Polyamines in buds of apple as affected by temperature and their relationship to bud development

L.H. Zhu*,1, J. Tromp, A.C. van de Peppel, O. Borsboom

Department of Horticulture, Wageningen Agricultural University, Haagsteeg 3, 6708 PM Wageningen, Netherlands

Accepted 27 March 1999

Abstract

The time course of changes in the concentrations of arginine and polyamines in spur buds of apple (Malus domestica Borkh.) was studied. The trees were exposed to six temperature treatments: 13°C and 13°C; 13°C then 20°C; 20°C and 20°C; 20°C then 27°C; 27°C and 27°C, and 27°C then 13°C in two successive periods of 6–7 and 12 weeks, starting at full bloom. Shoot growth and flowering were recorded as well. In the first six weeks, the arginine concentration in spur buds did not differ much between temperatures, but thereafter an increase occurred, especially at the 27–27°C and 20–27°C regimes. The drop in temperature from 27°C to 13°C at 6–7 weeks after bloom resulted in an increase of arginine that surpassed the 13–13°C and even the 27–27°C values.

In general, the time course of changes in the concentration of total polyamines (PAs) was not pronounced and lacked consistency. The relation to temperature was the inverse of that of arginine; the level became lower with the rise in temperature. More than 80% of PAs were accounted for by spermidine and spermine. The pattern for concentrations for spermidine and putrescine in relation to temperature was generally similar to that for total polyamines, but the reverse holds for spermine. The rise of temperature at 6–7 weeks after bloom reduced the contents of spermidine and putrescine, but did not influence spermine. Conversely, the drop of temperature (27°C to 13°C) increased the spermidine and putrescine levels, and produced a decline in spermine.

The results are discussed in terms of a ‘passive’ storage function for arginine and a more ‘active’ function for polyamines in modulating bud growth activity. The relationship between polyamines and flower-bud formation is also discussed. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Apple; Arginine; Flower-bud formation; Polyamines; Temperature
1. Introduction

Polyamines (PAs), mainly spermidine (Spd), spermine (Spm) and putrescine (Put), are ubiquitous in plant tissues and have been shown to be related to a number of physiological and morphogenic processes in plants including reproductive development (Altman et al., 1983; Smith, 1985; Kakkar and Rai, 1993). Kaur-Sawhney et al. (1988) found that flower formation in thin-layer tissue cultures of tobacco depended on a high Spd level. Kakkar and Rai (1993) and Rey et al. (1994a) suggested that Spm accumulation may be used as a physiological marker in flower induction. Rey et al. (1994b) found that high endogenous levels of Spd and Spm and low levels of Put in buds and leaves of hazelnut trees seemed to be related to flowering, rapid growth and leaf expansion, while the opposite levels seemed to be associated with the initiation of dormancy. In citrus, reproductive structures contained 80% of the total PAs (Kushad et al., 1990). In young apple trees application of PAs via cut pedicels enhanced the number of flower buds (Rohozinski et al., 1986). Spraying PAs also favoured flower-bud formation of apple (Costa and Bagni, 1983), but in a later experiment this result could not be confirmed (Costa et al., 1986). Recently, in an experiment with apple, wherein flower-bud formation was manipulated by shoot bending and by spraying gibberellins, Verheij (1996) could not find support for the view that changes in PAs in buds were involved in flower-bud formation.

As reported in more detail by Zhu et al. (1997), there is ample evidence that temperature affects flower-bud formation in apple. The aim of the present study was to evaluate the effect of temperature on the time course of the concentration of spermidine, spermine and putrescine in buds of young apple trees in relation to bud development and flowering. Since the amino acid arginine is an important precursor in the synthesis of PAs (Adigo and Prasad, 1985; Smith, 1985; Faust and Wang, 1992) the content of arginine was estimated as well. The data on flowering and shoot growth were mainly discussed in a preceding paper (Zhu et al., 1997).

