FLOWER INDUCTION IN THE HASS AVOCADO: IS IT INFLUENCED BY NH_2-NH_4^+? CAN IT BE REGULATED WITH FOLIAR UREA?

A Lecture Presented By

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This is the second of two lectures on the regulation of flowering in tropical and subtropical fruit tree crops. The first part of this lecture presented the results of research in my lab on the biochemical changes in key nitrogen compounds during induction and initiation of flowering in *Citrus* species. In addition, it provided evidence demonstrating that flower number can be manipulated by foliar application of low biuret urea in *Citrus* species. Please read the first lecture, entitled "Biochemical Changes in Key Nitrogen Compounds During Induction and Ontogeny of 'Washington' Navel Orange Flowers," to learn the full potential of our approach to the regulation of flowering.

The second half of my lecture presented the results of our research on the induction and initiation of flowering in the Hass avocado. Please note that we have not been studying the regulation of flowering in avocado for as long as we have been studying regulation of flowering in *Citrus*.

Flowering in citrus and avocado is recurrent under tropical and subtropical conditions unless synchronized into a well-defined period of concentrated bloom by external environmental conditions. Flower formation in citrus is promoted by drought and low temperature stress, followed by restoration of climatic conditions favorable for growth. In avocado, flowering usually follows a period of low temperature stress.

The impetus for our work with avocado stems from our studies with citrus, which provides evidence that changes in nitrogen metabolism influence stress-induced flowering in this species. Hopefully, you have read the lecture on regulation of citrus flowering. The results are summarized below:

(1) In citrus, leaf concentrations of NH_3-NH_4^+, but not total N or NO_3^-, increased in a manner that paralleled the duration or severity of low temperature and water-deficit stress (Lovatt *et al.*, 1988. Israel J. Bot. 37:181-188).

(2) Floral intensity was directly proportional to the concentration of NH_3-NH_4^+ accumulating during stress (Lovatt *et al.*, 1988. Israel J. Bot. 37:181-188).

(3) Foliar application of low biuret urea to artificially raise the NH_3-NH_4^+ content of trees subjected to minimal stress treatment (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.*, 1988. Israel J. Bot. 37:181-188).

(4) Neither the starch, nor 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.*, 1988. Proc. Int. Soc. Citriculture: In press).

The objectives of the research with avocado were to answer the following questions:

(1) Can flowering be induced in Hass avocado trees on clonal Duke 7 rootstock two years from budding by low-temperature stress at 15 to 18° C, 8-h day (500 $\mu E/m^2 \cdot sec$) and 10 to 13° C, 16-h night for 4 or 8 weeks?

(2) Does NH_3-NH_4^+ accumulate in the leaves of Hass avocado in response to low-temperature treatment?

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

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

(5) If number 4 above is affirmative, does artificially raising the NH_3-NH_4^+ content of the tree with foliar urea increase the flowering response of the Hass avocado?

Floral intensity was the same for trees treated for four or eight weeks of low temperature stress: Compare 1210 ± 258 flowers to 1253 ± 591 flowers per tree (x ± STD DEV, N-16 trees) subjected to four and eight weeks of low temperature,

respectively. 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).

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 during the second week of treatment for Hass avocado trees subjected to a total of four and eight weeks of low temperature, respectively. Maximum accumulation of NH_3-NH_4^+ in citrus was at the end of the low temperature stress and was 2636 μg per g dry wt. This is approximately 900 μg per g fr wt citrus leaf tissue. More than 10-fold more NH_3-NH_4^+ accumulates in citrus leaf tissue compared with avocado leaves. Leaf NH_3-NH_4^+ content remained high during the third week of stress and then decreased to the initial, pretreatment level of 35 μg per g fr wt leaf tissue. For trees subjected to either four or eight weeks of low temperature treatment, leaf NH_3-NH_4^+ content increased 2-fold to approximately 75 μg per g fr wt leaf tissue by week nine and remained significantly higher through the four-week period which culminated in full bloom ($p < 0.05$) (Figure 1). In citrus, leaf NH_3-NH_4^+ content is

maximum at the end of the low temperature treatment and decreases continuously during the four weeks of warm temperature leading to full bloom.

During the fourth week of low temperature treatment, there was a five-fold increase ($p < 0.05$) in the activity of the pathway for the *de novo* biosynthesis of arginine in the leaves of the Hass avocado. The increase in activity occurred two weeks after the initial increase in leaf NH_3-NH_4^+ content. For trees receiving four weeks of low temperature treatment this rate was 1.7 ± 1.1 nmoles $NaH^{14}CO_3$ incorporated into arginine. For trees receiving eight weeks of low temperature treatment, the rate was 2.1 ± 0.8 nmoles $NaH^{14}CO_3$ incorporated into arginine per g fruit leaf tissue during a 3-h incubation period. Leaves of 'Washington' navel orange trees subjected to four and eight weeks of low temperature treatment incorporated 5 and 20 nmoles $NaH^{14}CO_3$ into arginine per g fr wt leaf tissue during the 3-h incubation period. During week 10, trees previously subjected to either four or eight weeks of low temperature stress 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 (Figure 1). The *de novo* biosynthesis of arginine in citrus leaves decreased from the first week after transfer from the low temperature treatment to the warm temperature and continued to decrease during period leading to full bloom.

