperature stress but that carbohydrate utilization is reduced, causing starch accumulation during the stress. We found photosynthetic rates to be the same between warm-temperature control trees and low-temperature stress trees. Since starch remains constant during the stress period, carbohydrate utilization, like photosynthesis, must also be proceeding at the normal rate during the stress. The low temperature treatment did not cause leaf abscission. Therefore, the amounts of glucose and starch available to the tree for the flowering process were the same both before and after the stress. For glucose, this concentration was $1.25 \mu\text{g}$ glucose/g fr. wt. leaf tissue. Leaf starch concentrations were highly variable. For the more than 100 trees used in the many experiments conducted values ranged from 1.4 to 150 mg starch/g fr. wt. leaf tissue.

Despite the fact that leaf carbohydrate content did not change in response to low-temperature stress, nor in a manner that paralleled floral intensity within an experiment. Linear regression analysis of all the trees from all the treatments provided evidence that starch content of the youngest, fully expanded

leaves correlated significantly with number of floral shoots initiated ($p < 0.0001$; $r=0.75$). Leaf starch content was not correlated with the number of vegetative shoots produced. This is a significant finding. It says that starch is not simply serving as an energy source to increase bud break, but is actually influencing floral shoot development.

Neither total N nor the NO_3^- content of the leaves changed during or after the stress. Total N measured by Kjeldahl was 25.5 ± 3.8 mg/g dry wt. leaf tissue. Leaf NO_3^- content was 3% of the total N level both during and after the low temperature treatment. However, there was a significant increase in leaf $\text{NH}_3\text{-NH}_4^+$ content during the low-temperature stress. $\text{NH}_3\text{-NH}_4^+$ accumulated in a manner that paralleled the duration of the stress ($p < 0.01$; $r=0.605$). In addition, the amount of $\text{NH}_3\text{-NH}_4^+$ that accumulated was directly proportional to the number of flowers produced per tree ($p < 0.0001$; $r=0.803$). Leaf $\text{NH}_3\text{-NH}_4^+$ content had no effect on the number of vegetative shoots initiated per tree.

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 the $\text{NH}_3\text{-NH}_4^+$ status of the trees by foliar application of low biuret urea at the rate of 1.5 g/tree. For trees receiving only four or six weeks of low temperature stress, foliar application of low biuret urea increased the leaf $\text{NH}_3\text{-NH}_4^+$ content 66 and 115% over the value of the trees without added urea and gave a concomitant and equal increase on flower number per tree of 94 and 130% of the value of the trees not receiving urea, respectively. 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 2636 $\mu\text{g/g}$ dry wt. leaf tissue. The corresponding number of flowers for each tree was

4 to 3065. These results indicate that there is a threshold level of $\text{NH}_3\text{-NH}_4^+$ below which flowering will not take place in the 'Washington' navel orange. That value is approximately 400 $\mu\text{g/g}$ dry wt. leaf tissue.

From the results of our work, a simple linear regression model was developed to predict the influence of leaf starch and $\text{NH}_3\text{-NH}_4^+$ content on flower production in the 'Washington' navel orange. The model predicts that 66 new floral shoots will be initiated for each increase in 10 mg starch per g. fr. wt. youngest, fully expanded leaf tissue ($p < 0.0001$; $r=0.8$). The model predicts 208 new flowers will be produced for each increase in 100 μg $\text{NH}_3\text{-NH}_4^+$ per g. dry wt. youngest, fully expanded leaf tissue that accumulates by the end of the low temperature ($p < 0.0001$; $r=0.8$).

To determine (i) if the cause and effect relationship between floral initiation and tree $\text{NH}_3\text{-NH}_4^+$ status of the 'Washington' navel orange was unique to the 'Washington' navel orange or a general response of Citrus species and (ii) if $\text{NH}_3\text{-NH}_4^+$ accumulation could be caused by stresses other than low temperature, we also used water-deficit stress to induce flowering in 16-year-old Frost Lisbon lemon trees on 'Troyer' citrange rootstock under commercial production in the San Joaquin Valley, California. Trees were subjected to water-deficit stress during July and August, the hottest months of our summer, and rewatered at the end of August to give maximum bloom by the end of September. Trees were subjected to water-deficit stress treatments of differing severity. Well-watered control trees ($\psi > 1$ MPa) had the lowest leaf $\text{NH}_3\text{-NH}_4^+$ content (519 $\mu\text{g/g}$ dry wt.) and produced only 14 flowers per tree. One set of trees was subjected to severe water stress of short duration. The trees were taken to $\psi = -3$ MPa in 30 days and rewatered. The leaf $\text{NH}_3\text{-NH}_4^+$ of these trees increased to 646 $\mu\text{g/g}$ dry wt. and flower number per tree increased to 53. One set of trees received severe water stress followed by moderate water stress.