2. Materials and methods

Three-year-old potted apple trees cv. Summerred (rootstock M.9) were divided into six equivalent groups of 12 trees each. From May 4, one week after full bloom, until September 5 they were exposed to the following temperature treatments:

<table>
<thead>
<tr>
<th>Code</th>
<th>May 4–Jun 12</th>
<th>Jun 12–Sep 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>13–13</td>
<td>13°C</td>
<td>13°C</td>
</tr>
<tr>
<td>13–20</td>
<td>13°C</td>
<td>20°C</td>
</tr>
</tbody>
</table>
The experiment was conducted in three identical controlled-environment rooms which were illuminated by a mixture of high-pressure sodium (Philips SON-T) and high-pressure iodine lamps (Philips HPI-V) giving an irradiance of ca. 45 \( \text{W m}^{-2} \) (PAR) at plant height at the start of the experiment. Day length was 14 h, and the vapour-pressure deficit of the air was ca. 0.90 kPa.

Starting at May 24 until the beginning of September, at intervals ranging from 7 to 14 days, terminal buds of two spurs (<5 cm) or short shoots (5–10 cm) were taken at random from each of the four trees of each treatment. After freeze-drying, the dry weight of each two-bud-replicate was determined. The samples were kept at \(-20^\circ\text{C}\) until analysis.

The length of six arbitrarily chosen shoots per tree was determined at the time when the change in temperature occurred and at the end of the experiment when growth had ceased nearly completely. In the next spring, flowering records were taken from four trees that had not been used for bud sampling. To that end, during September the temperature in the rooms was gradually lowered to \(13^\circ\text{C}\). After defoliation, from the beginning of October, the trees were kept in a cold storage room at \(2^\circ\text{C}\). In February they were brought into flowering in a mildly heated greenhouse. The data were subjected to analysis of variance and the significance of differences was determined by Duncan’s multiple range test.

For polyamine and arginine analysis, ca. 15 mg of freeze-dried material of each of the four replicates was extracted in 900 \(\mu\text{l}\) 0.2 N perchloric acid (PCA) to which 100 \(\mu\text{l}\) 1.6-hexanediamine was added as internal standard. To prepare the extract for HPLC-analysis the procedure described by Smith and Davies (1985) was used with the following modifications. After dansylation, the PAs were extracted with 2 ml ethylacetate, and, after centrifugation, the organic phase was evaporated under a stream of air. The residue was dissolved in 250 \(\mu\text{l}\) 5 M KOH in methanol, and then kept at 50°C for 30 min. Thereafter, 2.25 ml \(\text{H}_2\text{O}\) and 2 ml ethylacetate were added for further extraction of the dansylamines. After centrifugation for 5 min, the organic phase was again evaporated to dryness. The residue was dissolved in 1 ml methanol for HPLC-analysis. For arginine analysis the PCA soluble fractions were dansylated according to the method of Tapuhi et al. (1981).

The HPLC-system consisted of a Pharmacia 2249 gradient pump (Pharmacia, Sweden), a Marathon autosampler (Spark, Holland) and a 470 fluorescence detector, equipped with a 250-4 LiChrospher 100 RP-18 (5 mm) column having a guard column (Merck, Darmstadt, FRG). For fluorescence the excitation and emission wavelengths were 365 and 510 nm, respectively. Detector output was
collected and analysed using a Baseline 810 workstation (Dynamic Solutions). Polyamines were eluted from the column at a flow rate of 1 ml min$^{-1}$ with solvent A (95% methanol: 5% 0.01 M Tris–HCl buffer pH = 7.7) and solvent B (25% methanol: 75% 0.01 M Tris–HCl buffer pH = 7.7) according to the following gradient programme (% solvent A : B): 0–5 min linear gradient from 70 : 30% to 78 : 22%, 5–19 min from 78 : 22% to 100 : 0%, and 19–20 min 100 : 0%. Arginine was eluted under the same conditions with the following gradient programme (% solvent A : B): 0–5 min from 30 : 70% to 33 : 67%, 5–7 min from 33 : 67% to 37 : 63% and 7–10 min from 37 : 63% to 55 : 45%.

