RESEARCH PAPER

The role of polar auxin transport through pedicels of *Prunus avium* L. in relation to fruit development and retention

Mark A. Else*, Anna P. Stankiewicz-Davies†, Carol M. Crisp and Christopher J. Atkinson

East Malling Research, West Malling, Kent ME19 6BJ, UK

Received 11 February 2004; Accepted 24 May 2004

Abstract

It was investigated whether premature fruit abscission in *Prunus avium* L. was triggered by a reduction in polar auxin transport (PAT). The capacity of pedicels to transport tritiated IAA ([3H]-IAA) via the PAT pathway was measured at intervals throughout flower and fruit development. The extent of passive diffusion, assessed by concurrent applications of [14C]-benzoic acid ([14C]-BA), was negligible. Transported radioactivity recovered from agar blocks eluted at the same retention time as authentic [3H]-IAA during HPLC fractionation. The capacity for PAT was already high 7 d before anthesis and increased further following the fertilization of flowers at anthesis. PAT intensity was greatest immediately following fertilization and at the beginning of the cell expansion phase of fruit growth; the transport intensity in fruitlets destined to abscind was negligible. The amount of endogenous IAA moving through the PAT pathway was greatest during the first 3 weeks after fertilization and was again high at the beginning of the fruit expansion stage. IAA export in the phloem increased following fertilization then declined below detectable levels. ABA export in the phloem increased markedly during stone formation and at the onset of fruit expansion. TIBA applied to pedicels of fruit *in situ* promoted fruitlet abscission in 2000 but not in 2001, despite PAT capacity being reduced by over 98% in the treated pedicels. The application of TIBA to pedicels did not affect fruit expansion. The role of PAT and IAA in relation to the development and retention of *Prunus avium* fruit is discussed.

Key words: Abscission, auxin, cherry, fruit, intensity, PAT, pedicel, *Prunus*, TIBA, velocity.

Introduction

Abscission of developing fruits shortly before harvest is a recurring constraint to the efficient production of fruit crops. The extent of premature abscission in *Prunus avium* L. (sweet cherry) varies greatly from year to year and is likely triggered by unfavourable climatic factors. Abscission is preceded by the activation of an abscission zone (AZ) at the base of the subtending organ. During ‘June drop’ in sweet cherry, this occurs between the pedicel and spur. The flow of IAA through the AZ apparently regulates the sensitivity of cells in the abscission layer to ethylene (van Doorn and Stead, 1997). Responsiveness to ethylene is increased when the flux of IAA through the AZ is reduced (Addicott, 1982), perhaps via changes in ethylene receptor levels (Zhou *et al.*, 1996).

In addition to transport within phloem sieve elements, basipetal IAA transport also occurs via the polar auxin transport pathway (PAT) (Rubery and Sheldrake, 1974; Raven, 1975) through cells in the cambium and its most recent derivatives (Morris and Thomas, 1978; Rubery, 1987). As well as unmediated diffusion of undissociated IAA molecules across the plasma membrane, saturable uptake and efflux carriers transport auxin into and out of competent cells (Lomax *et al.*, 1995). Recent cloning efforts have identified several putative uptake and efflux carriers (Friml and Palme, 2003). Plants are able to regulate the basipetal flow of IAA by modifying the number and activity of these carriers (Delbarre *et al.*, 1996) during growth, development, and in response to the environment (Lomax *et al.*, 1995). The flux of IAA through the PAT pathway is a function of both the rate of transport (velocity) and the number of cells capable of polar transport (intensity).

If IAA transport is reduced or inhibited, the organ may abscind (Bangerth, 1989) or growth may be slowed, since

* To whom correspondence should be addressed. Fax: +44 (0)1732 849067. E-mail: mark.else@emr.ac.uk
† Present address: RHS Garden Wisley, Woking, Surrey GU23 6QB, UK.

