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Changes in starch and ammonia metabolism during low temperature stress-induced flowering in Haas avocado -A preliminary report

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ABSTRACT

Hass avocado and clonal Duke 7 rootstock, two years from budding, were induced to flower by subjecting the trees to four and eight weeks of low temperature, 8-h day (500 µE/m^2 sec) at 15 to 18°C/16-h night at 10 to 13°C, and then transferring the trees to 12-h day (500 µE/m^2 sec) at 24°C/12-h night at 19°C. Floral intensity was the same for trees treated for four or eight weeks, approximately 1200 flowers per tree. Control trees maintained at the warm temperature did not flower. Leaf NH_3-NH_4^+ content increased to a maximum concentration during the second week of low temperature treatment and remained high through the third week of stress. For trees subjected to either four or eight weeks of low temperature treatment, leaf NH_3-NH_4^+ content increased more than two-fold by week nine and remained significantly higher throughout the four-week period which culminated in full bloom. Leaf starch content decreased during this period to a minimum value, coincident to peak bloom. Neither tree NH_3-NH_4^+ status nor flowering could be increased by foliar application of low biuret urea. Uptakes of [^{14}C] urea and urease activity of Hass avocado leaves were insignificant.

UITTREKSEL

Hass avokado's en klonale Duke 7-onderstam, twee jaar na knopvorming, was geinduseer vir blomvorming deur die bome te onderwerp aan lae temperatuurbe-handeling vir vier en agt weke vir 'n 8-uur dag (500 µE/m^2 sek) by 15 tot 18°C; 16-uur nag by 10 tot 13°C en dan vir 'n 12-uur dag (500 ^E/m^2 sek) by 24°C; 12-uur nag by 19°C. Blomintensiteit was dieselfde vir borne wat vir vier of agt weke behandel was; ongeveer 1200 blomme per boom. Kontrole borne wat by die warm temperatuur gehou was het nie geblom nie. Blaar NH_3-NH_4^+ -inhoud het verhoog tot 'n maksimum konsentrasie gedurende die tweede week van lae tempertuurbe-handeling en het hoog gebly gedurende die derde week van spanning. By borne wat onderwerp was vir vier of agt weke van lae temperatuurbehandeling, het die blaar NH_3-NH_4^+ -inhoud tweevoudig toegeneem tydens die negende week en dit het betekenisvol hoér gebly gedurende die vier-week periode wat 'n hoogtepunt bereik het met blomvorming. Blaarstyselinhoud het verlaag gedurende die genoemde periode tot 'n minimum waarde wat saam geval het met optimale blomvorming. Geeneen van die boom NH_3-NH_4^+ -inhoud of blomvorming kon verhoog word deur blaartoediening van lae biureet ureum nie. Opname van [^{14}C] *ureum-en urease-aktiwiteit van Hass avokadoblare was onbeduidend.*

INTRODUCTION

Regulation of flowering in fruit tree crops has long been a goal of growers, horticulturalists and plant physiologists. The ability to increase or decrease floral intensity would permit manipulation of crop load to obtain maximum yield at an optimal fruit size and quality. In addition, it would provide a tool for evening out alternate bearing. Benefits would also be derived from the ability to shift the time of flowering, ie, to capture a more lucrative marketing window, and to permit crop production ¡n marginal climatic areas by insuring an adequate flower number.

Flowering in tropical and subtropical tree crops is recurrent, unless synchronized into a well-defined period of concentrated bloom by external environmental conditions. Spring bloom in avocado is a result of lower winter temperatures. The use of water-deficit stress or low temperature stress to induce flowering in tropical and subtropical tree crops is well-known. In our hands, the Washington navel orange and Frost Lisbon lemon flowered in response to low temperature and water-deficit stress, respectively. Floral intensity was directly proportional to the amount of NH_3-NH_4^+ that accumulated in the leaves during the stress treatment (Lovatt *et al* 1988 a, b).

