The inhibitory effect of gibberellic acid on flowering in Citrus

J. L. Guardiola, C. Monerri and M. Agusti


The application of gibberellic acid (GA$_3$) at any time from early November until bud sprouting, resulted in a significant inhibition of flowering in the sweet orange (Citrus sinensis (L.) Osbeck) and the Satsuma (Citrus unshiu Marc.) and Clementine (Citrus reticulata Blanco) mandarins. Two response peaks were evident: the first occurred when the application was timed to the translocation of an unknown flowering signal from the leaves to the buds. The second occurred during bud sprouting, at the time the flower primordia were differentiating. From the pattern of flowering, it appears that the mechanism of inhibition was similar irrespective of the timing of GA$_3$ application. There was an initial reduction in bud sprouting affecting selectively those buds originating leafless inflorescences. An additional inhibition resulted in a reduction in the number of leafy inflorescences with an increase in the number of vegetative shoots, suggesting the reversion of a floral to a vegetative apex. The inhibited buds sprouted readily in vitro but invariably vegetative shoots were formed. A continuous influence of the sustaining branch is necessary to keep the flowering commitment of the buds; irreversible commitment occurs when the petal primordia are well differentiated.

Additional key words - Bud dormancy, bud sprouting, Citrus reticulata, Citrus sinensis, Citrus unshiu.

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Introduction

As in many fruit trees, flowering in Citrus is inhibited by the application of exogenous GA$_3$. Whilst a practical use of this property is being made to regulate flowering in sweet orange (Moss and Bevington 1977), the mechanism of inhibition is not fully understood. An interference with flower initiation has been suggested (Monselise and Halevy 1964, Moss 1970), since GA$_3$ is most effective during the winter rest period, at the time when some flowering factor is translocated from the leaves to the buds (Furr and Armstrong 1956, Ayalon and Monselise 1960, Sánchez-Capuchino and Casanova 1973), or during a period of water stress, which readily induces flowering in lemon (Nir et al. 1972). However, later applications of GA$_3$, timed to bud sprouting when flower differentiation is detectable under the microscope, are also effective (Nir et al. 1972, Goldschmidt and Monselise 1972, Guardiola et al. 1980). This response may involve the reversion of a flower bud to a vegetative apex, and the reduction in flowering achieved is related to the increase in shoot length. In contrast, the earlier GA$_3$ applications do not influence vegetative growth.

Thus, there is a possibility that GA$_3$ may inhibit flowering by acting at two different stages of flower formation. This has been tested in the present study by comparing the response of three Citrus species to GA$_3$ applications from early November to bud sprouting. Special attention was paid to the anatomical changes occurring in the buds and to their behaviour when excised from the tree in order to gain more insight into the mechanism of action of GA$_3$ at the bud level.

Abbreviations - 2,4-D, 2,4-dichlorophenoxyacetic acid; GA$_3$, gibberellic acid.

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Sizing in Citrus

The inhibitory effect of gibberellic acid (16-142) on flowering in the sweet orange (Citrus sinensis [L.] Osbeck) and the seedless Clemene-
tine (C. reticulata Blanco) and Owari Satsuma (Citrus unshiu Marcovitch) mandarins. Adult trees about 15-25 years old growing outdoors and budded on sour orange rootstocks were used in every experiment. GA3 sprays were periodically applied to the whole tree from early November until the time of bud sprouting. Each treatment consisted of a single GA3-spray applied to 5 or 6 replicates of whole-tree plots in a randomized block design. The concentration of the GA3 solution was 100 mg l-1 in the sweet orange experiment and 10 mg l-1 for the mandarins. A non-ionic wetting agent (Mojafar, manufactured by Quimiver), at a concentration of 0.1% (v/v) was included, this wetting agent itself having no effect on flowering.

Bud sprouting was measured in early spring, a few days before flower opening. Six to ten branches distributed uniformly around the tree and having more than 2,000 buds were selected beforehand, and the shoots initiated were counted and classified as previously reported (Guardiola et al. 1977). From the number of shoots developed and the number of flowers per shoot, the total number of flowers could be calculated. The results were expressed in shoots and flowers per 100 nodes to compensate for the differences in size of the branches selected for counting. Only the buds being less than 24 months old at the time of sprouting were considered for the countings and calculations, since the contribution of older buds to the spring flush was negligible. The influence of the age of the bud on sprouting was determined in the sweet orange experiment. An additional experiment was performed using Navelate orange trees (Tab. 2). GA3 was tested at the times of maximal sensitivity, in mid-November and at sprouting, at lower concentrations, 25 mg l-1 and 10 mg l-1, respectively. Ten whole tree replicates were used, the rest of the experimental conditions being as above.