3. Results

3.1. Shoot growth and flowering

When the temperature was kept the same throughout, shoots were significantly longer at 20$^\circ$C than at 13$^\circ$C, but a further increase to 27$^\circ$C was not more effective (Table 1). Initially, growth at 27$^\circ$C even lagged behind. The rise in temperature at 6–7 weeks after bloom (13–20$^\circ$ and 20–27$^\circ$C) stimulated growth to a level exceeding that of the treatments where the temperature was kept the same (20–20$^\circ$ and 27–27$^\circ$C). Lowering the temperature from 27$^\circ$ to 13$^\circ$C halfway through the growing season (27–13$^\circ$C) halted growth nearly completely. Growth in the 13–13$^\circ$ treatment stopped early, but growth continued until the end of the experiment (>4 months after bloom) in the treatments 13–20$^\circ$, 20–27$^\circ$ and 27–27$^\circ$C (Table 1). The remaining two treatments occupied an intermediate position.

When the temperature was kept constant throughout, flower production greatly increased with temperature (Table 1). Raising the temperature from 13$^\circ$ to 20$^\circ$C,

<table>
<thead>
<tr>
<th>Temperature ($^\circ$C)</th>
<th>Shoot length$^a$ (cm)</th>
<th>Weeks to cessation of growth</th>
<th>Percentage of flower clusters$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 46</td>
<td>end</td>
<td></td>
</tr>
<tr>
<td>13–13</td>
<td>16.6b</td>
<td>19.3d</td>
<td>10</td>
</tr>
<tr>
<td>13–20</td>
<td>17.8b</td>
<td>37.7ab</td>
<td>19</td>
</tr>
<tr>
<td>20–20</td>
<td>27.2a</td>
<td>29.7bcd</td>
<td>15</td>
</tr>
<tr>
<td>20–27</td>
<td>24.8a</td>
<td>48.7a</td>
<td>19</td>
</tr>
<tr>
<td>27–27</td>
<td>16.5b</td>
<td>31.2bc</td>
<td>19</td>
</tr>
<tr>
<td>27–13</td>
<td>21.0ab</td>
<td>21.0bc</td>
<td>15</td>
</tr>
</tbody>
</table>

$^a$ Values within each column followed by different letters differ significantly ($p = 0.05$).
six to seven weeks after bloom, favoured flowering markedly, but a similar increase from 20º to 27ºC had no effect. The severe drop of temperature from 27º to 13ºC reduced flowering dramatically.

3.2. Arginine and polyamines

In the first six weeks after bloom, the arginine content in the buds did not differ much between temperatures (Fig. 1(a)). Thereafter, an increase occurred which was very marked at 27ºC, but little pronounced at 13ºC. The 20ºC-treatment was in between, but not much different from 13ºC. The temperature shift from 13º to 20ºC enhanced the arginine content only very slightly (Fig. 1(b)). A similar shift from 20º to 27ºC was reflected in a substantial increase to the level at 27–27ºC (Fig. 1(c)). Initially, arginine slightly remained behind by the temperature drop from 27º to 13ºC, but after Day 90 it clearly outstripped the 27–27ºC level (Fig. 1(d)).

In the constant-temperature treatments, the concentration of total polyamines (Spd + Spm + Put) showed a dip at 40 to 50 days after bloom, but in general its course in relation to time was rather variable and lacked consistency (Fig. 2(a)). The relationship with temperature was just the inverse of that of arginine: the level was lower the higher the temperature. In agreement with that, the rise of temperature from 13º to 20ºC and from 20º to 27ºC lowered the polyamine level (Fig. 2(b) and (c)), while the drop of temperature from 27º to 13ºC produced an increase (Fig. 2(d)).

With respect to the individual polyamines, Spd and Put were lower with the rise of temperature (Fig. 3(a)). Roughly speaking, however, the inverse holds for Spm although later in the experimental period the concentrations of Spm in the 20–20º and 27–27ºC treatments were similar (Fig. 4(a)). There was no distinct, consistent effect of time of sampling (Figs. 3(a) and 4(a)). The data suggest that Spd was maximal at the start and at the end of the season and that, in general, Spd and Spm were slightly reduced 40 to 50 days after bloom. The rise of temperature from 13º to 20ºC and from 20º to 27ºC lowered the contents of Spd and Put to the levels at 20–20º and 27–27ºC, respectively (Fig. 3(b) and (c)), but did not have any effect on Spm (Fig. 4(b) and (c)). The drop of temperature from 27º to 13ºC produced a marked increase in the Spd and Put concentrations up to the 13–13ºC level (Fig. 3(d)), and a decline to that level for Spm (Fig. 4(d)). As can be derived from Figs. 3 and 4, >80% of total polyamines was accounted for by Spm and Spd. The contribution of Put was always small. At 13ºC Spd predominated over Spm, but at 27ºC Spm prevailed.