Journal of Experimental Botany, Vol. 55, No. 405, © Society for Experimental Biology 2004; all rights reserved
IAA is postulated to have a direct effect on assimilate partitioning (Patrick, 1979; Agusti et al., 2002). Moreover, perturbations to auxin homeostasis using inhibitors of PAT had marked effects on leaf expansion (Ljung et al., 2001). These authors suggest that normal leaf expansion is dependent on optimal leaf IAA concentrations and that tissue-specific regulatory mechanisms exist to control IAA homeostasis.

The stimulatory effect of IAA on xylem development and cambial activity in woody species is well established (Digby and Wareing, 1966; Aloni, 1995).UGA and et al. (1998) provided evidence that the radial width of the IAA concentration gradient within the cambial meristem correlated well with cambial growth rates. Thus, IAA is able to stimulate the production of its own transport pathways (Goldsmith, 1977; Sundberg et al., 2000). The basipetal flow of IAA may enhance the development of vascular tissues in the pedicel (Aloni, 1995; but see Koizumi et al., 2000), therefore increasing the capacity of the transport pathway through which growing fruit receive leaf-derived assimilates and water. The capacity of the vascular system in the pedicel could be a factor limiting the availability of essential photoassimilates; a strong correlation between fruit size and pedicel cross-sectional area was reported in Citrus fruit (Stewart et al., 1952; Bustan et al., 1995). The greater retention of fruits on leafy or multi-flower leafless inflorescences was attributed by Emer (1989) to the increased capacity of the branch’s vascular system, presumably mediated by the supply of IAA.

In this paper, the characteristics of PAT and the amount of endogenous IAA moving through the PAT pathway in pedicels of P. avium, are determined in relation to flower and fruit development and abscission. The effect of blocking polar transport pathway through pedicels of developing fruits in situ on fruit retention and expansion is also examined.

Materials and methods

Plant material

The experiments were conducted on fruits from ‘Summit’ scions on ‘Tabel’ (Edabriz) rootstocks, planted in the spring of 1993 at HRI, East Malling. Experiments were carried out between anthesis and final harvest during 1999, 2000, 2001, and 2002.

Measurement of fruit fresh weight and pedicel diameters

A sample of 40 fruits was collected from randomly chosen ‘Summit’/‘Tabel’ composite trees at intervals from before anthesis to final harvest. Fruits were sealed immediately into plastic bags and their average fresh weight determined within 20 min of excision from the tree. The diameter of the pedicels at a position 4 mm from the junction with the fruit was measured twice, 90° apart, with digital callipers. Average diameters were converted to cross-sectional areas for presentation.

Pollination with compatible and incompatible pollen

Four, two-year-old branches on each of eight ‘Summit’/‘Tabel’ trees were bagged at the beginning of May to ensure that pollination did not occur. The cv. ‘Summit’ is a self-sterile cultivar and requires pollen from compatible varieties such as ‘Lapins’ if the flowers are to be fertilized. Pollen was collected annually from ‘Lapins’ and ‘Summit’ anthers and stored in a dessicator at 4°C until required. In vitro germination tests of the ‘Lapins’ and ‘Summit’ pollen collected showed that over 95% of pollen grains from both cultivars germinated within 24 h at 20°C.

On the day of anthesis, unopened flowers and flowers that had opened on previous days were removed from each branch. Branches were demarcated into two such that there were similar numbers of flowers on the top and bottom halves of each branch. The flowers on either the top or bottom half of each branch were pollinated by hand using the ‘Summit’ (incompatible) pollen collected earlier. The remaining half of the flowers were pollinated with ‘Lapins’ (compatible) pollen. To ensure that compatible pollen was not introduced on to those flowers pollenated with ‘Summit’ pollen, the bags were replaced on the branches until pedicels were harvested for IAA transport experiments. Auxin transport assays were carried out 7 d before anthesis, at anthesis, and at 3, 7, and 14 d after hand pollination to determine whether pollination and/or fertilization increased the capacity for PAT.

