Title: The effect of the graft union on hormonal and ionic signalling between rootstocks and scions of grafted apple (*Malus pumila* L. Mill.)

Authors: Mark A. Else, June M. Taylor, Stephen Young, Christopher J. Atkinson

PII: S0098-8472(18)30499-4
DOI: https://doi.org/10.1016/j.envexpbot.2018.07.013
Reference: EEB 3509

To appear in: *Environmental and Experimental Botany*

Received date: 3-4-2018
Revised date: 29-6-2018
Accepted date: 15-7-2018

Please cite this article as: Else MA, Taylor JM, Young S, Atkinson CJ, The effect of the graft union on hormonal and ionic signalling between rootstocks and scions of grafted apple (*Malus pumila* L. Mill.), *Environmental and Experimental Botany* (2018), https://doi.org/10.1016/j.envexpbot.2018.07.013

Environmental and Experimental Botany

The effect of the graft union on hormonal and ionic signalling between rootstocks and scions of grafted apple (*Malus pumila* L. Mill.)

Mark A. Else[1*], June M. Taylor[1], Stephen Young[2] and Christopher J. Atkinson[2]

[1]NIAB EMR, New Road, East Malling, Kent ME19 6BJ, UK
[2]Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent, ME4 4TB, UK

*Corresponding author;

Email addresses: mark.else@emr.ac.uk (M.A. Else), june.taylor@emr.ac,uk (**J.M. Taylor**), Stephen.Young@greenwich.ac.uk (**S. Young**), c.j.atkinson@gre.ac.uk (**C.J. Atkinson**)

#Tables: 9

Figs: 5

Highlights

- We report the results of an original investigation that examines the role of ionic and hormonal signalling in the dwarfing effect of apple rootstocks. Although the graft union was shown to alter the xylem sap ionic composition, no evidence was found that a sub-optimal supply of essential mineral ions was involved in the dwarfing response. We show that the dwarfing capacity of M.9 apple rootstock is linked with a reduced capacity for polar auxin transport, combined with the greater export of ABA compared to the semi-invigorating MM.106 rootstock.

Abstract

The transport of chemical signals between rootstocks and grafted scions is implicated in the dwarfing capacity of apple (*Malus pumila*) rootstocks. This study investigated whether the intensity of putative ionic and hormonal signals between a dwarfing rootstock (M.9) and a semi-invigorating rootstock (MM.106) grafted with 'Queen Cox' scions was altered by the graft union. The capacity of rootstocks with different dwarfing potential for polar auxin

transport (PAT) was also compared. Split-top pressure chambers were used to collect xylem sap samples at a range of flow rates from above and below the graft union in composite M.9 and MM.106 trees. Concentrations of hormones, anions and cations were quantified in expressed xylem sap. The effects of the graft union, flow rate and rootstock on xylem sap solute concentrations and deliveries were compared. Rootstocks of different dwarfing capacities maintained in micro-propagation were used to estimate and compare acropetal auxin transport; the velocity and intensity of PAT were determined using radiolabelled auxin. Sap osmolality and Ca^{++} concentrations were reduced by passage through the M.9 graft union at very low flow rates. At transpirational flow rates, Ca^{++} and Mg^{++} concentrations were increased, and those of Na^{+} and NO_3^{-} decreased, by passage through the graft union. Deliveries of anions and cations from roots into shoots of M.9 composite trees were always similar or greater to those of MM.106 composite trees. Sap zeatin and zeatin riboside concentrations were reduced above the graft in both rootstocks. No evidence was found that a sub-optimal supply of essential mineral ions was involved in the dwarfing effect of M.9. Root- and shoot-specific deliveries of ABA in M.9 composite trees were significantly higher compared to MM.106 trees, and the greater import of ABA correlated temporarily with earlier shoot growth termination in M.9 composite trees. Intensity of PAT through micro-propagated stem tissue was lower in the dwarfing rootstock compared to more invigorating rootstocks.

Keywords: dwarfing; graft union; hormone, ions; *Malus*; polar auxin transport, rootstock; signalling; xylem sap

1. Introduction

The ways in which dwarfing apple (*Malus*) rootstocks impart growth control over grafted scions have not been fully elucidated (Atkinson and Else, 2001; Aloni et al., 2010, Gregory et al., 2013). Shoots grafted onto dwarfing rootstocks extend more slowly and shoot growth terminates earlier in the season, but internode length remains largely unchanged (Costes and Garcia-Villanueva, 2007; Seleznyova et al., 2008). While some of the effects conveyed

by dwarfing rootstocks, such as the transition from the vegetative to reproductive phase and early shoot termination are via genes homologous to FLOWERING LOCUS T (Foster et al., 2014), the mechanisms that confer dwarfing remain unclear.