3.3. The ratio (Put + Spd)/Spm

As shown in Fig. 5(a), the ratio (Put + Spd)/Spm decreased with increasing temperature and the differences between 13º, 20º and 27ºC were statistically
Fig. 1. The arginine content of apple spur buds throughout the growing season at a number of temperature regimes. Arrows indicate the time that the temperature was changed. SE bars are shown when larger than the symbols.
Fig. 2. The content of total polyamines of apple spur buds throughout the growing season at a number of temperature regimes. Arrows indicate the time that the temperature was changed. SE bars are shown when larger than the symbols.
Fig. 3. The spermidine and putrescine contents of apple spur buds throughout the growing season at a number of temperature regimes. Arrows indicate the time that the temperature was changed. SE bars are shown when larger than the symbols.
Fig. 4. The spermine content of apple spur buds throughout the growing season at a number of temperature regimes. Arrows indicate the time that the temperature was changed. SE bars are shown when larger than the symbols.
Fig. 5. The ratio (Put + Spd)/Spm of apple spur buds throughout the growing season at a number of temperature regimes. Arrows indicate the time that the temperature was changed.
An increase in temperature from 13°C to 20°C or from 20°C to 27°C halfway through the experiment caused a reduction in the ratio compared with that at 13–13°C and 20–20°C, respectively (Fig. 5(b) and (c)). The reduction was more pronounced for the 13–20°C treatment. On the other hand, lowering the temperature from 27°C to 13°C resulted in a significant increase in the ratio (Fig. 5(d)).

4. Discussion

The effect of temperature on flower-bud formation and its relation to shoot growth was discussed in detail by Zhu et al. (1997). The present discussion will be mainly restricted to the pattern of arginine and polyamine concentrations in spur buds as affected by temperature and their role in bud development.

The general increase in arginine, as found in all of the present temperature treatments, agrees with the data of Verheij (1996) for spur buds of apple, but does not fit in with the results of Tromp (1970) and Wang and Faust (1993) who found for perennial tissues of apple a decreasing trend until August whereupon an increase occurred again in September. These conflicting results can be reconciled when it is realised that arginine is an important component of the storage nitrogen of the tree which is gradually used up for new growth in spring and early summer (Tromp, 1970, 1983; Tromp and Ovaa, 1971; Titus and Kang, 1982). In the new buds, on the contrary, the situation is just opposite. Since bud growth ceases early and any outgrowth does not occur in the current year, the time that the supply of ‘new’ nutrients exceeds demand will be reached early in the season, resulting in a gradual increase of storage nitrogen including arginine. A similar reasoning holds for the enhanced arginine content at higher temperatures. Assuming that increasing the temperature throughout all or part of the season favours the nitrogen nutrition of the tree, in spur buds this ‘extra’ nitrogen can only manifest itself in raising the nitrogen, i.e. arginine storage level (Fig. 1(a)–(c)), whereas for the tree as a whole it is reflected in growth stimulation (Table 1, longer shoots that stop growth later). The general increase of arginine with temperature might also be related to the increased flower-bud formation (Table 1, the higher the temperature, the higher percentage of flower clusters). The drop of temperature from 27°C to 13°C six to seven weeks after bloom blocked shoot growth completely. Probably, all nitrogen already absorbed in the 27°C-period might be stored, thus resulting in higher arginine levels which even exceed that when the temperature was kept at 27°C throughout the experimental period (Fig. 1(d)).

In contrast to the findings of Wang and Faust (1993) for one-year old shoot tissue, the polyamines in spur buds of the current year did not exhibit the same seasonal pattern as arginine. Obviously, although there is ample evidence that arginine is a precursor of polyamines (Adigo and Prasad, 1985; Smith, 1985;
Faust and Wang, 1992), there is no close quantitative link. Likewise, it even remains to be seen whether there is a functional relationship between the two. The role of arginine seems to be ‘passive’; it just accumulates when for some reason there is a (local) surplus of nitrogen. On the other hand, polyamines are more directly involved in modulating plant development (Faust and Wang, 1992; Tiburcio et al., 1993).