The trees were taken to $\psi = -3$ MPa in 20 days then maintained at $\psi = -2$ MPa for 40 days with deficit irrigation. These trees accumulated the highest endogenous level of $\text{NH}_3\text{-NH}_4^+$ (728 $\mu\text{g/g}$ dry wt. leaf tissue) and produced the highest number of flowers (611 per tree). One set of trees was subjected to moderate water stress. The trees were maintained at $\psi = -2$ MPa for 50 days by deficit irrigation. Half of the trees received a foliar application of low biuret urea 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 ($p < 0.05$; $r=0.56$).

The idea that nitrogen might play a key role in the regulation of flowering became popular within the context of the Kraus-Kraybill Hypothesis. The potential regulation of flower initiation by the balance of carbohydrate to nitrogen or by a calculated mathematical ratio of carbohydrate divided by nitrogen is internationally known 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. Our data, however, do not support a role for a carbohydrate-to-nitrogen ratio in flower initiation and demonstrate that only specific metabolic forms of carbohydrate (starch) and nitrogen ($\text{NH}_3\text{-NH}_4^+$) influence flower number, while other metabolic species do not. Glucose, total N, and NO_3^- do not influence flower initiation. Starch and

$\text{NH}_3\text{-NH}_4^+$ appear to influence flowering independently of each other, except when one or the other is limiting to floral initiation.

Based on these results, Lovatt proposed that starch and $\text{NH}_3\text{-NH}_4^+$ do not influence flower initiation directly, but serve as substrates for the synthesis of key metabolites that act alone or through plant hormones at the genetic level to initiate the flowering process. Thus, studies were undertaken to elucidate the pathways of starch and $\text{NH}_3\text{-NH}_4^+$ metabolism critical to flowering. They focused on metabolic pathways which exhibit accelerated activity in response to stress and, specifically, in response to the accumulation of $\text{NH}_3\text{-NH}_4^+$. Thus, the research investigation focused on arginine and polyamine metabolism.

The activity of the pathway for the de novo biosynthesis of arginine measured in youngest, fully expanded leaves during the first week after transfer of the trees from the low-temperature treatment to the warm temperature increased with the length of the low temperature treatment as follows: 0 weeks (warm temperature control), 4,6,and 8 weeks of low temperature treatment gave rates of arginine biosynthesis of 5,7,11, and 27 nmoles $\text{NaH}^{14}\text{CO}_3$ incorporated into the guanido carbon of arginine per g fr. wt. leaf tissue during a 3-h incubation period, respectively. Changes in leaf polyamine content in response to increasing duration of low temperature stress and after transfer from the low temperature stress to the warm temperature treatment leading to full bloom were quantified. In addition, the time and rate of export of arginine and polyamines from the leaf to the buds was also monitored. Final results are not completely analysed statistically.

For convenience and for development of commercial analyses in the future, we have monitored changes in carbohydrate and nitrogen metabolism in leaves, specifically the youngest, fully expanded leaves to avoid the secondary effects of leaf senescence and mineral nutrient deficiencies. Previous work in my lab

has demonstrated that senescence and mineral nutrient deficiencies cause $\text{NH}_3\text{-NH}_4^+$ to accumulate in leaves. Based on our results, it is clear that leaf starch and $\text{NH}_3\text{-NH}_4^+$ concentrations can be developed into a tool of value in monitoring flower initiation in Citrus and predicting floral intensity. Whether the leaves contribute to flower initiation or simply reflect parallel changes occurring in the buds was also investigated.

In addition to determining if $\text{NH}_3\text{-NH}_4^+$, arginine, and/or polyamines were produced in the leaves and translocated to the bud or produced in the bud itself, we wanted to determine whether arginine and/or polyamines play a role in flower and ovary development in citrus. Finally, we wanted to determine if the metabolism of these key nitrogen compounds was different in flowers born on leafy versus leafless inflorescences. Previous work in my lab demonstrated that the activity of the pathway for the de novo biosynthesis of arginine was accelerated in response to increased $\text{NH}_3\text{-NH}_4^+$ content of leaves of several species and hybrids of citrus (Rabe and Lovatt, 1986, Plant Physiol 81:774-779). Arginine is the predominant precursor of polyamines synthesized in plants. Polyamines are a class of nitrogen compounds that accumulate during stress and a class of nitrogen compounds that are being recognized as important factors in cell division and organogenesis. For example, in Tomato, fruit growth was reduced by inhibition of polyamine synthesis. Resupplying polyamines restored normal fruit development (Cohen, Arad, Heimer and Mizrahi, 1982, Plant Physiol 70:540-543).