A number of hypotheses have been put forward to explain the 'dwarfing effect' and involve changes to hydraulic, ionic or hormonal signalling within the composite tree (rootstock and scion) (Gjamovskia and Kiprijanovskib, 2011; Gregory et al., 2013)). Roots of dwarfing rootstocks often have a lower hydraulic conductivity (Atkinson et al., 2003; Tombesi et al., 2010, Gregory et al., 2013), and it has been suggested that an altered shoot water balance could underpin the dwarfing effect (Cohen and Naor 2002; Solari and DeJong, 2006; Nardini et al., 2006; Goncalves et al., 2007). In *Malus*, the diametric growth of the graft union generally increases with the dwarfing potential of the rootstock and the convoluted xylem tissues within the union often run contrary to the axis of the stem (Simons, 1986; Soumelidou et al., 1994a). Similar observations have been made in unions of dwarfing *Prunus* rootstocks (Olmstead et al., 2006). It has been suggested that this distorted xylem arrangement imposes an axial resistance to water flow resulting in shoot water deficits which limit shoot growth (Warne and Raby, 1938; Beakbane, 1956; Tubbs, 1973a,b, Olien and Lasko, 1984). However, expressing the hydraulic conductivity of the union relative to scion canopy area showed no difference between semi-invigorating (MM.106) and dwarfing (M.9) *Malus* rootstocks (Atkinson et al., 2003), although M.9 root hydraulic conductivity was lower (Gregory et al., 2013). Root system conductance was also lower in Olea dwarfing rootstocks (Nardini et al., 2006). Swelling of the graft union with age may be a compensatory response to overcome the hydraulic limitations induced by altered xylogenesis at the union (Atkinson et al., 2003).

The stimulatory effect of indole acetic acid (IAA) on xylem development and cambial activity in woody species is well established (Sundberg et al., 2000) and altered xylogenesis within the graft union may arise from an accumulation of scion-derived IAA) in and above the graft union due, in part, to a reduced ability of the rootstock to transport IAA to the roots (Simons, 1986). A reduced IAA flux to a dwarfing rootstocks could also account for the

greater phloem-to-xylem ratio observed in more dwarfing rootstocks (Beakbane, 1952), as low concentrations of IAA preferentially induce differentiation of phloem rather than xylem (Aloni, 1995). In addition to disrupted phloem IAA transport across the graft union, two studies of polar auxin transport (PAT) in apple rootstocks indicate that transport velocity is reduced in more dwarfing rootstocks compared to more invigorating ones (Soumelidou et al., 1994b; Kamboj et al., 1997). Although Zhang et al. (2015) reported lower expression of MdPIN8 in inter-stems of M.9, transcript levels were not reduced in M.9 rootstock tissue compared to Fuji scion tissue. It is, however, unclear what the true flux of IAA through the PAT stream is without knowledge of both rate of transport (velocity) and the number of cells capable of polar transport (intensity).

Dwarfing rootstocks often have less extensive root systems than their more invigorating counterparts which led to the suggestion that the dwarfing effect may be imparted by sub-optimal supply of essential minerals (Jones, 1971; Lliso et al., 2004). Neilsen and Hampson (2014) reported a correlation between leaf P and tree vigour during establishment in a field trial involving 31 dwarfing and semi-dwarfing apple [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] rootstocks. However, foliar concentrations of the major nutrients are not often altered by the growth-controlling capacity of the rootstock and so the importance of ionic signalling is unclear. A role for the graft union in sequestering xylem sap minerals and other solutes has been proposed and Jones (1974) reported that sap concentrations of N, P and K in osmotically-exuded xylem sap were lowered after passage through the graft union. Furthermore, the extent of the ‘filtering’ correlated with the dwarfing capacity of the rootstock. Whether this sequestration operates at faster sap flows that match those of daytime transpiration rates and the possible impact on the availability to the shoot of essential mineral ions has not yet been determined.

An alternative and widely-held hypothesis is that the differential ability to synthesise, or metabolise endogenous plant hormones underpins the “dwarfing effect” (Lockard and Schneider, 1981; Jones, 1974; Soumelidou et al., 1994a, b; Atkinson and Else, 2001; Sorce et al., 2002, 2007; van Hooijdonk et al., 2011; Zhang et al., 2015) The idea that the export

of growth-promoting hormones (auxin, gibberellins, cytokinins) is lowered from dwarfing rootstocks, and/or that export of growth-inhibiting hormones (abscisic acid, ethylene) to the canopy is increased in composite trees has been tested many times, but definitive evidence for the hormonal control of scion vigour is still lacking. Often, the intensity of the putative signal may differ from extremely dwarfing and very invigorating rootstocks, but can be similar from semi-dwarfing and semi-invigorating rootstocks, despite a marked difference in tree stature (e.g. van Hooijdonk et al., 2011). The lack of definitive evidence is due, in part, to the difficulties of extrapolating hormone concentrations measured in slowly-flowing sap to hormone deliveries into canopies of intact, transpiring, composite trees. To determine accurately the passage of signals from roots to shoots in the xylem sap, information on both the concentration and the delivery rate of putative signals is needed (Else et al., 1995). It is also important to elucidate the role of the graft union on the intensity of root- and shoot-sourced hydraulic and chemical signalling (Gregory et al., 2013).

The main aims of this paper were to (1) identify differences in the intensity of putative hormone and ionic signals in sap xylem exported at realistic transpiration rates from dwarfing (M.9) and semi-invigorating (MM.106) *Malus* rootstocks, (2) to determine whether the graft union modifies xylem sap composition and (3) to quantify the velocity and intensity of PAT in micro-propagated apple rootstocks of different dwarfing capacity. Our experiments with composite trees were carried out immediately prior to the cessation of shoot growth in M.9 composite trees to try to identify differences in the intensity of putative xylem-borne signals from dwarfing M.9 and semi-invigorating MM.106 rootstocks.