The impetus for investigating changes in starch and NH_3-NH_4^+ metabolism during low temperature stress-induced flowering ¡n the Hass avocado stems from our previous research with citrus. Our results demonstrated that changes in tree NH_3"NH_4^+ status directly influenced stress-induced flowering in citrus and provided evidence that despite the fact that leaf starch content did not change significantly during the stress treatment, the basal level of starch available did influence floral, but not vegetative, shoot production:

- In citrus, leave concentrations of NH_3-NH_4^+, but not NO_3^- or total N, increased in a manner that paralleled the duration of severity of low temperature and water-deficit stress (Lovatt *et al*, 1988a).
- Floral Intensity was directly proportional to the concentration of NH_3-NH_4^+ accumulating during each stress (Lovatt *et al* 1988a, b).
- Foliar application of low biuret urea to artificially raise the NH_3-NH_4^+ content of trees subjected to mini mal stress treatment, ie, one that did not induce significant flowering, increased floral intensity to a number equal to that of the trees receiving maximum stress (Lovatt *et al* 1988a).
- Neither the starch, nor the glucose content of the leaves changed during or after stress, but the basal level of starch available for the flowering process was demonstrated to be significantly correlated to the number of floral shoots induced by stress (Lovatt et al 1988b).

In the light of these results, the objective of the work presented here was to answer the following questions:

a) Can flowering be induced in Hass avocado trees on clonal Duke 7 rootstocks after four or eight weeks of the same low temperature treatment that induces flowering in citrus?

b) Does NH_3-NH_4^+ accumulate in the leaves of Hass avocado in response to low

temperature treatment?

c) Is there a statistically significant correlation between NH_3-NH_4^+ content and floral intensity in avocado?

d) Does foliar application of low biuret urea increase the NH_3-NH_4^+ con tent of the Haas avocado, i.e., is urea taken up by Hass avocado leaves and do they possess an active urease?

e) If d) above is affirmative, does raising the NH_3-NH_4^+ content of the tree artificially with foliar urea increase the flowering response of the Hass avocado?

MATERIALS AND METHODS

Hass avocados on clonal Duke 7 root-stock, two years from budding, were placed in a growth chamber and subjected to low temperature treatment of 8-h day (500 µ/E/m2 sec) at 15 to 18°C; 16-h night at 10 to 13°C. At the end of four or eight weeks, trees were transferred to a greenhouse and maintained at approximately 24°C, 12-h day (500 µE/m2 sec) 19°C, 12-h night. Control trees were maintained under these conditions throughout the experiment. Trees were watered twice a week with half-strength Hoagland's solution. Plant water potential was determined at midday using a pressure bomb. Flowers were counted weekly for each tree.

The youngest, fully expanded leaves were used to assess changes in NH_3-NH_4^+, starch, and activity of the *de novo* arginine biosynthetic pathway.

Leaf NH_3-NH_4^+ levels were determined in a 10 per cent trichloroacetic acid extract, using a Wescan Ammonia Analyzer (Carlson, 1978). Leaf starch content was measured by a modification of the glucose oxidase-peroxidase-o-dianisidine method as described by Hamid *et al* (1985). The activity of the *de novo* arginine biosynthetic pathway was determined by the method of Lovatt and Cheng (1984).

To test the capacity of Hass avocado leaves to take up foliarly applied in urea, areas of a leaf (1 cm X 1 cm) were painted with a single application of 10µl of [^{14}C]urea (12 X 10^3 dpm/ nmol). These areas were collected with a cork borer after 1 h, 3 h, 6 h, 12h, 24 h, 48 h and five days. Sample areas were swabbed with distilled water until no radioactivity remained on the surface. The sample was placed in 80 per cent methanol and bleached. The content of radioactivity was determined using a Beckman LS100 liquid scintillation counter.

