DEMONSTRATION OF A CHANGE IN NITROGEN METABOLISM INFLUENCING FLOWER INITIATION IN CITRUS

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ABSTRACT
Changes in the leaf NH$_3$-NH$_4^+$ content were monitored during floral induction in Citrus. Five-year-old rooted cuttings of 'Washington' navel orange (Citrus sinensis) were induced to flower by subjecting the trees to 8 weeks of low temperature, 8-h day (500 μE m$^{-2}$ s$^{-1}$) at 15-18°C/16-h night at 10-13°C and by transferring them afterwards to 12-h day (500 μE m$^{-2}$ s$^{-1}$) at 24°C/12-h night at 19°C. Maximum bloom occurred 4 weeks after the transfer to the warmer temperature. The number of flowers and the NH$_3$-NH$_4^+$ content of leaves increased significantly (p < 0.05 and p < 0.01, respectively) with the length of the low temperature treatment. Sixteen-year-old 'Frost Lisbon' lemon trees (Citrus limon) on 'Troyer' clementine rootstocks (C. sinensis x Poncirus trifoliata) under commercial production were subjected to water-deficit stress of increasing severity. The intensity of flowering and the leaf NH$_3$-NH$_4^+$ content increased with the severity of the stress. The foliar application of low biuret urea to trees subjected to less than 8 weeks of low temperature stress or to moderate water-deficit stress, i.e., to treatments resulting in a low degree of flowering, increased the NH$_3$-NH$_4^+$ content of the leaves and doubled the intensity of flowering.

In this paper, a brief summary of the current knowledge on flowering in citrus is presented, a new hypothesis on the regulation of flower initiation is postulated, and some results of experiments testing this hypothesis are reported along with discussion of future research. As we review the literature, the reader cannot help noting the considerable contribution of Prof. Shaul Monselise to our understanding of flowering in citrus.

In Citrus sinensis, flowers are borne in cymose inflorescences of one to many flowers with zero to many leaves (Lord & Eckard, 1985; Monselise, 1985). Typical inflorescences terminate with a flower and contain ca. six nodes with zero to five axillary flowers and zero to five developing leaves (Lovatt et al., 1984; Lord & Eckard, 1987). Shoots of ten nodes terminating in a flower rarely have more than nine flowers or nine leaves under conditions occurring in Riverside, California, USA (Lovatt et al., 1984) and Mildura, Victoria, Australia (Sauer, 1951). Citrus inflores-

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ences arise by elongation of axillary buds borne principally on 1-year-old wood, although substantial numbers arise on 2-year-old wood. Flowering on older wood is limited (Sauer, 1951).

Under tropical and subtropical conditions, flowering is recurrent due to the tropical phylogenetic background of Citrus species (Monselise, 1985), unless synchronized into a well-defined period of concentrated bloom by external conditions (Monselise & Goren, 1969; Goldschmidt & Monselise, 1972; Monselise, 1978). Flower formation in Citrus species is promoted by drought or low temperature, followed by restoration of climatic conditions favorable to growth (Monselise & Halevy, 1964; Monselise & Goren, 1969; Monselise, 1978, 1985; Southwick & Davenport, 1986). The action of these abiotic factors, together with that of cultural practices that promote flowering in Citrus species, i.e., girdling (Monselise, 1985), graft incompatibilities resulting in weak rootstocks (Mosse, 1962), confining of root systems in small pots (Furr et al., 1947), and root pruning (Monselise & Halevy, 1964; Monselise, 1985), support the idea that the cessation of root growth is an essential prerequisite to flowering in Citrus as first proposed by Monselise (1947). Subsequent research employing growth regulators and antimitabolites has established the inhibiting effects of gibberellins (GA) on flowering in Citrus species (Monselise & Halevy, 1964; Moss, 1970; Nir et al., 1972; Goldschmidt & Monselise, 1972; Monselise, 1978; Guardiola et al., 1977, 1982; García-Luis et al., 1986; Lord & Eckard, 1987). On this ground, Goldschmidt and Monselise (1972) suggested that from their inception in the adult phase, all Citrus buds are determined to flower, but the presence of GA may inhibit their flowering. This hypothesis is supported by the fact that compounds inhibiting plant growth and those reducing GA biosynthesis promote flowering (Monselise et al., 1966; Nir et al., 1972). In addition, a link between the abiotic factors promoting flowering in Citrus species and a reduced GA content was established by the work of Nir et al. (1972) demonstrating that GA3 inhibits drought-induced flowering in 'Eureka' lemons, while the growth retardant Cycocel (trimethylammoniumchloride) can replace the water-stress treatment and promote flowering in lemons. Extrapolation led to the idea that inhibition of root activity results in a reduction of the synthesis or transport of GA (Monselise, 1978, 1985). However, since antimitabolites of nucleic acid and protein synthesis such as chloramphenicol, succinate, 5-fluorodeoxouridine, and bromacil (5-bromo-3-sec butyl-6-methyluracil) enhance flowering in Citrus sinensis (Goren & Monselise, 1969; Jona et al., 1971), the role of drought, low temperature, and inhibitors of GA biosynthesis in promoting flowering may be due to their more general effect of overall growth inhibition (Iwasaki et al., 1959). This would cause a temporary inhibition of mitosis followed by rapid division conducive to flower bud formation (Bernier et al., 1970; Stebbins, 1965) once the inhibiting factor has been removed, as suggested by Goren in 1978 (Monselise, 1985).