Thus, a study was undertaken: (1) to determine if flower initiation by low temperature stress results in elevated levels of $\text{NH}_3\text{-NH}_4^+$ in developing flowers of the 'Washington' navel orange; (2) to define the relationship between the pool size of available $\text{NH}_3\text{-NH}_4^+$, the activity of the novo arginine biosynthetic pathway, and tissue concentrations of polyamines; and (3) to determine whether

there is a difference in the metabolism of these key nitrogen compounds in flowers born on leafy versus leafless inflorescences and in flowers born in apical versus subapical position. The apical or terminal flower of an inflorescence is precocious and exerts dominance over the flower buds below, retarding their development. The flower in the subapical position, the one just below the terminal flower, is the most delayed in its development. There is a controversy in the literature as to which sets more fruit.

In the apical flower borne on a leafy inflorescence, the highest concentrations of $\text{NH}_3\text{-NH}_4^+$ and putrescine and the greatest rate of de novo arginine biosynthesis are all found in the earliest (youngest) stage of flower development. This is consistent with the accumulation of $\text{NH}_3\text{-NH}_4^+$ in flower buds as well as leaves during the low temperature induction of flowers in Citrus. Flower $\text{NH}_3\text{-NH}_4^+$ content, de novo arginine biosynthesis, and putrescine content all decreased in parallel from Stage I of flower development to Stage V, petal fall. These changes are not the result of dilution as the flower increased in biomass as Stage II and Stage III are the same size and weight, one is a closed flower, the other is an open flower. However, Stage III flowers have significantly ($p < 0.05$) higher levels of $\text{NH}_3\text{-NH}_4^+$, arginine synthesis, and putrescine than Stage IV flowers. Parallel decreases in all three parameters through flower development were also recorded for subapical flowers and for flowers borne on leafless inflorescences.

To determine whether $\text{NH}_3\text{-NH}_4^+$ availability influences amino acid metabolism in general, or only the synthesis of specific amino acids, arginine in general, or only the synthesis of specific amino acids, arginine in particular, changes in the activity of the shikimic acid pathway for the de novo biosynthesis of tyrosine were also quantified. The activity of the tyrosine biosynthetic pathway in developing flowers changes in a manner that is totally different from that of

the arginine biosynthetic pathway. This suggests that the parallel changes in flower $\text{NH}_3\text{-NH}_4^+$ content, arginine biosynthesis, and polyamine content are causally related.

The research also investigated the influence of flower position and inflorescence type on the metabolism of these key nitrogen compounds. During early flower development, terminal flowers had higher levels of $\text{NH}_3\text{-NH}_4^+$ than subterminal flowers ($p < 0.05$). For each flower type, the flower borne on the leafy inflorescence had the higher $\text{NH}_3\text{-NH}_4^+$ content ($p < 0.05$). One week after petal fall, these differences were insignificant at the 5% level. De novo arginine biosynthesis followed this same pattern ($p < 0.05$), except one week after petal fall, there was an increase in de novo arginine biosynthesis in the fruit. This increase in de novo arginine biosynthesis resulted in a marked increase in the putrescine content of the fruit one week after petal fall ($p < 0.05$), with the highest level of putrescine found in flowers borne on leafy inflorescences.

Putrescine is the first polyamine synthesized from arginine. It is subsequently converted to spermidine, which is converted to spermine. Quantification of the sum of these three polyamines demonstrated a dramatic increase in total polyamines at one week after petal fall. Fruit on leafy inflorescences exhibited the greater concentration of total polyamines. This is consistent with the fact that fruit borne on leafy inflorescences exhibit faster-growth rates and a greater potential to set and survive to harvest and with the fact that polyamines promote cell division and growth of ovaries. It is interesting to note that the diameters of persisting versus abscising fruit of the 'washington' navel orange were not significantly different at petal fall, growth rates of two groups of fruit were significantly different at the 5% level

(Dugger, Harris, Lovatt, and Tomlinson, 1983, Summary of Citrus Research, UC-Riverside, pp.8-9).