Urease activity was determined by incubating 500 mg fr wt leaf blade tissue in Shive's nutrient solution, supplemented with [^{14}C]urea (12 X 10^3 dpm/nmol) for 3 h in a waterbath-shaker at 37°C. The reaction was terminated by the addition of 1 ml 6 N $HClO_4$, and the samples were returned to the waterbath-shaker at 37°C for 1 h. $^{14}C0_2$ released by the enzymic hydrolysis of urea was collected on a filter paper wick saturated with 0,3 ml 20 per cent KOH in a plastic center, well suspended from the rubber stopper sealing the reaction flask. The center well and its contents were transferred quantitatively to a scintillation vial containing 4 ml H_2O and the sample was diluted with 13 ml liquid scintillation cocktail. The content of radioactivity was determined using a Beckman LS100 liquid scintillation counter.

RESULTS

Low temperature-induced flowering in avocado

Floral intensity was the same for Hass avocado trees receiving four or eight weeks of low temperature treatment: compare 1210 ± 258 flowers per tree subjected to four weeks of low temperature versus 1253 ± 591 flowers per tree subjected to eight weeks of low temperature (X ± STD DEV; N = 10 trees).

Control trees, maintained at the warm temperature for the duration of the experiment, did not flower.

Low temperature treatment did not induce water-deficit stress. The average mid-day water potential of low temperature treated Hass avocado trees was not significantly different from that of the warm temperature control trees: compare -2,5 ± 0,6 versus -2,2 ± 0,9 MPa (X ± STD DEV; N = 30; N = 16), respectively.

It is important to note that floral shoot development in trees receiving four or eight weeks of low temperature induction followed the same timetable, with the exception of flower opening which was one week later in trees receiving eight weeks of low temperature treatment (Figure 1).

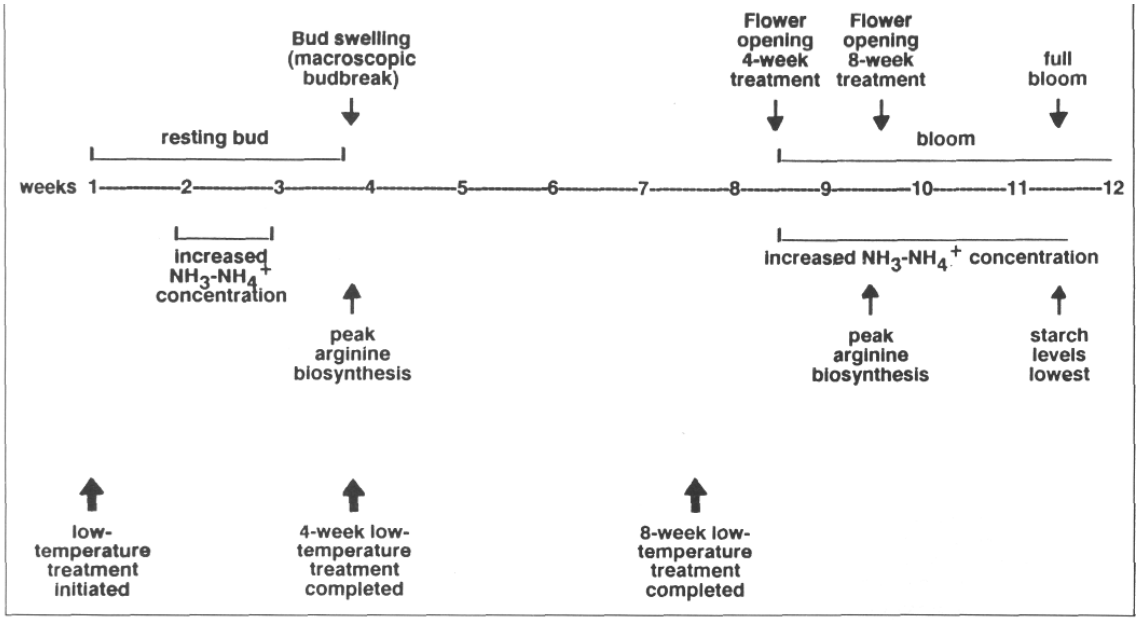

Fig 1 Low-temperature induction/initiation of flowering in Hass aocado. All events occurred simultaneously for trees receiving 4 or 8 weeks of low-temperature treatment unless otherwise stated.