In our laboratory, we have investigated the hypothesis that any stress inhibiting the growth of a plant will result in the accumulation of ammonia (measured as the combined pool of \( \text{NH}_3-\text{NH}_4^+ \)) to an extent directly correlated with the severity or duration of the stress. Four observations suggested that this phenomenon may be related to the induction of flowering in Citrus and in other tropical and subtropical
evergreen tree crops: (1) plants growing in a stressful environment often flower prior to death; (2) in *Citrus* and in other tropical and subtropical tree crops, flowering is promoted by low temperature and water-deficit stress; (3) the degree of flowering in *Citrus* is directly proportional to the severity or duration of the stress (Southwick & Davenport, 1986); and (4) nitrogen fertilization applied at the moment of stress removal enhances the degree of flowering (Monselise, 1985).

We proposed and tested the following hypotheses: (1) ammonia accumulation is an important factor in the regulation of flower initiation in *Citrus* species; (2) ammonia accumulation is an early stress-linked event common to low temperature and water-deficit stress induced flowering in *Citrus*; and (3) the intensity of flowering can be increased under minimal stress conditions if the ammonia content of the tree is artificially raised by foliar application of low biuret urea.

**MATERIALS AND METHODS**

*Low temperature stress.* Five-year-old rooted cuttings of the ‘Washington’ navel orange (*Citrus sinensis* [L.] Osbeck) grown in pots containing ca. 19 l of University of California soil mix were induced to flower by subjecting the trees to low temperature, 8-h day (500 µE m⁻² s⁻¹) at 15–18°C/16-h night at 10–13°C, for 4, 6, or 8 weeks and by transferring them afterwards to 12-h day (500 µE m⁻² s⁻¹) at 24°C/12-h night at 19°C (Moss, 1969). Control trees were maintained under the warmer conditions described above for the total length of the experiment. Each experiment consisted of 10 trees per treatment. Trees were watered once a week with half-strength Hoagland's nutrient solution and as needed with distilled water. Urea-treated trees received one foliar application of low biuret urea at the rate of 1.5 g per tree upon transfer to the warmer temperature. Maximum bloom occurred 4 weeks thereafter.

*Water-deficit stress.* Sixteen-year-old trees of 'Frost Lisbon' lemon (*Citrus limon* [L.] Burm. f.) on 'Troyer' citurage rootstocks (*C. sinensis* × *Poncirus trifoliata* [L.] Raf.) under commercial production in the San Joaquin Valley, California, were subjected to the following treatments beginning mid-June:

1) No treatment: well-watered control trees (w > −1 MPa);
2) Severe water stress followed by moderate water stress: water was withheld and the trees were stressed to w less than −3 MPa for 20 days, maintained at less than −2 MPa for further 40 days, and then rewatered quickly;
3) Severe water stress of short duration: water was withheld and the trees were stressed to approximately −3 MPa for 30 days, and then rewatered quickly;
4) Moderate water stress: water was withheld and the trees were stressed to approximately −2 MPa for 10 days, irrigated for 50 days at 25% of the rate of well-watered control trees, and then rewatered quickly;
5) Same as treatment 4, with foliar application of low biuret urea at the rate of 0.1 kg nitrogen per tree at the end of the 10 days without water.

The experiment was performed with single tree replicates, four trees per treatment. Maximum bloom occurred 4 weeks after removal of the stress.
Physiological parameters. The leaf NH$_3$–NH$_4^+$ content was monitored weekly according to the method of Rabe and Lovatt (1986a); the water status of the trees was monitored as predawn water potential (ψ) by a pressure bomb; the total number of flowers per tree was determined by counting every flower on each tree. For 16-year-old trees, counts were made in parallel vertical sectors from the ground to a height of 2 m. The number of flowers was confirmed by determining the percentage of fruit harvested per total flowers produced on tagged branches (10 per tree) and the total number of fruit harvested per tree.