Bud sprouting in vitro

Bud explants, consisting of the whole bud without the sustaining leaf together with a 15 mm long flap of bark and wood, were planted under sterile conditions on a solid (2% agar) Murashige and Skoog (1962) medium and grown at 26 ± 1°C under continuous illumination (circa 25 W m-2) provided by Sylvania Gro-lux lamps. In view of the inhibitory effect of high temperatures on flower development (Moss 1969), growth at a lower temperature (19 ± 1°C) was tried as well. A 2 cm2 piece of leaf lamina was kept in some explants to determine its influence on bud sprouting.

While this procedure was adequate to compare the characteristics of the shoots sprouted, the large number of contaminated explants made the determination of the time-course of sprouting uncertain. Neither the use of a more drastic procedure for surface-sterilization (Giladi et al. 1979), nor the previous application to the tree of antibiotics and fungicides proved useful with our plant material. Finally, the time course of sprouting was followed by forcing the buds on a bed of sterile moist vermiculite. At variance with the observations of Altman and Goren (1977), we did not find sucrose essential for the initial bud emergence. The stage of bud dormancy was estimated from the time necessary to reach 50% sprouting. In the case where some buds had already initiated growth at the time of planting, these were considered to have sprouted in 0 days in the calculations.

Histological observations

Coinciding with the GA3-treatments, five branches per tree from the autumn flush of growth were collected and fixed in ethyl alcohol-glacial acetic acid (3:1, v/v). The five most apical buds were excised and sectioned with a freezing microtome or embedded in paraffin prior to cutting (Johansen 1940), and stained with safranin and fast green.

Results

Anatomical changes during bud sprouting

The resting bud is a complex structure consisting normally of three buds of different sizes with well differentiated leaf primordia surrounded by phylls, and usually of a thorn. The biggest bud, of 250 ± 10 μm long up to the top of the apex and with 7-8 leaf primordia which covered completely the apex, was invariably the first to sprout on each node, and the one used in the measurements. Neither increase in size nor indication of flower differentiation could be detected for the first three samplings (Fig. 1). By the fifth sampling the most terminal bud had enlarged significantly. In later samplings sprouting gradually moved towards the base of the branch.
The differentiation of the flower parts was related to the size of the sprouting shoot. The primordia of the sepals were clearly visible when the shoots were 650 μm long, and at that moment the biggest leaf primordia had enlarged up to about 1 mm. Petal primordia were well differentiated on 1.3 mm long shoots, the biggest leaf primordia being at least 2–3 mm long.

**GA₃ effects on flowering**

The application of GA₃ at any time from late October up to the bursting of buds reduced flowering significantly in the three species studied (Fig. 2). Two response peaks were evident. The first occurred during the winter rest period and it was earlier for *C. sinensis* and *C. reticulata* (end of November and early December) than for *C. unshiu* (end of December and early January). At that time the dormancy of the buds was deepest as measured by the time it took to sprout in vitro (Fig. 1). Microscopic examination did not show evidence of growth nor flower differentiation. The second response peak occurred during bud sprouting, when the developing shoots were about 1.0 mm long; shortly afterwards they became insensitive to GA₃ as regards flowering (not shown).

**GA₃ effects on sprouting**

Following a GA₃ application there was a reduction in the number of shoots developed during the natural spring flush, peak effects coinciding with the effects on flowering (Fig. 2). This effect was mainly due to a reduction in the proportion of buds sprouted. The influence of GA₃ on the number of shoots developed per sprouted bud was much smaller and not significant (Tab. 1). This inhibition of bud sprouting was observed irrespective of bud age, a factor which had a marked influence on natural sprouting.