PAT assay

Thirty fruits were excised from randomly selected trees at the distal end of the pedicel with a sharp razor blade on each sampling date; 20 fruits with attached pedicels were chosen at random as a source of experimental material. After excision from the fruits under deionized water, two 4 mm sections were cut from the apical end of the pedicels (proximal to the fruitlet) with two parallel razor blades mounted in a cutting block. The apical sections were discarded and the subsequent sections used for IAA transport assays. The diameter of the remaining pedicel was measured twice, 90° apart at the mid-point, with digital callipers, and fruitlet fresh weight was recorded. The time between fruitlet removal from the trees and the start of the IAA transport assays was typically less than 20 min.

The 4 mm pedicel sections were placed with their morphologically basal end into micro-vials (Dynatech Laboratories Inc, VA, USA) containing 400 μl of 0.85% (w/v) Gauze C255 (Sigma-Aldrich Company Ltd, Poole, UK) in 10 mM MES buffer adjusted to pH 5.2 with 100 mM KOH. In experiments to determine the extent of acropetal transport, the 4 mm pedicel sections were placed in reverse orientation into the agar blocks. Routinely, a 0.1 μl droplet of [3H]-IAA (specific activity 777 GBq mmol⁻¹; Nycomed Amersham plc, Bucks., England) and [14C]-benzoic acid (specific activity 229 MBq mmol⁻¹; Sigma, St Louis, Missouri, USA) was applied to the apical cut surface of each pedicel section using a Hamilton syringe. The radioactivity was applied to the section within 12 min of excision from the fruitlet to preclude the loss of polar transport capacity of the sections (Morris and Johnson, 1990). The addition of [3H]-IAA as a microdroplet avoided the intensive IAA metabolism at the cut surface that can occur when IAA is added via a donor agar block (Sanchez-Bravo et al., 1988). Each pedicel section was transferred to a new agar block and the clock time noted. Each micro-vial was racked, covered, placed in a dish lined with damp paper covered with cling film, and incubated for up to 3 h in the dark at 20°C in an incubator. At 15 min or 30 min intervals, the agar blocks at the basal end of the pedicel sections were renewed. At the end of the transport period, the pedicel sections were removed from the agar receiver blocks and sectioned into the apical 1 mm segment and two 1.5 mm sections using a razor blade. Care was taken to avoid the transfer of radioactivity between segments by rinsing the razor blade with deionized water after every cut.

Measurement of radioactivity

The agar receiver blocks were placed into plastic scintillation vials with screw lids containing 2 ml of 100% MeOH, and [3H]-IAA and...
any [14C]-benzoic acid were extracted at room temperature for 24 h. Fifteen millilitres of Optima Gold scintillation fluid was added to each vial and, after vortexing, [3H]-IAA and any [14C]-benzoic acid were counted using a scintillation counter (Beckman Instruments, Fullerton, CA, USA) on a dual label [3H]/[14C] channel. Disintegrations per minute were converted to Bq for presentation. Each stem segment was placed into plastic scintillation vials with screw lids containing 2 ml of 100% MeOH and [3H]-IAA and [14C]-benzoic acid were extracted at room temperature for 24 h. Counts of [3H]-IAA and [14C]-benzoic acid in the tissue segments were determined as above.

**Calculation of IAA transport velocities and intensities**

The intensity (amount of IAA transported per unit time) and velocity (rate of IAA transport) of PAT in *P. avium* pedicel sections were determined according to the method devised by Van der Weij (1932) (see insert in Fig. 2A). Briefly, the cumulative radioactivity in the agar blocks over the transport period for each pedicel section was calculated and plotted against time. Times were adjusted to account for the time taken to apply the microdroplets to the pedicel sections. Linear regression lines were fitted to the transport curves using the least squares method. The slope of the regression line represents the transport intensity (Bq h⁻¹). Individual *I* values were determined for each tissue section and averaged for each treatment. The intercept with the time axis was calculated from the regression equation, this indicates the time at which [3H]-IAA first entered the agar receiver blocks. Transport velocities (mm h⁻¹) were then determined for each tissue section by dividing *T*₀ by the length of the tissue sections. Velocities were averaged for each treatment.