2. Materials and methods

2.1. Plant material

Three-year-old M.9 and MM.106 rootstocks in 25 L pots of compost, grafted with ‘Queen Cox’ scions were maintained in a glasshouse at 20/15 °C day/night temperature. Trees were watered automatically using a drip irrigation system; RH was uncontrolled. The majority of the experiments were conducted in August, at the time when shoot growth in M.9 composite trees was slowing compared to the semi-invigorating MM.106.

Stem sections from micro-propagated plants were used to investigate the capacity for PAT in a range of rootstocks that encompassed the vigour of M.9 and MM.106: rootstocks 7.33 (dwarfing), Pi.80 (semi-invigorating) and M.7 (invigorating) were chosen since each has "Malling rootstock" as a parent. Micro-propagated shoots were maintained in vials in a growth room at 20 °C with a photoperiod of 16 h under a PAR of 100 μmol m^{-2} s^{-1} from fluorescent lamps (Philips, Surrey, UK). After four weeks on proliferation medium, individual shoots were transferred to medium without GA_3 or BAP, but with an increased concentration of IBA (3 mg m^{-3}) to initiate rooting. After three days, plantlets were transferred to individual vials containing medium without hormones. Thirty days after the start of the root initiation protocol, 94% of shoot cultures had rooted. Twenty rootstocks of each variety were subsequently selected for uniformity of size and used as a source of experimental material.

2.2. Collection of xylem sap from composite apple trees

Water loss from whole trees was determined gravimetrically for 24 h before xylem sap collection. Xylem sap samples were then collected from above and below the graft union using specially designed split-top root pressure chambers (Else et al., 1995). A decreasing series of pneumatic pressures was applied to the roots such that sap flow rates from the cut stem encompassed the whole-tree transpiration rates measured earlier. After each reduction in pressure, 5 min were allowed for xylem sap flows to stabilise before samples were collected on ice for 5 min, weighed and frozen in liquid nitrogen before storage at -78 °C. The stem of the rootstock was then cut 50 mm below the graft union and the sap collection procedure repeated. Sap collection from pressurised, detopped roots was completed within 3 h. Samples of sap exuding osmotically were then collected on ice at intervals over the next 24 h from each rootstock. Total shoot leaf area and root dry weights (after careful extraction) were determined for each tree.

2.3. Hormone analysis - internal standards

A solution of internal standards, containing 100 ng μl^{-1} of [$^2H_6$]-ABA; 50 ng μl^{-1} of [phenyl-$^{13}C_6$]-IAA (Promochem Ltd., Welwyn Garden City, UK); 25 ng μl^{-1} of [17,17 $^2H_2$]-gibberellins A_1, A_3, A_4, A_5, A_8, A_9, A_{12}, A_{19}, A_{20}, A_{29}, A_{44} and A_{53} (from Prof. L.N. Mander, Australian National University, Canberra, Australia) was prepared in ethanol (EtOH). These GAs constitute the major bioactive gibberellins, their precursors and their metabolites in apple (Yamaguchi, 2008). A solution of deuterated cytokinin standards containing 50 ng μl^{-1} each of [$^2H_5$]-zeatin (Z), [$^2H_5$]-zeatin riboside (ZR), [$^2H_3$]-dihydrozeatin (DHZ), [$^2H_3$]-dihydrozeatin riboside (DHZR), [$^2H_6$]-isopentenyladenine (IPA) and [$^2H_6$]-isopentenyladenosine (IPAR) (Apex Organics ltd., Honiton, UK) was prepared in dimethyl sulphoxide (DMSO).

2.4. Quantification of xylem sap hormones

After the addition of deuterated internal standards, xylem sap hormones were extracted and purified using anion exchange (SAX) (Isolute IST Ltd, UK) and C_{18} 'Sep-Pak' cartridges (Waters Associates, USA). Following derivatisation with ethereal diazomethane, methyl esters of ABA, IAA and GAs were quantified using an Agilent 6890 gas chromatogram equipped with a split/splitless injector coupled to an Agilent 5973 mass selective detector (MSD). Following the addition of trimethylphenylammonium hydroxide in methanol (TMAH) (Supelco USA), permethyleated cytokinins were produced in the injector of the GC-MS by heating to 305 °C. Concentrations of ABA, IAA, GAs and cytokinins were computed from calibration curves relating molar ratios of the endogenous compound and its deuterated analogue to ion intensities of their respective molecular ions.

2.5. Mineral analyses and osmolality

Calcium and Mg ion concentrations ([Ca], [Mg]) were determined in sap samples by AAS (1100B, Perkin Elmer, Bucks, UK), after the addition of lanthanum chloride; K and Na ion concentrations were determined by flame photometry. Phosphate, NO_3, SO_4 and malate ion ($C_4H_4O_5^{--}$) concentrations were determined in 10 mm^3 xylem sap samples by anion-

exchange chromatography using an IonPac AS4 analytical column (250 mm x 4 mm i.d.) with a AG4-SC guard column and a Dionex Conductivity Detector-3 (Dionex UK Ltd, Camberley, Surrey, UK). High background conductivity was lowered prior to chromatography by an Anion Self-Regenerating Suppressor-1 (Dionex (UK) Ltd). A standard solution of anions was chromatographed to establish retention times. Anions were eluted with a buffer containing 2.24 mol m^{-3} sodium hydrogen carbonate and 1.8 mol m^{-3} sodium carbonate (BDH Chemicals Ltd, Poole, UK) at a flow rate of 2 x 10^3 mm^3 min^{-1}. Osmolality of 50 mm^3 xylem sap samples was determined by depression of freezing point using a Roebling Digital Micro-Osmometer (Camlab Ltd., Cambridge, UK).