A similar pattern of inhibition was obtained irrespective of the timing of the GA₃ application (Tab. 2). As previously reported for *C. sinensis* (Guardiola et al. 1977), this inhibition was maximal for leafless shoots and for leafy multiflowered ones. Some reduction in the number of leafy single flowered shoots was observed in *C. unshiu*, a response which we have confirmed in additional experiments.

**Fig. 1.** Seasonal change in dormancy, as measured by the time to sprout in vitro, and natural bud emergence as affected by the position of the bud on the branch, in the Navelate sweet orange. The buds have been numbered from the apex of the branch. Mean data for GA₃-treated and untreated trees, the effect of the hormone on these parameters not being significant.

**Fig. 2.** The influence of the time of GA₃ application on the sprouting (O) and flowering (Δ) in the Washington navel orange and the Satsuma and Clementine mandarins. The application was timed to bud sprouting. The standard error of the mean is given for the number of shoots developed.
The influence of bud age on spring sprouting and its inhibition by GA$_3$ in the Washington navel orange. Buds located on branches formed during the spring (12 months old), summer (8 months old) and autumn (5 months old) flushes of growth. Only sprouted buds were used to calculate the number of shoots developed. NS — not significant.

<table>
<thead>
<tr>
<th>Bud age at sprouting</th>
<th>5 months old</th>
<th>8 months old</th>
<th>12 months old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sprouted buds (%)</td>
<td>Shoots per sprouted bud</td>
<td>Sprouted buds (%)</td>
</tr>
<tr>
<td>Untreated</td>
<td>86</td>
<td>1.86</td>
<td>52</td>
</tr>
<tr>
<td>GA$_3$-treated</td>
<td>73</td>
<td>1.84</td>
<td>36</td>
</tr>
<tr>
<td>Level of significance</td>
<td>$P \leq 0.01$</td>
<td>NS</td>
<td>$P \leq 0.01$</td>
</tr>
</tbody>
</table>

Tab. 1. The influence of the level of flowering on the pattern of shoot sprouting in GA$_3$-treated Navelate orange trees. GA$_3$ applications as a full coverage spray of the whole tree at a concentration of 25 mg l$^{-1}$ on Nov. 29 or Dec. 15. Results, in thousands of flowers and shoots per tree, are the mean from 9 replications ± SE.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flowers</th>
<th>Shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vegetative</td>
<td>Mixed-type</td>
</tr>
<tr>
<td>Control (untreated)</td>
<td>114±9</td>
<td>3.5±0.4</td>
</tr>
<tr>
<td>GA$_3$, Dec. 15</td>
<td>80±5</td>
<td>3.7±0.2</td>
</tr>
<tr>
<td>GA$_3$, Nov. 29</td>
<td>62±4</td>
<td>5.1±0.7</td>
</tr>
</tbody>
</table>

Tab. 3. The influence of GA$_3$ application on shoot sprouting. The standard error of the number of shoots developed.

Bud sprouting in vitro

Excised buds readily sprouted in vitro, the time required to achieve 50% sprouting depending on the time of collection (Fig. 1). For the buds collected in mid-January bud sprouting was enhanced and the final percentage of sprouting increased by raising temperature from 19°C to 26°C and by the presence of the leaf lamina in the explant (Fig. 3). The latter also increased slightly the number of shoots initiated per bud explant (data not shown). Almost invariably vegetative growth was obtained in vitro, although more than 95% of the...
Tab. 4. The percentage of bud sprouting on the tree and in vitro for the six most apical buds from autumn branches of untreated and GA<sub>3</sub>-treated Navelate orange trees. Sprouting percentages in vitro are the means from the six collections shown ± SE of the mean.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Attached</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>96±1</td>
<td>86±2</td>
</tr>
<tr>
<td>GA&lt;sub&gt;3&lt;/sub&gt;-treated</td>
<td>87±2</td>
<td>89±2</td>
</tr>
</tbody>
</table>

shoots sprouted from these buds on the tree bore flowers. Only for buds collected in mid-January were flower buds formed in vitro in less than 2% of explants; at that time more than 50% of the buds already showed visible growth when collected in the field. In the nutrient medium the shoots grew vigorously in several weeks, reaching a maximal elongation of about 2 cm (Fig. 4). On moist vermiculite, leaf expansion was markedly reduced and the developing leaves showed characteristic epinastic curvature. Buds from control and GA<sub>3</sub>-treated trees gave the same percentage of sprouting in vitro (Tab. 4). Final sprouting was unaffected by dipping the buds in a 100 mg l<sup>-1</sup> GA<sub>3</sub> solution prior to planting, but this treatment enhanced the sprouting of buds collected in mid-January when grown at 26°C (Fig. 3). This treatment exerted its typical effect on internode elongation while leaf expansion was almost completely suppressed (Fig. 4).