Accumulation of NH_3-NH_4^+ in response to stress

The low temperature treatment caused leaf NH_3-NH_4^+ content to increase to a maximum concentration of 72 ± 9 and 77 ± 13 µg per g fr wt leaf tissue (X ± STD DEV; N = 8 trees), during the second week of treatment for Hass avocado trees subjected to a total of four and eight weeks of low temperature, respectively (Table 1). Leaf NH_3-NH_4^+

content remained high during the third week of stress and then decreased to the initial, pretreatment level. For trees subjected to either four or eight weeks of low temperature treatment, leaf $NH_3^-NH_4^+$ content increased two-fold by week nine and remained significantly higher through the four-week period which culminated in full bloom (P=0,05) (Table 1, Figure 1). Leaf $NH_3^-NH_4^+$ content during bloom was equal to the level observed to accumulate during weeks two and three of the low temperature treatment (Table 1).

TABLE 1 Change in leaf $NH_3^-NH_4^+$ content and *de novo* arginine biosynthesis during low temperature-induced flowering in Hass avocado*

	Four-week low-temperature treatment											
	Cold temperature				Warm temperature							
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}
$NH_3^-NH_4^+$ g/g fr wt leaf tissue	37,28 ± 13,7 d	72,00 ± 9,1 ab	66,99 ± 16,2 bc	36,80 ± 12,5 d	34,70 ± 5,5 d	31,05 ± 4,8 d	34,66 ± 8,3 d	29,55 ± 7,5 d	70,32 ± 25,9 abc	56,30 ± 7,5 c	82,55 ± 25,1 a	58,84 ± 6,4 bc
nmol $NaH^{14}CO_3$ incorporated into arginine/g fr wt leaf tissue 3 h	0,331 ± 0,28 de	0,619 ± 0,65 cde	0,381 ± 0,04 de	1,720 ± 1,1 a	0,184 ± 0,22 e	0,802 ± 0,17 bcd	0,972 ± 0,08 bc	0,792 ± 0,32 bcd	0,593 ± 0,45 cde	1,280 ± 0,69 ab	0,855 ± 0,15 bcd	0,739 ± 0,29 cd
	Eight-week low-temperature treatment											
	Cold temperature								Warm temperature			
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}
$NH_3^-NH_4^+$ ug/g fr wt leaf tissue	40,65 ± 15,5 e	76,69 ± 12,6 a	63,21 ± 16,8 bc	36,09 ± 15,1 ef	32,38 ± 5,3 efg	21,96 ± 6,7 g	25,73 ± 4,1 fg	23,49 ± 2,6 g	51,90 ± 10,7 d	64,20 ± 12,4 b	69,32 ± 13,9 ab	53,00 ± 5,3 cd
nmol $NaH^{14}CO_3$ incorporated into arginine/g fr wt leaf tissue 3 h	0,426 ± 0,46 de	0,226 ± 0,09 ef	0,468 ± 0,08 de	2,07 ± 0,83 a	0,043 ± 0,02 f	0,920 ± 0,23 bc	0,559 ± 0,14 de	0,593 ± 0,25 de	0,356 ± 0,43 def	1,21 ± 0,32 b	0,706 ± 0,15 cd	0,950 ± 0,13 bc

* Data are × ± STD DEV; N = 10 trees. Average values in horizontal rows followed by different letters are significantly different at P < 0,05 by Duncan's multiple range test.

De novo arginine biosynthesis

During the first week of low temperature treatment, the activity of the *de novo* arginine biosynthesis pathway increased significantly (P=0,05) in leaves of Haas avocado. The increase in activity occurred two weeks after the initial increase in leaf $NH_3^-NH_4^+$ content. During week 10, trees previously subjected to either four or eight weeks of low temperature treatment exhibited increased *de novo* biosynthesis of arginine (P = 0,05). The increase in activity occurred one week after the increase in leaf $NH_3^-NH_4^+$ content and prior to the beginning of bloom (Table 1, Figure 1).