**HPLC fractionation**

At the end of some experiments, the 2 h agar blocks were shaken in 2 ml of cold MeOH at 4 °C for 3 h. Two hundred microlitres of MeOH were removed from each replicate and pooled within treatments. The pooled sample was injected into a C₁₈ reverse-phase HPLC (Hewlett Packard series 1050) fitted with a 4.6 i.d. × 250 mm column containing Hypersil ODS. The column was eluted at a flow rate of 1 ml min⁻¹ with 5% MeOH for 5 min, followed by a linear gradient to 100% over 45 min (all solvents contained 50% acetic acid). Samples were collected into glass centrifuge tubes every minute using a fraction collector. Two millilitres of each fraction were dispensed into scintillation vials, mixed with 10 ml of ‘Optima Gold’ scintillation fluid and their radioactivity determined as above. Authentic [3H]-IAA was fractionated and counted as above.

**Measurement of endogenous IAA and ABA flows through PAT and phloem**

*P. avium* fruit were excised from the tree and the pedicels immediately re-cut under deionized water. The basal ends of the pedicels were then immersed into solutions containing either: (i) 10 mM MES buffer, pH 7.2, 100 μM CaCl₂, 100 mM TIBA; (ii) 10 mM MES buffer, pH 5.2, 100 μM CaCl₂; (iii) 10 mM MES buffer, pH 7.2, 10 mM EDTA, 100 mM TIBA. Fruit were placed in an incubator maintained at 20 °C with lighting from fluorescent tubes. After a 7 h transport period, fruit fresh weight was measured and the solutions stored at −20 °C until analysis. After the addition of 10 ng [phenyl-13C]IAA and [3H]-ABA, IAA and ABA were extracted from the solutions using C₁₈ Sep-Pak reverse phase cartridges (Waters Associates, Watford, UK). After derivatization using ethereal diazomethane, the IAA and ABA methyl esters were quantified using a Hewlett-Packard 5890 Series II gas chromatograph coupled to a ThermoQuest Trio-1 mass spectrometer.

**Effect of TIBA on fruitlet abscission**

Lanolin paste, containing 1, 5, or 10 mM TIBA, was applied to pedicels of 10 fruits on each of seven trees as a 1 mm wide ring about 8 mm from the junction of the pedicel and the fruit. Lanolin without TIBA was applied to similar fruits as a control. These treatments were applied just before stone formation on 18 May 2000, 2 weeks before visible signs of impending fruitlet abscission were apparent. A 10 mm length of silica rubber tubing (i.d. 5 mm) was then placed around the pedicels to prevent the lanolin being washed off by rain. The appearance and the number of treated fruit remaining was recorded at 2 week intervals.

In 2001, a 1 mm ring of lanolin containing 0, 1, 5, or 10 mM TIBA was applied as above just before stone formation. Care was taken to ensure that each lanolin application amounted to a volume of 0.1 ml and, therefore, the amount of TIBA applied was consistent within treatments. Numbers of retained fruit within each treatment on each tree were recorded at weekly intervals until final harvest. Values were averaged within the same treatments between trees. Fruit length and diameter were measured and fruit fresh weight predicted using a regression equation of fruit weight against fruit volume, calculated at weekly intervals. To ensure that TIBA applied in this way effectively blocked PAT, pedicels treated with 0, 1, 5, and 10 mM TIBA were harvested on 19 May and 11 June 2001 and an IAA transport assay was performed.

**Results**

**Fruit growth and pedicel cross-sectional area (CSA)**

During the cell division phase (stage I), fruitlet fresh weight increased slowly at first then rose sharply until the beginning of stone formation (stage II; Fig. 1). During stage II, no discernible increases in fruitlet fresh weight were apparent. In those fruit that were to be retained, fresh weight increased rapidly during the cell enlargement phase (stage III; Fig. 1). Visual symptoms of pending fruitlet abscission first became apparent on 3 June and coincided with a slight reduction in fruitlet fresh weight (Fig. 1). Thereafter, fruit fresh weight remained constant until the fruit abscinded during the middle 2 weeks of June.