Starch content of Hass avocado leaves was very variable and did not change during the low temperature treatment. The average leaf content of starch during the stress was 31.0 ± 12.0 and 28.8 ± 8.5 mg per g fr wt leaf tissue for trees receiving four and eight weeks of low temperature treatment, respectively. Leaf starch content decreased during bloom with the lowest starch concentration coincident with full bloom (3.0 mg per g fr wt) (Figure 1).

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. Maximum uptake represented only 2.1% of the urea applied to the leaf surface. More than 90% of the [^{14}C]urea applied to the leaf was recovered in the water used to wash the surface of the leaf before counting the leaf. Thus, the urea was not metabolized by surface microorganisms on the leaf before it could be taken up by the leaf.

Consistent with the failure of Hass avocado leaves to take up [^{14}C]urea, foliar application of low biuret urea at the rate of 1.5 g 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) from trees treated with and without urea. Foliar application of 1.5 g low biuret urea to five-year-old rooted cuttings of the 'Washington' navel orange of comparable size increased the leaf NH_3-NH_4^+ conent of trees subjected to four or six weeks of low temperature 1.7- and 2.2-fold, respectively (Lovatt *et al.*, 1988. Israel J. Bot. 37:181-187).

To determine if leaves of Hass avocado could metabolize urea if we could get it into the leaf, we measured the activity of urease in Hass leaves. For both young and mature leaves from greenhouse and field grown trees, urease activity was insignificant. In all cases, urease activity was less than 0.05 nmoles $^{14}CO_2$ released from [^{14}C]urea per g fr wt leaf tissue during a 3-h incubation period. Urease activity assessed by the same method in citrus tissue was 138 nmoles $^{14}CO_2$ released from [^{14}C]urea per g fr wt. In a subsequent experiment we tried to induce urease activity in leaf tissue of the Hass avocado by incubating leaf discs in the presence of 10 mM

urea for 1h, 2h, 4h, 6h, 12h, 24h, 48h, and 5 days. Urease activity for all the different induction treatments was not significantly different from the time zero control value of 0.05 nmoles $^{14}CO_2$ per g fr wt leaf tissue. Thus, urease activity could not be induced in Hass avocado leaf tissue in the presence of urea.

The results of this research provide evidence that low-temperature stress induces flowering in the Hass avocado, demonstrate that the same temperature regime that is effective in inducing flowering in *Citrus* species is effective in avocado and suggest that avocado, like *Citrus*, requires no more than 4 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, it did not increase in a manner that paralleled the duration of the 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 or severity of the stress. Avocado is the first crop we have studied in which this was not the case.

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 consistent with our previous reports that the arginine *de novo* pathway is responsive to changes in tissue concentrations of NH_3-NH_4^+ (Rabe and Lovatt. 1986. Plant Physiol 81:774-779) and with our proposal that the *de novo* arginine biosynthetic pathway serves as a homeostatic mechanism to prevent ammonia from accumulating to toxic levels. The activity of the pathway for *de novo* biosynthesis of arginine in the Hass avocado is very low 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

$NaH^{14}CO_3$ incorporated into arginine per g fr wt leaf tissue of the 'Washington' navel orange (*Citrus sinensis*); 35 nmol for summer squash (*Cucurbita pepo*); 15 nmol for *Phaseolus vulgaris*; and 17 nmol for *Phaseolus acutifolius*.

Whether the leaf content of NH_3-NH_4^+, or a metabolite therefore, is important to the induction of flowering in the Hass avocado cannot be determined from the results of this research. Consistent with our demonstration that urea uptake and urease activity are probably too low in the leaves of the Hass avocado to be of physiological significance, we were unable to raise the NH_3-NH_4^+ of the tree through foliar application of low biuret urea at the same concentration that is effective with *Citrus* species. 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*. We are currently attempting to increase the NH_3-NH_4^+ content of Hass avocado trees through soil application of urea or NH_4NO_3, trunk drenches of urea, and foliar application of NH_4NO_3 during minimal stress treatments to determine the effect of tree NH_3-NH_4^+ status on flowering in avocado.

There have been conflicting reports regarding the ability of avocado leaves to take up foliar-applied urea. In a California study of young Hass avocado trees grown both in the field and in the lathhouse, increasing concentrations of urea were applied through two repeated applications. Total N content of the leaves was not significantly different among treatments (Galindo-Tovar, 1983). However, Aziz *et al.* reported in 1975 that urea sprays were effective in raising total nitrogen levels with 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 sprays did not improve the limited uptake of urea by leaves of the Hass avocado (Galindo-Tovar, 1983). Our results support those of Embleton and Galindo-Tovar that urea uptake and metabolism by Hass avocado leaves is so minimal that it is of little physiological significance.

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. If you will permit me to be a little facetious, the results of our research strongly suggest that comparing flowering in avocados and citrus is like comparing "apples and oranges."

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