Changes in carbohydrate status in response to low temperature stress

Starch content of Haas avocado leaves was very variable. As we observed for the Washington navel orange, leaf starch content of Haas avocado trees subjected to four or eight weeks of low temperature did not change in response to stress (Table 2). Leaf starch content tended to be lower during bloom with the lowest leaf starch concentration

coincident with full bloom (week 12) (Table 2, Figure 1).

TABLE 2 Leaf starch content during low-temperature-induced flowering of the Hass avocado*

	Four-week low-temperature treatment											
	Cold temperature				Warm temperature							
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}
Starch mg/g fr wt	18,63 ± 9,6 cd	36,96 ± 13,5 ab	22,91 ± 9,3 c	42,62 ± 23,8 a	24,03 ± 10,6 bc	13,87 ± 10,7 cde	6,30 ± 6,6 de	21,24 ± 21,3 cd	7,43 ± 6,3 de	15,74 ± 14,1 cde	14,26 ± 14,9 cde	3,21 ± 3,9 e
	Eight-week low-temperature treatment											
	Cold temperature								Warm temperature			
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}
Starch mg/g fr wt	29,94 ± 5,9 ab	30,15 ± 13,8 ab	26,24 ± 18,2 ab	28,36 ± 17,3 ab	26,09 ± 27,3 ab	24,53 ± 17,5 bc	19,84 ± 11,5 bc	45,57 ± 14,9 a	28,49 ± 14,9 ab	15,25 ± 10,2 bc	18,07 ± 14,4 bc	2,98 ± 3,4 c

* Data are × ± STD DEV; N = 10 trees. Average values in horizontal rows followed by different letters are significantly different at $P < 0,05$ by Duncan's multiple range test.

Urea uptake and metabolism

Movement of [^{14}C]urea applied to the upper surface of Hass avocado leaves into the leaf was minimal. The amount of [^{14}C]urea recovered in the leaf increased with the length of exposure. Maximum uptake occurred after two days and was not improved by an additional three days of exposure to the [^{14}C]urea (Table 3). Maximum uptake represented only 2,1 per cent of urea applied to the leaf surface.

TABLE 3 Uptake of foliar-applied [^{14}C]urea by Hass avocado

Time after application	dmp/cm²	Uptake as a percentage of the total [^{14}C]urea applied to the leaf
0 hours (control)	36,55	0,3
2 hours	34,86	0,3
4 hours	49,85	0,4
6 hours	123,36	1,0
12 hours	143,47	1,2
24 hours	189,95	1,6
2 days	254,76	2,1
5 days	240,01	2,0

In addition, urease activity of Hass avocado leaf tissue was insignificant (Table 4).

TABLE 4 Urease activity in Hass avocado leaves

Source of leaves	Relative age	nmol $^{14}CO_2$ recovered / g fr wt leaf tissue 3 h
Greenhouse	young	0,044
	old	0,038
Field	young	0,043
	old	0,049

Consistent with the failure of Hass avocado leaves to take up urea or hydrolize it, foliar application of 1,5g low biuret urea per tree failed to increase the leaf $NH_3^-NH_4^+$ content: compare 23,6 ± 5,8 versus 20,1 ± 5,7 µg $NH_3^-NH_4^+$ per g fr wt leaf tissue (X ± STD DEV; N = 3 trees per treatment) from trees treated with and without urea, respectively. Foliar application of 1,5 g low biuret urea per tree to five-year-old rooted cuttings of the Washington navel orange of comparable size, increased the leaf $NH_3^-NH_4^+$ content of trees subjected to four, six or eight weeks of low temperature 1,7-, 2,2and 1,3-fold, respectively (Lovatt *et al*, 1988a).

DISCUSSION

The results of this research provide evidence that low temperature stress induces flowering in the Hass avocado. This demonstrates that the same temperature regime that is effective in inducing flowering in citrus is effective in avocado and suggests that avocado, like citrus, requires no more than four weeks of low temperature stress for floral induction.