Pedicel CSA increased gradually throughout stages I and II until the beginning of stage III, after which it remained reasonably constant until final harvest (Fig. 1). Pedicel CSA in fruitlets destined to abscind declined once symptoms of pending abscission became apparent (Fig. 1).

**Basipetal and acropetal IAA transport**

[3H]-IAA applied to the morphologically apical end of the pedicel was transported basipetally through 4 mm long pedicel sections (Fig. 2A). The linear portions of individual transport curves were used to calculate IAA transport intensity and velocity. Average transport intensity was 261 Bq h⁻¹ and average velocity was 11.3 mm h⁻¹. 38% of applied radioactivity was transported through the pedicel section during the 3 h transport period. By contrast, transport of [3H]-IAA applied to the morphologically basal end of the pedicel amounted to 0.5% of that applied; 84% remained in tissue within 1 mm from the [3H]-IAA application site (data not shown).

**Active versus passive transport**

[14C]-benzoic acid was applied to pedicels to assess the extent to which passive diffusion contributed to the total
auxin basipetal movement. Only 0.05% of the [14C]benzoic acid applied diffused through pedicel tissue into agar receiver blocks, most (97%) stayed in apical tissue within 1 mm from the application site (Fig. 2B).

**HPLC fractionation of transported tritium**

The tritium extracted from the receiver blocks at the end of the 2 h transport period coeluted with authentic [3H]-IAA during HPLC fractionation (Fig. 3). The absence of radioactivity in any other fractions suggests that the transported [3H]-IAA remained unmetabolized.

**Effect of fertilization on PAT capacity**

The capacity for polar IAA transport was already high 7 d before anthesis (Table 1) and increased further following pollination with compatible or incompatible pollen (Table 1). Thereafter, both transport intensity and velocity decreased in pedicels of flowers pollinated with incompatible pollen. Transport velocity in pedicels of flowers pollinated with compatible pollen was at its highest value 14 d after pollination; transport intensities were similar to those of unfertilized flowers at this time (Table 1).

**PAT through pedicels of fruit destined to abscind**

Polar transport in pedicels of fruit destined to abscind was measured and compared with that in pedicels of fruit assumed to be retained (Table 2). The assay was conducted towards the end of stone formation, when fruit destined to abscind had just begun to show morphological changes such as an early red colouration. Only 0.7% of applied [3H]-IAA was transported to receiver blocks through pedicel sections taken from fruit destined to abscind, with 72% remaining in tissue within 1 mm from the application site (Table 2).

**Time-course of PAT capacity over the season**

Transport intensity (I) and velocity (V) were determined for proximal 4 mm long pedicel sections shortly after initial set (30 April) to final set (5 July 1999) (Fig. 4). The intensity of polar IAA transport through pedicel sections was high during the last week in April and the first week in May, fell briefly during mid-May before returning to initial values throughout stone formation (Fig. 4A). Transport I reached its maximum value immediately following stone formation then declined gradually towards final harvest (Fig. 4A). The pattern of transport V was generally more variable (Fig. 4B), V was highest during stone formation then eventually decreased prior to final harvest.

**Endogenous IAA and ABA flows through PAT and phloem**

The amount of endogenous IAA moving through the PAT pathway increased from anthesis to the beginning of stone formation (Fig. 5A). PAT was low during stone formation then increased at the beginning of the cell expansion phase. The amount of IAA moving through the phloem was similar to that moving through the PAT pathway during the first 2 weeks after fertilization (Fig. 5A). Export of IAA in the phloem fell below detectable levels during stone formation and the early stages of fruit expansion (Fig. 5A).