RESULTS

NH$_3$–NH$_4^+$ accumulated in the leaves of *C. sinensis* in a manner parallel to the duration and severity of the low temperature stress (Table I). After 4, 6, and 8 weeks of treatment, the leaves of ‘Washington’ navel orange trees maintained at the lower temperature contained an amount of NH$_3$–NH$_4^+$ higher by 103 μg, 127 μg, and 216 μg per g dry weight leaf tissue, respectively, than that in the leaves of trees maintained at the warmer temperature (p < 0.01; paired analysis by Student’s *t* test).

In the commercial lemon trees subjected to the most severe water stress treatment (maintained by withholding water at predawn water potentials as low as −3 MPa for 20 days and then for 40 days at about −2 MPa), the leaf NH$_3$–NH$_4^+$ content was higher by 209 μg per g dry weight leaf tissue than in well-watered controls. In the trees subjected to severe water stress only for a short interval, i.e., stressed to −3 MPa for 30 days and rewatered, and in the trees subjected to moderate water stress (−2 MPa) for

<table>
<thead>
<tr>
<th>Duration of low temperature stress (weeks)</th>
<th>Leaf NH$_3$–NH$_4^+$ content$^1$</th>
<th>Average number of flowers per tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without urea$^2$</td>
<td>With urea$^3$</td>
</tr>
<tr>
<td>0</td>
<td>456a</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>559b</td>
<td>166%</td>
</tr>
<tr>
<td>6</td>
<td>583b</td>
<td>215%</td>
</tr>
<tr>
<td>8</td>
<td>672c</td>
<td>134%</td>
</tr>
</tbody>
</table>

$^1$Determined during the first week after transfer to the warmer temperature.

$^2$Values within a column followed by different letters are significantly different at *p* < 0.05 by Duncan’s multiple range test.

$^3$Low biuret urea was applied at the rate of 1.5 g per tree at the end of the low temperature treatment. Figures represent percentages of the values recorded in the trees where no urea application had been performed.
60 days, leaf NH$_3$-NH$_4^+$ levels were higher by 127 µg and 164 µg per g dry weight leaf tissue, respectively, than in the well-watered control trees (Table II).

**TABLE II**
Effect of water-deficit stress and of foliar application of urea on the leaf NH$_3$-NH$_4^+$ content and on the flowering of lemon

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average leaf NH$_3$-NH$_4^+$ content during stress (µg/g dry weight)$^1$</th>
<th>Average number of flowers per tree$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: no water stress</td>
<td>519c</td>
<td>14b</td>
</tr>
<tr>
<td>($\psi &gt; -1$ MPa)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe water stress of short duration</td>
<td>646b</td>
<td>53b</td>
</tr>
<tr>
<td>($\psi = -3$ MPa for 30 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe followed by moderate water stress</td>
<td>728b</td>
<td>611a</td>
</tr>
<tr>
<td>($\psi = -3$ MPa for 20 days, then</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\psi = -2$ MPa for 40 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate water stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>($\psi = -2$ MPa for 50 days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without application of urea</td>
<td>683b</td>
<td>165b</td>
</tr>
<tr>
<td>With application of urea$^3$</td>
<td>863a</td>
<td>426a</td>
</tr>
</tbody>
</table>

$^1$Values within a column followed by different letters are significantly different at $p < 0.05$ by Duncan's multiple range test.

$^2$Foliar application of urea at a rate of 0.1 kg nitrogen per tree.

The intensity of flowering was also related to the duration and severity of the stress. Five-year-old rooted cuttings of the 'Washington' navel orange maintained at the warmer temperature for 8 weeks produced six flowers per tree, while in trees subjected to the lower temperature for 4, 6, and 8 weeks, the number of flowers per tree increased with the length of the treatment ($p < 0.05$) (Table I).

In lemon trees subjected to water-deficit stress, the intensity of flowering increased with the severity and duration of the stress and with the increase in leaf NH$_3$-NH$_4^+$ content (Table II). The total number of flowers per tree correlated significantly ($p < 0.05$) with the average concentration of NH$_3$-NH$_4^+$ per g dry weight leaf tissue during the stress period. In addition, the yield from the bloom induced by water-deficit stress was significantly correlated ($p < 0.05$) with the average leaf NH$_3$-NH$_4^+$ content during stress.