2.6. Polar auxin transport assay

The intensity and velocity of PAT through micro-propagated rootstock stem tissues were determined according to Else et al., (2004). Briefly, 0.2 mm^3 of [^{3}H]-IAA (3.33 kBq; 777 GBq $mmol^{-1}$; Nycomed Amersham Plc, Bucks, UK) was applied to the apical surfaces of stems of 7.33, Pi.80, M.7 rootstocks; in some experiments, [^{14}C]-benzoic acid (0.1 kBq; 229 MBq $mmol^{-1}$; Sigma-Aldrich Company Ltd, Poole, UK) was also applied. Following transport through the rootstock stems, the accumulation of radioactivity in basal agar receiving blocks was determined at 0.5 h intervals over 6 h. At the end of the transport period, the stem sections were removed from the agar receiver blocks and cut into smaller sections with a razor blade. The amount of [^{3}H]-IAA and any [^{14}C]-benzoic acid in the agar blocks and stem tissue sections were counted on a dual label ^{3}H/^{14}C channel using a Beckman 3801 LS scintillation counter (Beckman Instruments, Fullerton, CA, USA).

The cumulative radioactivity in the agar blocks over the transport period for each stem section was calculated and plotted against time. A linear regression was fitted to the central part of the transport curves using the least squares method. The slope of the regression equations represents the transport intensity, I (Bq h^{-1}); individual I values were determined for each tissue section and averaged within each rootstock. The intercept with the time axis was calculated from the regression equation, this indicates the time (T_0) at

which [^{3}H]-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 section. Velocities were averaged within each rootstock.

2.7. Statistical analyses

Typically, cation and anion delivery was proportional to flow rate on a log/log plot, implying a power law relationship (Fig. 1). The three data points used for subsequent comparative analysis between rootstocks were obtained from each data set (analysis from a single tree replicate) at low, median and high flow rates, substituting log standardised flow rates into the associated regression equation (Fig. 1). The standardised flow rates were the median flow rate for the whole study, the 25th percentile, and the 75th percentile flow rates; these were 40, 18, and 71 mm^3 s^{-1} respectively. These sap flow rates correspond to transpiration flows which would occur at the beginning and end of the photoperiod [low], over most of the photoperiod when stomata are fully open [median] and at the maximal midday-early pm transpiration rates (high) (see transpiration rate data in Fig. 2). The data were then analysed using a mixed effects model (Bates et al., 2015) allowing for the three repeated measures from each tree replicate. Means and standard errors were extracted from this model, which had rootstock and flow rate as fixed effects, and replicate as a random effect. Statistical inference was conservative, using the relevant number of true replicates (trees) to work out the residual degrees of freedom.

The relationship between [ABA] and sap flow rate was modelled by fitting an exponential decay function of the form:

$$[ABA] = a + m.e^{-(flow/a)}$$

where *a* is the asymptotic concentration at high flow rates, and m is the downwards slope at low flow rates. Curves were fitted to each replicate using a non-linear least squares method (R package: Bates and Watts, 1988). Average values for these parameters were calculated from all the combined replicates and presented as the mean [ABA] (± SE) for flow values in 10 (units) bins. Analysis of variance was also used to determine relationships between sap

flow, [ABA] and delivery rates above and below the graft union. For this comparative analysis, the range of xylem sap flow rates were averaged over the three flow categories. ABA data are means from six replicate composite trees of each rootstock.

3. Results

3.1. Effects of the graft union and flow rates on xylem sap solute concentrations

Despite rootstock differences in transpiration flow rate (Fig. 2) and leaf transpiration rates per unit area of canopy, the graft union did not impose an axial resistance to sap flow as reported in Gregory et al., (2013). However, xylem sap composition was altered by passage through the M.9 graft union at all flow rates tested. The osmolality of sap collected at the lowest flow rate (~5 $mm^3\ s^{-1}$) was significantly reduced above the graft union in M.9, but not in MM.106 rootstocks (Fig. 3). However, sap osmolality was not altered by passage through the graft union when sap flowed at rates that encompassed daytime transpiration rates in composite trees with either rootstock (Fig. 3). At the lowest flow rate, [Ca^{++}] was also higher in sap collected below the M.9 graft union than above it (see inset in Fig. 3), implying sequestration of Ca^{++} in slowly flowing sap by the tissues in the graft union of M.9 composite trees.

Analysis of xylem constituents in sap flowing at low (18 $mm^3\ s^{-1}$), median (40 $mm^3\ s^{-1}$) and high (71 $mm^3\ s^{-1}$) rates showed highly significant effects of the graft union, sap flow rate and rootstock on the ionic composition of the expressed xylem sap (Table 1). In M.9 composite trees, [Ca^{++}] and [Mg^{++}] were significantly higher in sap collected at all flow rates above the graft union than below it, while [Na^{+}] were significantly reduced above the graft union (Table 1). Nitrate concentrations were reduced significantly in sap collected above the graft union, whilst [K^{+}], [$C_4H_4O_5^{--}$], [PO_4^{--}] and [SO_4^{--}] were not influenced by sap flow rate or by passage through the graft union (Table 1). In MM.106 composite trees, [Ca^{++}], [Mg^{++}] and [NO_3^{-}] were influenced by flow rate but not by passage through the graft union (Table 1). Sap concentrations of K^{+} and $C_4H_4O_5^{--}$] were higher above the MM.106 graft union, whilst sap concentration of [SO_4^{--}] was lowered by passage through the graft union (Table 1).