ABA was transported only in the phloem (Fig. 5B). The amount of ABA exported from the developing fruit was...
greatest during stone formation and the beginning of fruit expansion and declined markedly as fruit began to mature (Fig. 5B). The low ABA flows found in treatments where phloem transport had been blocked by adding CaCl₂, confirmed that the flows of IAA through the PAT pathway were not adulterated by phloem-mobile IAA.

Effect of TIBA on fruitlet retention

Two weeks after application of lanolin, only 24% of fruit treated with lanolin alone remained attached (Table 3). This loss of fruit coincided with the main wave of fruit abscission that occurred at the beginning of the cell enlargement stage; the extent of premature fruitlet abscission was particularly high during 2000. After this stage, most fruit were retained until final harvest (Table 3). However, 1 mM TIBA exacerbated the extent of fruit loss following stone formation; only 7% of those fruit treated with TIBA were retained (Table 3). Higher concentrations of TIBA (5 mM and 10 mM) did not increase abscission rates further (data not shown).

In 2001, fruit retention was not altered by the application of several concentrations of TIBA to the pedicels (Fig. 6A). Similarly, the predicted fresh weight of fruit whose pedicels
were treated with TIBA was not different to that of control fruit (Fig. 6B). PAT assays carried out 1 d and 3 weeks after TIBA application confirmed that the flow of IAA through the PAT pathway was reduced by 98% and 50%, respectively, following treatment with 5 mM or 10 mM TIBA (data not shown).

**Discussion**

The capacity of pedicels for PAT throughout *Prunus avium* flower and fruit development was characterized and the flow of endogenous IAA moving through the PAT pathway and exported from the fruit in the phloem was measured. It was also tested whether fruitlet retention and development was influenced by a reduction in the capacity for PAT.

PAT was determined in 4 mm long pedicel sections; transport velocities of 11.3 ± 1.1 mm h⁻¹ were within the range considered typical (5–20 mm h⁻¹) for excised tissue from a number of species (Lomax *et al.*, 1995). Transport intensities averaged 261 ± 34.3 Bq h⁻¹. The intensity of basipetal transport is often not determined, but these values are 5-fold higher than those reported for etiolated lupin hypocotyls (Sanchez-Bravo *et al.*, 1992). This great capacity for basipetal transport is reflected in the high percentage (33%) of the applied radioactivity that was transported basipetally, compared to the 4–5% in the work of McCready and Jacobs (1963) and Sanchez-Bravo *et al.* (1992). Acropetal movement of IAA was barely detectable and passive diffusion accounted for less than 1% of the IAA transported. Tritium extracted from the receiver blocks eluted in the same fraction as authentic [³H]-IAA during HPLC fractionation, suggesting that the majority of the transported tritium was [³H]-IAA and not a metabolite or conjugate of IAA. Despite the obvious limitations of using excised tissue sections, these results suggest that the capacity for PAT was not adversely affected during these short-term measurements.

**Table 1.** PAT intensities and velocities through pedicels of *P. avium* flowers pollinated with ‘Summit’ (incompatible) and ‘Lapins’ (compatible) pollen

<table>
<thead>
<tr>
<th>Days from anthesis</th>
<th>Intensity (Bq h⁻¹)</th>
<th>Velocity (mm h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>‘Summit’</td>
<td>‘Lapins’</td>
</tr>
<tr>
<td></td>
<td>184.84 ± 14.1</td>
<td>12.3 ± 1.5</td>
</tr>
<tr>
<td>0</td>
<td>211.2 ± 13.5</td>
<td>13.5 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>327.5 ± 13.0</td>
<td>11.1 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>201.6 ± 25.2</td>
<td>8.9 ± 0.8</td>
</tr>
<tr>
<td>14</td>
<td>193.8 ± 27.6</td>
<td>9.9 ± 0.9</td>
</tr>
</tbody>
</table>

**Table 2.** Distribution of [³H]-IAA and [¹⁴C]-benzoic acid through 4 mm long pedicel sections of *P. avium* fruits destined to be retained or to abscind