It is important to note that the low temperature treatment did not induce water-deficit stress. Throughout the 8 weeks of exposure to low temperature, the average predawn water potential of 5-year-old rooted cuttings of 'Washington' navel orange was the same as in the warm temperature control trees: $\psi = -0.7 \pm 0.2$ MPa.

To provide additional evidence supporting the role of ammonia in flower initiation
in *Citrus* species, trees were subjected to minimal stress treatments that did not induce a significant number of flowers. For the 5-year-old rooted cuttings of the 'Washington' navel orange, the low temperature treatment was limited to only 4 or 6 weeks. The 16-year-old lemon trees in commercial production were maintained at −2 MPa by deficit irrigation. In half of the trees, the leaf NH$_3$–NH$_4^+$ content was increased artificially by foliar application of low biuret urea. In both experimental systems, the application of urea increased significantly the leaf NH$_3$–NH$_4^+$ content and the number of flowers per tree (Tables I and II).

**DISCUSSION**

The results of this research provide two additional examples supporting our previous hypothesis that any stress inhibiting the growth of a plant will cause the accumulation of ammonia (Rabe & Lovatt, 1986a). The findings also demonstrate that accumulation of ammonia (measured as NH$_3$–NH$_4^+$) is an early stress-linked event influencing floral initiation in *Citrus* species, in response to both low temperature and water-deficit stress.

The considerable variation in the flowering response to the two stress treatments may be due, in part, to differences in the nitrogen and carbohydrate status of the trees before treatment. For the several low temperature treatments conducted throughout a year, rooted 'Washington' navel orange cuttings were obtained from several different sources. It is now clear that the environmental conditions before the initiation of treatment can alter the basal level of ammonia and starch.

A linear regression analysis of all the treatments performed in 'Washington' navel orange trees, including the warm temperature controls, the trees induced to flower by low temperature stress, and the trees receiving urea sprays, demonstrated a significant correlation (p < 0.0001) between the total number of flowers per tree and the NH$_3$–NH$_4^+$ content of the youngest, fully expanded leaves during the first week after the transfer of the trees to the warmer temperature; the analysis covered more than 100 trees used in the experiments (including trees not mentioned in Table I). Leaf NH$_3$–NH$_4^+$ concentrations varied from 389 to 2636 µg per g dry weight and the number of flowers per tree ranged from 4 to 3065. Low temperature stress significantly increased the leaf NH$_3$–NH$_4^+$ content regardless of the basal level at the start of the low temperature treatment (p < 0.01). In addition, the linear regression analysis suggests that there is a level of ammonia below which flower initiation does not occur: ca. 389 µg per g dry weight leaf tissue.

Preliminary results concerning leaf starch content suggest that there is a threshold level for starch below which floral initiation does not occur despite the accumulation of NH$_3$–NH$_4^+$. It has been suggested previously that carbohydrate levels are a factor limiting flower formation in *Citrus* (Ogaki et al., 1963; Goldschmidt & Golomb, 1982; Goldschmidt et al., 1985). The changes in carbohydrate metabolism during stress-induced flowering in *Citrus* are also being studied in our laboratory.

For convenience and for the development of commercial analyses in the future, we have monitored changes in nitrogen and carbon metabolism occurring in leaves, using
mostly the youngest fully expanded leaves in order to avoid the secondary effects of leaf senescence and mineral nutrient deficiencies (Rabe & Lovatt, 1984, 1986a). Whether the leaves contribute to floral initiation or simply reflect parallel changes occurring in the buds remains to be determined and represents the subject of future research in our laboratory.

Currently, we are investigating the relationship between ammonia accumulation, increased de novo biosynthesis of arginine, and the subsequent conversion of arginine to polyamines. Previous work in our laboratory has demonstrated that conditions limiting the growth of a plant and resulting in the accumulation of ammonia also result in an increased activity of the pathway for the de novo biosynthesis of arginine (Rabe & Lovatt, 1986a,b).

It is well documented in the literature that in plants arginine is the precursor of polyamines, e.g., putrescine, spermidine, and spermine, which accumulate in plant tissues in response to stress (Galston, 1984). Recently, the levels of these polyamines have been shown to correlate with cell division and morphogenesis in many plant systems, as well as with flower initiation in apple (Edwards, 1986). Our current working hypotheses are: (1) the accumulation of ammonia during stress results in increased biosyntheses of arginine and polyamines and subsequently in an increased rate of cell division following the release of *Citrus* plants from stress; and (2) these physiological changes and the subsequent rapid increase in cell division are prerequisites to flower initiation in *Citrus*.

REFERENCES