To borrow a concept from the plant pathologists, we must now in essence "prove Koch's postulates" with polyamine metabolism. If polyamines are essential for faster-growing fruit and/or fruit set, we should be able to increase fruit abscission by inhibiting polyamine biosynthesis. Conversely, we should be able to improve fruit set by supplying fruit with polyamines. Our research suggests that we may be able to improve fruit set by improving the polyamine content of the fruit by increasing fruit $\text{NH}_3\text{-NH}_4^+$ content by foliar application of low biuret urea. We are now conducting research to determine if foliar application of urea can improve fruit set in the 'Washington' navel orange.

The role of polyamines in cell division and ovary (fruit) growth is one mechanism by which tree $\text{NH}_3\text{-NH}_4^+$ status through foliar applied urea can be used to influence fruit set. A second mechanism would be through increasing the number of leaves per inflorescence and the proportion of leafy inflorescence per tree.

Our preliminary results suggest that urea may prove beneficial in increasing the number of leafy inflorescence and number of leaves per inflorescence. When five-year-old rooted cuttings of the Washington' navel orange were induced to flower by 6 weeks of low temperature stress and were also given a foliar application of low biuret urea at the rate of 1.5 g. per tree, the number of leafless inflorescences doubled. However, the number of leafy inflorescences increased four-fold bringing the number of leafy inflorescences from a value one-half the total flower population to two-thirds of the total population. Thus, foliar application of low biuret urea caused a disproportionally greater increase in leafy inflorescences than leafless inflorescences. In addition, the foliar application of low biuret urea increased the average number of leaves per

inflorescence four-fold. While we still have much work ahead of us, our results do provide us with a testable, practical approach for improving fruit set in shy bearing Citrus varieties.

Most studies have examined changes in hormone content and carbohydrate allocation during flower initiation and flower development, fruit set, and fruit development. We also monitored the hormone content of leaves and roots by ELISA using kits commercially available from Idetek, Inc. during the low temperature stress treatment and during the four-week period after transfer of the trees to the warm temperature treatment that leads to full bloom. We were unable to detect any changes in the concentration of IAA, ABA, zeatin, or zeatin-riboside at any time during stress-induction, initiation, flower development, or bloom. Either our samples were not prepared for ELISA sufficiently or our method was not sensitive enough to detect differences in hormone content, or changes in the hormonal composition of leaves or roots do not occur during flowering.

I have since repeated this work, using a completely different method of tissue preparation and analysis in cooperation with Dr. Jonathan Cutting and Dr. Tohn bower, Citrus and Subtropical Fruit Research Institute, Nelspruit, RSA. In addition, we monitored several GAs and changes in the quantity of hormones moving from the roots to the shoots in the xylem. These analyses are not completed at this time.

Finally, the results of this research clearly demonstrate that foliarly applied low biuret urea can raise the tree $\text{NH}_3\text{-NH}_4^+$ status and increase flowering. This suggests that foliar applied urea can be used as a tool to manipulate floral intensity in Citrus species. In addition, the results of this research provide evidence suggesting that tree $\text{NH}_3\text{-NH}_4^+$ status, and thus, foliar-applied low biuret urea, influences fruit set in the 'Washington' navel orange by improving the polyamine content of the developing ovary at a time

critical to fruit set and by increasing the number of leaves per inflorescence and the proportion of leafy inflorescences per tree.

PUBLICATIONS

- Erner, Y. 1989. Citrus fruit set carbohydrate, hormone and leaf mineral relationship. (ed) C. J. Wright. Manipulation of fruiting. Nottingham, U.K.
- Sagee, O. and Y. Erner. Gibberlin and abscisic acid contents during flowering and fruit set of 'Shamouti' orange. Submitted for publication: Plant Growth Regulators
- Erner, Y. 1987. Effect of inflorescence type on early growth and mineral content of 'Shamouti' orange fruitlets. 84th Annual Meeting of A.S.H.S. Orlando Florida. Abstract.
- Sagee, O. and Y. Erner. 1987. Hormonal level and transport in 'Shamouti' orange inflorescence. 84th Annual Meeting of A.S.H.S. Orlando, Florida. Abstract.
- Erner, Y. Effect of inflorescence types on morphological and anatomical changes in 'Shamouti' orange and Grapefruit. To be presented at the 23rd I.H.C. Firenze. Itali. 1990.
- Bennet, S.R. and C.W. Coggins, Jr. Importance of inflorescence leaves in the apoplastic transport of H₂O and nutrient to developing inflorescences of the 'Washington' navel orange. J. Am. Soc Hort. Sci. (manuscript in preparation).
- Lovatt, C.J., Y. Zheng, and K.D. Hake 1988. Demonstration of change in nitrogen metabolism influencing flower initiation in Citrus. Israel J. Bot. 37:181-188.