<table>
<thead>
<tr>
<th>Distance from pedicel apex</th>
<th>[³H]-IAA</th>
<th>[¹⁴C]-BA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retain</td>
<td>Abscind</td>
</tr>
<tr>
<td>0–1 mm</td>
<td>42.5 ± 1.9</td>
<td>72.4 ± 2.5</td>
</tr>
<tr>
<td>1–2.5 mm</td>
<td>11.3 ± 0.2</td>
<td>24.3 ± 2.1</td>
</tr>
<tr>
<td>2.5–4 mm</td>
<td>13.4 ± 0.5</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Receiver block</td>
<td>32.8 ± 1.9</td>
<td>0.7 ± 0.1</td>
</tr>
</tbody>
</table>

Fruits were chosen immediately after stone formation when fruitlets destined to abscind showed slight alterations in colour. Data are means of eight replicates with associated standard errors.
incompatible pollen and these flowers failed to set fruit. The transport capacity of pedicels pollinated with compatible ‘Lapins’ pollen remained high throughout the experimental period, however, the values for transport velocity and intensity did not remain constant. PAT intensity is influenced mostly by the number of cells that are capable of polar IAA transport, while the transport velocity is influenced most strongly by the activity of the influx and efflux carriers. These data suggest that once the number of transporting cells stabilized, the capacity for IAA transport was regulated by the activity of the influx and efflux carriers. The activity of these enzymes may have been altered by IAA emanating from the developing ovary since IAA has been postulated to affect the turnover of efflux carrier proteins (Wilkinson and Morris, 1994).

Generally, the estimates of PAT capacity correlated well with the determinations of the amounts of endogenous IAA moving through the PAT pathway (compare Figs 4A, B and 5A). The amount of IAA moving basipetally was greatest during the first 3 weeks after fertilization (Gruber and Bangerth, 1990), and coincided with the period of rapid pedicel expansion (CSA) and elongation (data not shown). These changes in pedicel CSA occurred concurrently with the secondary thickening of the vascular system in the pedicels. The amount of IAA supplied by the PAT pathway to the cambial region controls the extent of cell divisions in the cambial meristem and, therefore, xylem production (see references in the Introduction). Presumably, IAA transported through the PAT pathway stimulates vascular development in the pedicels before and after anthesis to ensure that the capacity of the vascular system to transport essential substances does not limit the growth of the developing fruit. Patterns of xylem development were not obviously affected in pedicels of fruits destined to abscind (data not shown). It is, therefore, unlikely that premature fruitlet abscission in *Prunus avium* is triggered by the limited capacity of the vascular system to transport essential substances to the growing fruit.

Immediately following stage II, the PAT intensity in fruits destined to be retained was at its highest. The amount of endogenous IAA moving through the PAT pathway also

---

**Fig. 4.** Time-course of PAT (A) intensity and (B) velocity of 4 mm long pedicel sections of *P. avium* fruits between initial and final set, plotted with the increase in fruitlet fresh weight over the same period.
increased at this time. This pattern of transport capacity in retained fruit corresponds with that described for IAA concentrations in developing Prunus avium fruit (Kondo et al., 2000; authors’ unpublished results) and Prunus persica (Westwood, 1993) seeds; during stage II, IAA concentrations were low but increased immediately following stone formation. Reduced levels of endogenous IAA, either through reduced synthesis or increased conjugation, decarboxylation, or compartmentalization, can adversely affect the capacity for PAT (Morris and Johnson, 1990). The resumption of basipetal auxin transport may, therefore, reflect this change in endogenous IAA levels in the developing seed, and ultimately dictate whether fruit enter the cell expansion phase (stage III), or abscind (see below). This notion is supported by the very low capacity for PAT in pedicels of fruit destined to abscind.