Concentrations of ABA were measured in xylem sap collected over a range of flow rates and showed significant differences with respect to flow (p <0.0001), the graft union (p ≤0.014) and the rootstock (p≤0.002). The asymptote of the relationship between [ABA] and flow rate in M.9 composite trees was significantly higher than that for MM.106 composite trees (Fig. 4, Table 2) and indicates that sap [ABA] was greater above the graft union in M.9 composite trees, compared to MM.106 composite trees.

Concentrations of IAA in xylem sap from both rootstocks were very low and could not be quantified reliably in many samples, although the [phenyl-$^{13}C_6$]-IAA internal standard was always recovered. Where quantification was possible, [IAA] in sap collected from the same point were averaged within rootstocks, irrespective of the xylem sap flow rates at which they were collected (Table 3). These limited quantifications indicate that xylem [IAA] were apparently lower in sap collected above the graft union in M.9 composite trees, compared to values in sap below the graft union, but the results were not significantly different due to high sample variability. There was no significant effect of the graft union on sap [IAA] in MM.106 composite trees, although values were higher in sap collected above the graft union (Table 3).

Concentrations of GAs in all of the xylem sap samples collected from both M.9 and MM.106 composite trees were too low to quantify accurately (detection limit = 10 pg) even after bulking samples to increase the amount of GAs present; again deuterated internal standards were always recovered. Xylem concentrations of Z and ZR were similar in sap collected above the graft union from M.9 and MM.106 composite trees (Table 4). However, [Z] and [ZR] were significantly higher in sap collected from below the graft union in both rootstocks (Table 4).

3.2. Effect of the graft union on xylem sap ion delivery

Solute deliveries were calculated by multiplying sap concentrations by sap flow rates. The delivery of all ions in both rootstocks was significantly influenced by sap flow rate, with faster flows resulting in greater ion deliveries; this result was expected given the less-than-

proportional dilution of xylem sap solutes with increasing flow (Figs 3 & 4). In M.9, deliveries of Ca^{++}, Mg^{++}, Na^{+}, K^{+}, $C_4H_4O_5^{--}$ and PO_4^{-} were significantly higher above the graft union, delivery of NO_3^{-} was significantly lower above the graft union, while that of SO_4^{-} was unaffected by passage through the graft union. In MM.106, deliveries of Na^{+}, K^{+} and $C_4H_4O_5^{--}$ were significantly higher above the graft union, delivery of NO_3^{-} was significantly lower above the graft union, and Ca^{++}, Mg^{++} and SO_4^{-} deliveries were similar above and below the graft union (Table 5).

3.3. Hormone delivery from M.9 and MM.106 rootstocks

In M.9 and MM.106 composite trees, [ABA] were dependent on sap flow rates (Fig. 4) and so hormone delivery rates (sap concentration x flow rate) were calculated to enable meaningful comparisons of ABA output from M.9 and MM.106 rootstocks (Fig. 5). Using a combined ANOVA derived from both rootstocks and all flows and positions, the Tukey comparisons based on rootstocks above and below the graft union, but within each flow rate, show that at low flow, ABA delivery below the graft union from M.9 was significantly higher than all other comparisons at low flow. At median flows ABA delivery above the graft union in MM.106 was significantly lower than all other comparisons. At high flow rates there were no differences in ABA delivery rate.

3.4. Accounting for the effects of different root masses and leaf areas on xylem sap solute delivery into 'Queen Cox' scions from M.9 and MM.106 rootstocks

The root masses and leaf areas of composite M.9 trees were significantly smaller than those of composite MM.106 trees (Table 6). To account for these differences, the deliveries of ions and hormones from M.9 and MM.106 rootstocks into 'Queen Cox' scions were expressed in terms of nmol of substance delivered per unit dry weight of root or per unit area of shoot per unit time. These 'root' and 'shoot' specific deliveries were used to determine whether the intensity of putative signals differed between M.9 and MM.106 rootstocks. These specific deliveries were compared at the three different flow rates between rootstocks

and *p* values for sap flow rate, rootstock and rate x rootstock interaction are given in Table 7.

Export of Ca^{++} was significantly higher from M.9 than MM.106 rootstocks, and the corresponding import of Ca^{++} into the shoots was again significantly higher in M.9 composite trees (Table 7). Export of Mg^{++} was significantly higher from M.9 rootstocks, but the higher import into M.9 scions was just short of statistical significance. Export of NO_3^- was significantly higher from M.9 rootstocks and import into scions significantly greater than in MM.106 composite trees, while the export of PO4^{--} was greater from M.9 rootstocks but import into scions was similar, irrespective of rootstock. Similar patterns were seen with K^+ and with Na^+ (Table 7). Root- and shoot-specific deliveries of malate^{--} and SO_4^{--} were similar, irrespective of rootstock (Table 7).