- Lovatt, C.J., Y. Zheng, and K.D. Hake. 1988. A new look at the Kraus-Kraybill Hypothesis and Flowering in Citrus. Proc. Int. Citriculture: in press.
- Sagee, O. and C.J. Lovatt. 1989. Putrescine content parallels ammonia and arginine metabolism in developing flowers of the 'Washington' navel orange. Plant Physiol: in press.
- Sagee, O. and C.J. Lovatt. Number of inflorescence leaves influences the polyamine content of young navel orange fruit. Plant Physiol: in preparation.

BENEFITS TO AGRICULTURE

The knowledge about carbohydrate translocation, sink competition, mineral element and growth regulator contents will enable the citrus researcher to better understand the phenomena of fruit set physiology. This can serve as an advanced basis for further research in this important sector of citriculture. The abscission of fruitlets borne on LS may be caused by an increase in ABA contents in the fruitlet. Irrigation manipulation could possibly reduce water stress at midday and therefore, reduce LS fruitlet abscission. This idea has to be tested by further experiments.

The ability to shift inflorescence type toward the Leafy instead of the Leafless will increase fruit set. The combination of GA + BA has been experimentally proven capable of this shift, but has to be examined on half-commercial scale. Mini-experiments have showed that this is viable and needs further economic examinations.

Foliar-applied low biuret urea raises the $\text{NH}_3\text{-NH}_4^+$ content of the tree with a concomitant and proportional increase in flower number. Thus, foliar-applied urea can be used as a tool to manipulate floral intensity in Citrus species.

For example, in area or years where there is inadequate chilling or water-deficit stress prior to spring bloom, urea could be applied to raise the $\text{NH}_3\text{-NH}_4^+$ status of the orchard and improve flowering. We are doing this in California to augment low temperatures that precede spring bloom. A former student of mine, Dr. Etienne Rabe, is using foliar applied urea in South Africa to improve spring flowering in the navel orange and several cultivars of easy-peelers in areas where spring bloom is in response to water-deficit stress.

In the San Joaquin Valley of central California, Farm Advisors, Joseph Maranto and Kater Hake, are experimenting with the use of deficit irrigation to induce a second bloom in the fall which will produce a crop of summer or "verdelli" lemons. This is in addition to the winter crop which is harvested from the normal spring bloom. The summer crop of "verdelli" lemons is highly desirable because it has a five-to-ten-fold greater market value than the winter crop. The use of water-deficit stress to induce a summer lemon is commonly carried out in Israel.

Previously, without the employ of deficit irrigation, trees were stressed to -3 MPa. This meant that some trees were stressed to as low as -4 MPa which can cause them to collapse, especially if there are *Phytophthora* spp. present. Some soils just do not dry out well enough to take the trees to -3 MPa or the orchard dries out unevenly. The use of deficit irrigation to maintain trees at a moderate stress of -2 MPa plus a foliar application of low biuret urea results in floral intensity equal to trees receiving the maximum stress of -3 MPa. Thus, deficit irrigation combined with foliar application of low biuret urea extends the types of soils and climates under which the Verdelli process can be carried out and

gives more uniform results through the field and from year to year. Furthermore, analyzing the leaf $\text{NH}_3\text{-NH}_4^+$ content during the stress provides a tool for monitoring its progress and predicting its success.

In addition to influencing flowering, fruit set is another practical problem, especially with regard to shy bearing varieties, like the 'Washington' navel orange, 'Shamouti' orange, and varieties of mandarins, that we feel might be influenced by changes in $\text{NH}_3\text{-NH}_4^+$, arginine, and polyamine metabolism. Thus, we feel that fruit set could be improved by foliar low biuret urea applied just before or at petal fall. In addition, urea sprays during floral initiation may increase the number of leafy inflorescences and, thus, improve fruit set.

Field trials are underway to develop the use of foliar-applied urea into a reliable tool manipulating floral intensity, leaf to flower ratio, and fruit set. In addition, we are presently undertaking field studies to develop guidelines for the use of leaf starch and $\text{NH}_3\text{-NH}_4^+$ analyses as tools for predicting floral intensity, fruit set potential, and yield of Citrus species.

The results of our research are already being extended to other crops. Field research is underway or proposed for lime, Clementine, and Ellendale citrus varieties, avocado mango, and litch in California, Mexico, Florida, the Caribbean, and Republic of South Africa.