Several reports suggest that IAA may regulate sink activity and resource partitioning (Patrick, 1979, 1987; Baker 2000; Agusti et al., 2002). A synthetic auxin, 3,5,6-TPA, stimulated fruit growth and increased the concentrations of hexoses in developing Satsuma mandarin when applied at the onset of the cell expansion period (Agusti et al., 2002). IAA effects on sink activity may be mediated via its promotive action on invertase activity and sucrose hydrolysis (Davies et al., 1997). It was tested whether IAA exported via the PAT pathway was necessary for fruit retention and development using the PAT inhibitor 2,3,5-triiodobenzoic acid (TIBA), which blocks the membrane-trafficking of the putative efflux carrier PIN1 (Geldner et al., 2001; Muday et al., 2003). Application of TIBA to pedicels prompted fruitlet abscission over and above that seen during the normal wave of fruitlet abscission in 2000. These data suggest that the ability of IAA to regulate the flow of assimilates to developing fruits (Patrick, 1979, 1987; Agusti et al., 2002) may be an important factor in determining whether fruit are retained. However, TIBA did not alter patterns of fruit retention when applied at several concentrations in 2001, even though the capacity for PAT
was greatly reduced. Furthermore, the predicted fresh weight of treated fruit was not different from that of control values. Thus, perturbations to PAT may exacerbate fruit abscission in some years when other, as yet unknown, factors predispose fruit to premature abscission.

Recent reports have demonstrated that cell division and cell expansion in developing leaves and elongating hypocotyl is influenced by the IAA pool size within the tissues (Ljung et al., 2001; Reed, 2001). Apparently, regulatory mechanisms exist that serve to maintain the IAA pool size at optimum levels for tissue growth. For example, the rapid increase in IAA content of expanding Arabidopsis leaves treated with an auxin transport inhibitor (NPA) was quickly followed by a sharp decrease, indicating feedback inhibition of IAA biosynthesis (Ljung et al., 2001). Thus, in Prunus avium fruit, export of IAA via the phloem and PAT pathway, coupled with IAA degradation and conjugation, may serve to maintain IAA concentrations within an optimum range to facilitate cell division during stage I and cell expansion during stage III. Further work is needed to substantiate this hypothesis.

Phloem export of ABA from developing fruit decreased markedly during the later stages of fruit expansion. This may reflect a general decrease in phloem export, but it could also contribute to the accumulation of ABA in the ripening fruit. Ripening of the non-climacteric Prunus

---

**Table 3. The effect of 1 mM TIBA applied to pedicels of P. avium fruit on fruitlet retention**

The TIBA was applied just before stone formation as a 1 mm wide lanolin ring around the pedicel of 10 fruits on each of seven trees. Results are means of 50 replicates with associated standard errors.

<table>
<thead>
<tr>
<th>Date</th>
<th>Percentage of fruit remaining</th>
<th>-TIBA</th>
<th>+TIBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 June</td>
<td>24±3</td>
<td>13±4</td>
<td></td>
</tr>
<tr>
<td>12 June</td>
<td>19±4</td>
<td>7±2</td>
<td></td>
</tr>
</tbody>
</table>

---

**Fig. 6.** (A) The effect of TIBA (1, 5, or 10 mM) on P. avium fruit retention during 2001. TIBA was applied in a 1 mm ring of lanolin around the pedicel just before stone formation. Data are means of 50 replicates, with associated standard errors. (B) The effect of TIBA (1, 5, or 10 mM) on fruit expansion during 2001. TIBA was applied just before the beginning of the cell expansion phase. Data are means of 50 replicates, with associated standard errors.
Acknowledgements

We thank Mrs June Taylor for excellent technical assistance, Dr Yannick Ford and Mr Patrick Blake for their comments on an earlier version of the manuscript and Dr Tony Webster for helpful discussions. This work was supported by funds from a MAFF-sponsored HORT LINK project (HORT LINK 6 (CSA 4116)) and from the East Malling Trust for Horticultural Research.

References


Kondo S, Tomiyama A. 1998. Changes of free and conjugated ABA in the fruit of ‘Satohishiki’ sweet cherry and the ABA metabolism after application of (s)-(+-)ABA. Journal of Horticultural Science and Biotechnology 73, 467–472.