While leaf $NH_3^-NH_4^+$ content of the Hass avocado increased in response to low temperature stress, the results of this research are unlike those we obtained with citrus, cucurbits, and alfalfa, and do not support our working hypothesis that $NH_3^-NH_4^+$ will accumulate in a manner that parallels the duration of severity of the stress.

The activity of the pathway for the *de novo* biosynthesis of arginine increased coincidently with the increased pool of available $NH_3^-NH_4^+$. This is coincident with our previous demonstration that the *de novo* arginine biosynthesis pathway is responsive to changes in leaf $NH_3^-NH_4^+$ content (Rabe & Lovatt, 1986) and our proposal that this pathway serves as a homeostatic mechanism to prevent ammonia from accumulating to toxic levels (Rabe & Lovatt, 1986). The activity of the pathway for *de novo* biosynthesis of arginine in the Hass avocado is very low (Table 1), relative to the basal activity of this pathway assessed by the same method in the youngest, fully expanded leaves of other plant species: 5 nmol $NaH^{14}C0_3$ incorporated into Arginine per g fr wt leaf tissue of the Washington navel orange *(Citrus sinensis);* 15 nmol for rough lemon *(Citrus limón);* 35 nmol for summer squash *(Cucúrbita pepo);* 15 nmol for *Phaseolus vulgaris;* and 17 nmol for *Phaseolus acutifolius.*

Whether the leaf content of $NH_3^-NH_4^+$ or a metabolite thereof, is important to the induction of flowering in the Haas avocado, cannot be determined from the results of this initial research. Four and eight weeks of low temperature stress caused equal concentrations of $NH_3^-NH_4^+$ to accumulate In Haas avocado leaves and resulted in equal floral intensity. Consistent with our demonstration that urea uptake and urease activity are probably too low in the leaves of the Haas avocado to be of physiological significance, we were unable to raise the $NH_3^-NH_4^+$ content of the tree through foliar application of low biuret urea at the same concentration that is effective with citrus. Thus, it was impossible to determine if floral intensity was influenced by the $NH_3^-NH_4^+$ status of the tree as it is in citrus.

Future research will determine if tree $NH_3^-NH_4^+$ status directly influences flowering in Hass avocado by artificially raising the $NH_3^-NH_4^+$ status of the tree through soil

application of urea or NH_4NO_3 or foliar application of NH_4NO_3.

There have been conflicting reports in the literature regarding the ability of leaves to take up foliar-applied urea. In a California study, young Hass avocado trees grown both in the field and in the lathhouse, received increasing concentrations of foliar applied urea. Total N content of the leaves was not significantly different among treatments (Galindo-Tovar, 1983). However, Aziz *et al* reported in 1974 that urea sprays were effective in raising total nitrogen levels of the Fuerte avocado. In 1987, Zilkar *et al,* in Israel, reported translocation of foliar-applied [^{15}N]urea from young leaves to the fruit of Fuerte and Hass avocados. The inconsistencies among reports may be explained by the choice of plant material or the method of urea application. New leaves may not have a well-developed cuticle. There may also be differences in the cuticles of Fuerte and Hass avocado leaves. Finally, using a brush to apply urea may disturb the surface waxes allowing greater permeability than would be obtained by simply spraying the urea on the leaf surface. Inclusion of a variety of different surfactants in the urea spray did not improve the limited uptake of urea by the leaves of the Hass avocado (Galindo-Tovar, 1983).

To resolve these discrepancies, a comparative study of the cuticle and urea metabolism of leaves of Fuerte and Hass avocado and Washington navel orange is underway in our laboratory.

CONCLUSION

Due to the lack of information regarding flowering in avocado, regulation of the flowering process is usually considered to be similar to that of citrus because both tree crops have a tropical phylogenetic background. The results of our research thus far demonstrates that NH_3-NH_4^+ accumulates during low temperature stress-induced flowering in Hass avocados as it does in citrus, but unlike citrus, the concentration of NH_3-NH_4^+ does not increase with time. In addition, leaf NH_3-NH_4^+ content of Haas avocado leaves doubled during bloom. This was not observed in citrus.

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