The root-specific delivery of ABA was significantly greater from M.9 rootstocks than from MM.106 rootstocks at low, median and high flow rates (Table 8). Within each rootstock, root-specific deliveries of ABA increased significantly at higher sap flow rates. Shoot-specific deliveries into grafted scions were significantly higher in M.9 composite trees, compared to MM.106 composite trees (Table 8), and also increased significantly as sap flow rate increased.

3.5. Transport intensity and velocity in stems of 7.33, Pi.80 and M.7, rootstocks

Polar auxin transport intensities and velocities were greatest in the most invigorating rootstock, M.7 (Table 9). Transport intensities were lowest in the most dwarfing rootstock, 7.33, while transport velocities were similar in 7.33 and Pi.80 (Table 9). Transport intensities were reduced in all rootstocks after treatment with 100 mmol m^{-3} TIBA, but to different extents with 7.33 declining by 25%, Pi.80 by 58% and M.7 by 87%. Transport velocities were only marginally altered after treatment with TIBA (Table 9).

4. Discussion

The ways in which dwarfing rootstocks impart their control over grafted scions are complex and result from the cumulative and interactive effects of numerous different

processes operating simultaneously. Our results suggest that these include a 'filtering effect' of the graft union at very low flow rates, augmentation of xylem sap constituents by passage through the union at faster flows, and an altered synthesis or metabolism of key rootstock-sourced hormone signals. Complementary experimental evidence using micro-propagated rootstocks with dwarfing capacities similar to those of M.9 and MM.106 suggests that altered auxin transport is also likely to be involved, with dwarfing rootstocks having a reduced capacity for PAT.

The simple hypothesis that export or synthesis of growth-promoting hormones such as GAs and CKs are reduced from dwarfing rootstocks has lacked supporting experimental data, especially in more recent work where GC-MS has enabled the unequivocal identification of hormones and their precursors (e.g. van Hooijdonk et al., 2011, but see Zhang et al., 2015). Much previous work has relied on interpretations of hormone concentrations in xylem sap exuding osmotically from detopped trees (e.g. Kamboj et al., 1999; Sorce et al., 2002), but this approach may mislead. Our work shows that solute concentration in sap flowing under osmotic forces collected below the graft union from detopped rootstocks was high compared to that of expressed sap. Presumably, these effects were due to a combination of very low flow rates and the influence of an increasing shortage of respirable carbohydrates over a lengthy collection period (Else et al., 1995; Schurr and Schulze, 1995). Furthermore, elimination of PAT has been shown to alter root-sourced cytokinin export (Bangerth, 1994) and so interpretation of solute concentrations in osmotically-flowing sap is problematic. The concentrating effect of slow flowing sap was also seen in expressed sap flowing at rates below 20 $mm^3\ s^{-1}$ (night time transpiration rates) but at faster flows, solute concentrations remain relatively constant (see Figs. 3 & 4). The negligible effect of faster flows on sap solute concentrations was also reported in work with large conifers where concentrations of xylem sap components were unrelated to diurnal changes in transpirational flow rates (Osonubi et al., 1988). Clearly, it is important to quantify signal intensity at a range of flows that encompass whole-tree transpiration rates.

The impact of the graft union on xylem sap osmolality also varied with sap flow rate. In

M.9 composite trees, xylem sap osmolality was significantly lowered by passage through the graft union at very low flow rates (~5 mm^3 s^{-1}), but no further effects were seen at low, median or higher flow rates. Similar results were found with xylem sap [Ca^{++}]. The graft union in MM.106 did not affect the total osmolality of the xylem sap at the range of flow rates tested. However, our analyses show that the concentration and delivery of individual xylem sap constituents was altered markedly by flow rate, the graft union and the rootstock.

In M.9, xylem [NO_3^-], [Z] and [ZR] were reduced by passage through the graft union, as was [Na^+]. These findings agree in part with the work of Jones (1971, 1974) who showed that xylem sap [solute], [N], [P] and [K] were depleted after passage through the graft union of M.9 composite trees. In these earlier studies, sap exuding under osmotic forces was used and flow rates of such sap would presumably have been less than 1% of the whole tree transpiration rates (see Figs. 2 & 3). Our work suggests that, for certain solutes (e.g. Ca^{++}), this "filtering effect" of the graft union was only manifested at very low flow rates (~5 mm^3 s^{-1}), while sap [Ca^{++}] was augmented by passage through the M.9 graft union at flows encompassing whole-plant transpiration rates. Our data suggest that the source of this Ca may be that sequestered at very low flows; this idea is supported by reports that Ca accumulated in the graft union of several rootstock/scion combinations (Simons, 1986). The mechanistic basis of this flow-rate dependent switch between sequestration and augmentation in the tissues of the graft union is, at present, unclear, but may result from the flow-rate dependent movement of xylem sap through the apoplastic and symplastic pathways (Steudle, 2000). The flow-rate dependent cumulative removal or augmentation of physiologically important solutes such as Ca^{++} and NO_3^- by the graft union over the life of the tree could conceivably alter scion growth and development and even post-harvest fruit quality, but in our work, specific deliveries of essential ions was similar or higher in M.9 composite trees, compared to MM.106 composite trees, and not lower (see below).