As found by Verheij (1996) for apple, among the three individual polyamines, spermidine predominated in the spur buds in spring and early summer. However, in the same period, spermine prevailed in stem tissue (Wang and Faust, 1993), while in buds collected in winter, putrescine was the major PA present (Wang and Faust, 1994). These different results may be due to the time of year when the samples were taken, but, in addition, the prevailing temperature may be an important factor as well. As shown in this study, when comparing Figs. 3(a) and 4(a), it is found that at 13°C spermidine predominated over spermine, while at 27°C the reverse is true. In view of the widely accepted involvement of PAs in stimulation of growth processes in plants, the negative effect of increasing temperature on PA concentration, as found for total PAs and for spermidine and putrescine separately, is rather surprising. However, it should be realised that, because shoot growth was enhanced at higher temperatures (Table 1), the extent of apical dominance of the growing shoot tips over the lower situated spur buds was greatly increased as well. It may be warranted, therefore, to suggest that spur bud activity (vegetative growth) was inhibited at higher temperatures which is reflected in a lower PA concentration. However, the flowering of spur buds was increased with increasing temperature. This might be related to the reduced ratio of (Put + Spd)/Spm at higher temperatures. Conversely, the drop in temperature from 27°C to 13°C, which stopped shoot growth completely, coincided with a rise in PA concentration in the spur buds. It is not unlikely that the supposed effect of shoot growth on bud activity also concerns the time that the bud enters endodormancy. The relationship between PAs and dormancy is well founded. Polyamines in dormant cherry buds were low but increased markedly when activity was resumed (Wang et al., 1985). Polyamine content of apple buds increased when chilling was increased and dormancy was gradually broken (Wang and Faust, 1994). For buds of hazel, Rey et al. (1994b) found much lower PA levels in October than in April when flowering occurred. In dormant tubers of Helianthus tuberosus PA concentration was low but it increased considerably when dormancy was broken (Bagni et al., 1980).

Verheij (1996) created obvious differences in flower production in young apple trees by application of gibberellins and by bending of shoots in the horizontal position, but could not find any correlation with the levels of PAs in buds. In the present study, raising temperature resulted in an increase of flower-bud formation and a decrease of total PA level, indicating a sort of negative relationship between the two. However, when comparing (Put + Spd)/Spm at the different temperature
treatments, it was shown that flowering increased but the ratio decreased with a rise of temperature (Table 1 and Fig. 5). This may suggest some kind of positive relationship between flower-bud formation and Spm concentration and a negative relation between Put and Spd concentrations. Nevertheless, this does not mean that a direct relationship between PAs and flowering has been established. A more indirect relationship via the implication of PAs in a diversity of physiological processes as cell division, dormancy and flower-bud formation is plausible as well. Even the stimulation of flowering by application of PAs mentioned in the introduction of this paper does not form an absolute proof for a direct involvement of PAs in the flower-bud formation process. As stated by Verheij (1996), the significance of PAs in flower formation in apple can only be certain if the rate of turnover of PAs is known. A further objection, probably, is that analysing whole buds is too rough. Analysis at the meristem level would be more appropriate but is very laborious, certainly when time series are needed as in the present study. However, a major problem with sampling spur buds is that any distinction between the vegetative and the generative stage can only be made later in the season when anatomical and morphological differences start to occur. This means that since the effect of temperature on flowering is never completely positive or negative, each bud sample contains leaf and flower buds in varying proportions, a fact that hampers detection of possible differences in chemical composition.

Acknowledgements

The financial support to the senior author by the Royal Academy of Sciences of The Netherlands (under the auspices of the exchange Research Programme between the People’s Republic of China and the Netherlands) and by a research fellowship of the Agricultural University of Wageningen is gratefully acknowledged.

References

Rey, M., Díaz-Sala, C., Rodríguez, R. Effect of repeated severe pruning on endogenous polyamine content in hazelnut trees. Physiol. Plant. 92 (1994a) 487–492.
Rey, M., Díaz-Sala, C., Rodríguez, R. Comparison of endogenous polyamine content in hazel leaves and buds between the dormancy and flowering annual phases of growth. Physiol. Plant. 91 (1994b) 45–50.