Discussion

The inhibition of flowering by exogenous GA<sub>3</sub> in Citrus plants presents two distinct maxima during the period extending from early November until bud sprouting begins. The first maximum is almost exactly timed with the beginning of the translocation of an unknown flowering signal from the leaves to the buds, a process which takes place one month later for C. unshiu than for the two other species studied (Sánchez-Capuchino and Casanova 1973). The second one, which corresponds to the late GA<sub>3</sub> effect reported by several authors (Goldschmidt and Monselise 1972, Nir et al. 1972, Guardiola et al. 1980), coincides with the onset of the morphological differentiation of the flowers. The smaller but significant inhibition found outside these periods of peak response could be due to the persistence of the GA<sub>3</sub> in the plant and/or an heterogeneity in the development of the buds. In agreement with previous reports (Abbott 1935, Ayalon and Monselise 1960), we found flower differentiation linked to bud sprouting. Irreversible commitment of buds to flower formation is apparently very late, well after the differentiation of the sepal primordia.

The similar flowering pattern, irrespective of the timing of the GA<sub>3</sub> application, suggest the existence of a common mechanism(s) of inhibition. As previously shown, selection of a mechanism depending on the position of the bud (Tab. 1) shoots compensates for GA<sub>3</sub>-inhibited flowering by stimulating the rest of the tree. This allometric effect is also observed in other trees and when the whole tree is GA<sub>3</sub>-inhibited.

Fig. 3. The influence of the culture conditions on bud sprouting in vitro in the Navelate orange. Top. - Leafy (○) and leafless (△) explants forced at 19°C. Bottom. - Leafless explants forced at 19°C (circles) and 26°C (triangles) with (filled symbols) and without (open symbols) the application of GA<sub>3</sub>. Buds collected on January 9.

Fig. 4. Growth of bud explants in the culture medium. Photograph taken after 24 days culture. Lower row: control buds. Upper row: buds dipped in a 100 mg l<sup>-1</sup> GA<sub>3</sub> solution prior to planting.
Flowering appears to be a complex phenomenon which may involve hormone and metabolite levels in the buds as well as an internal condition possibly related to its location on the tree. Morphological differences are not detectable until the development of flower primordia. Well developed leaf primordia are present even in those buds forming leafless shoots, detectable growth occurring before their final abortion. GA₃ may interfere with flower development at two different stages at least, although its role may not be unique since it is mimicked by 2,4-D, an auxin-like growth regulator (Guardiola et al. 1977). Our results are best explained if it is assumed that the potential development of the bud is committed before the period of time studied. The stimulus coming from the leaf allows, and the application of GA₃ interferes with, the expression of the bud potential. Any interference in the development of the buds results in the inhibition of sprouting and, for the less sensitive buds, in a reversal to the vegetative condition. Evidence for this hypothesis comes from the time-course of flower inhibition by the fruit (Guardiola, J. L., Almela, V. and Agustí, M., unpublished results). Thus, giberellin levels in the plant, whether endogenous or exogenous, may determine the intensity of flowering as suggested (Goldschmidt and Monselise 1972), but the shoot type to be formed would depend on another kind of endogenous control.

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References


Introduction

It is almost twenty years ago that Murashige and Skoog (1962) first reported that 10^{-4} M 6-(4-hydroxy-3-butenyl) adenine (HBA) was a highly effective plant growth regulator. In cultured cells of potato, it was reported to be active at a concentration as low as 10^{-10} M (Skoog et al. 1967). Since then, many other plant tissues have been shown to be highly responsive to HBA.

In the present study, the effects of HBA on the growth and development of Chinese cabbage (Brassica campestris L.) were investigated. The results indicate that HBA is a potent growth regulator for this crop. In particular, it was found that HBA can induce the formation of new shoots from dormant roots.

References


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