When comparing putative signal intensities between rootstocks, a meaningful expression of solute delivery in xylem sap has to be one that takes account of differences in root mass and canopy area into which the dissolved solute is dispersed. These

considerations are especially important when comparing signal intensity in composite trees of different rootstocks where root mass and length, and shoot leaf area are often very different. Having accounted for these inherent differences, the root-specific delivery rates of Ca^{++}, Mg^{++}, NO_3^-, PO_4^{--}, K^+ and Na^+ were greater from M.9 than from MM.106 rootstocks (Table 7). These data imply that the ability of the M.9 root system to acquire and export essential nutrients does not influence the termination of scion growth. Although we have shown that xylem sap ionic composition was altered via sequestration and augmentation as it passes through the graft union, the intensity of xylem-borne ions passing into the shoots of M.9 composite trees was similar or greater than in MM.106 composite trees (Table 7), and so we conclude that the graft union is not a major contributor to the dwarfing effect. A three-locus model for apple rootstock-induced dwarfing proposed by Harrison et al. (2016), in which 84% of the variance in dwarfing response could be accounted for by primary rootstock traits, also supports this view.

A differential capacity to synthesise or metabolise key hormones with subsequent effects on the intensity of xylem-borne signaling is likely to underpin the dwarfing response (e.g. Sorce et al., 2007; van Hooijdonk et al., 2011) but definitive evidence is lacking due largely to technical challenges associated with accurately sampling xylem sap, particularly so in woody plants. In this study, we have accounted for the potentially confounding effects of flow rate on xylem sap constituents and have generated novel data on the intensity of key putative hormone signals. Since sap flow rates were not affected by the graft union, the concentration of xylem sap constituents at low, median or high flows could be used to assess accurately the filtering effect implicated in the dwarfing mechanism. Statistical analysis of our limited [IAA] data shows no significant effect of the graft union at the time of sampling in either rootstock, but the trend is for depletion of xylem-borne IAA by passage through the graft union in M.9. In support of this, Simons (1986) suggested that the tissue within the union of M.9 trees accumulated scion-derived IAA (and see below). Concentrations of Z and ZR were also similar above the graft union in M.9 and MM.106 composite trees (see also van Hooijdonk et al., 2011) but were much higher below the graft

union in both rootstocks. Concentrations of key CKs and their precursors were actually highest in sap flowing from branch stubs in the canopy of scions grafted on to M.9 (Else, unpublished data) and overall, no evidence was found to suggest that the dwarfing capacity of M.9 rootstocks could be attributed to a reduced supply of CKs from the rootstock to the scion (but see Zhang et al., 2015). Concentrations of the major bioactive GAs and their precursors and metabolites were below the limits of detection (10 pg). In earlier work, levels of GA were shown to differ in ungrafted rootstocks (Yadava and Lockard, 1977) but the proposed role of GAs in the dwarfing response (van Hooijdonk et al., 2010) is unclear since van Hooijdonk et al., (2011) reported that concentrations of GA_{19} were similar in xylem sap collected throughout the growing season from scions grafted on to M.9 or MM.106 rootstocks. The involvement of a DELLA-mediated disruption of GA regulation (Zhu et al., 2008) is also unlikely given that DELLA homologues in apple are located on linkage groups different from those known to confer dwarfing (Gregory et al., 2013; Fazio et al., 2014).

The export of ABA from the roots and into the scion was significantly higher in M.9 composite trees than in MM.106 composite trees at each of the three flow rates tested, once the effects of leaf area and root mass were taken into account. The higher delivery of ABA into the canopy of M.9 composite trees (Figure 5, Table 2) compared to that in MM106 composite trees presumably triggered the onset of stomatal closure earlier in the M.9 composite trees and the resultant slowing of transpiration rates in the afternoon and evening (Fig. 2B). Corresponding measurements of leaf stomatal conductance confirmed that stomata were open for longer each day in MM.106 composite trees (data not shown).

Our finding that ABA export was greater from M.9 rootstocks is supported by previous work that showed [ABA] to be higher in the tissues of the more dwarfing rootstocks (Kamboj et al., 1999). ABA is generally considered to be a potent growth inhibitor (but see Sharp et al., 2000) and the temporal correlation between the greater delivery of xylem-borne ABA into the canopy of composite M.9 trees when shoot extension had ceased would support further work to try to establish causality. ABA has been shown to limit extension growth by supressing the accumulation of GA_1 (Benschop et al., 2005) but whether rootstock-sourced

ABA and scion-derived GAs interact to regulate shoot extension in grafted scions is not known. Repeated measurements of xylem sap constituents over the entire growing season (van Hooijdonk et al., 2011) using an untargeted metabolomics approach would yield valuable information on altered signalling from dwarfing rootstocks. This approach is likely to be more fruitful than measuring tissue concentrations of key hormones since these do not often correlate with measured differences in vigour (see Pearce et al., 2004).

In addition to rootstock-derived signals, a disruption to PAT has also been implicated in the dwarfing response. In support of this, van Hooijdonk et al., (2010) reported that when N-1-naphthylphthalamic acid (NPA), an auxin transport inhibitor, was applied to the stem of invigorating rootstocks, total shoot growth of the scion was decreased and the resulting architectural changes most closely resembled those imposed by M.9. Zhang and co-authors (2015) suggested that low expression of PIN gene family members was involved in triggering the dwarfing effect of M.9 interstems but not of M.9 rootstocks. In our work, PAT velocity was reduced in more dwarfing apple rootstocks compared to more invigorating ones, in agreement with reports by Soumelidou et al. (1994a). However, only simultaneous measurements of PAT velocity and intensity can provide information on the capacity of the PAT, the parameter most likely to impact on the physiological processes that regulate the dwarfing response. Our results from micro-propagated rootstock stem sections indicate that PAT is likely to be an important component of dwarfing since PAT intensity correlated with dwarfing capability. TIBA reduced PAT I transport capacities to different extents in the three rootstocks; the intensity of PAT in Pi.80 rootstocks was less affected by TIBA compared to the other two rootstocks. The reasons for these differences are not known but could include the number of cells capable of polar transport, the activity, turnover or membrane trafficking of the efflux carriers (Geldner et al., 2001), or the concentrations of, or sensitivity to, naturally-occurring polar transport inhibitors. Disruption of PAT may also contribute to the effects of grafted bark tissue on scion vigour, an effect attributed solely to the interruption of phloem-transported IAA by Lockard and Schneider (1981). Auxin moving through the PATS may be more important than phloem-borne IAA since the diffusion of IAA from the primary

shoot tips was similar in M.9 and MM.106 composite trees (van Hooijdonk et al., 2011).

Acknowledgements

This work was funded by Defra (formerly the Ministry of Agriculture Fisheries and Food – Project HH1614STF). We thank Mr Lez Saker, Ms Lorraine Taylor and Mr Aytekin Polat for their excellent technical assistance.

References

Aloni, R., 1995. The induction of vascular tissues by auxin and cytokinin. *In*: Davies P.J. (Ed.). Plant Hormones. Kluwer Academic Publishers, Netherlands, pp. 531-546.

Aloni, B., Cohen, R., Karni, L., Aktas, H., Edelstein M. 2010. Hormonal signalling in rootstock-scion interactions. Sci. Hort. 127, 119-126.

Atkinson, C.J., Else MA. 2001. Understanding how rootstocks dwarf fruit trees. *Com. Fruit Tree* 34, 46-49.

Atkinson, C.J., Else, M.A., Taylor, L., Dover, C.J., 2003. Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). J. Exp. Bot. 54, 1221-1229.

Bangerth, F., 1994. Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus vulgaris* L.) plants to decapitation and auxin treatment, and relationships to apical dominance. Planta 194, 439-442.

Bates, D.M., Watts, D.G., 1988. *Nonlinear Regression Analysis and Its Applications* John Wiley & Sons, Inc. Hoboken, New Jersey, USA.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67, 1-48.

Beakbane, A.B., 1952. Anatomical structure in relation to rootstock behaviour. *In* Proc. of the 13th Int. Hort. Cong., Vol. I. Ed. P.M. Synge. London, pp 152-158.

Beakbane, A.B., 1956. Possible mechanism of rootstock effect. Ann. App. Biol. 44, 517-521.

Benschop, J.J., Jackson, M.B., Guhl, K., Vreeburg, R.A.M., Croker, S.J., Peeters, A.J.M., Voesenek, L.A.C.J., 2005. Contrasting interactions between ethylene and abscisic acid in *Rumex* species differing in submergence tolerance. Plant J. 44, 756-768.

Cohen, S., Naor, A., 2002. The effect of three rootstocks on water use, canopy conductance and hydraulic parameters of apple trees and predicting canopy from hydraulic conductance. Plant Cell Environ. 25, 17–28.

Costes, E., Garcia-Villanueva, E., 2007. Clarifying the effects of dwarfing rootstock on vegetative and reproductive growth during tree development: a study on apple trees. Ann. Bot. 100, 347–357.

Else, M.A., Hall, K.C., Arnold, G.M., Davies, W.J., Jackson, M.B., 1995. Export of ABA, ACC, phosphate and nitrate from roots to shoots of flooded tomato plants. Accounting for effects of xylem sap flow rate on concentration and delivery. Plant Physiol. 107, 377-384.

Else, M.A., Stankiewicz-Davies, A.P., Crisp, C.M., Atkinson, C.J., 2004. The role of polar auxin transport through pedicels of *Prunus avium* L. in relation to fruit development and retention. J. Exp. Bot. 55, 2099-2109.

Fazio, G., Wan, Y.Z., Kviklys, D., Romero, L., Adams, R., Strickland, D., Robinson, T., 2014. Dw2, a new dwarfing locus in apple rootstocks and its relationship to induction of early bearing in apple scions. J. Amer. Soc. Hort. Sci. 139, 87-98.

Foster, T.M., Watson, A.E., van Hooijdonk, B.M., Schaffer, R.J., 2014. Key flowering genes including FT-like genes are upregulated in the vasculature of apple dwarfing rootstocks.

Tree Gen. Genomes 10, 189–202.
Geldner, M., Friml, J., Stierhof, Y-D., Jűrgens, G., Palme K., 2001. Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413, 425-428.
Gjamovskia, V., Kiprijanovskib, M., 2011. Influence of nine dwarfing apple rootstocks on vigour and productivity of apple cultivar 'Granny Smith'. Sci. Hort. 129, 742–746.
Goncalves, B., Correia, C.M., Silva, A.P., Bacelar, E.A., Santos, A., Ferreira. H., Moutinho-Pereira. J.M. 2007. Variation in xylem structure and function in roots and stems of scion–rootstock combinations of sweet cherry tree (*Prunus avium* L.). Trees 21, 121–130.
Gregory, P.J., Atkinson, C.J., Bengough, G.A., Else, M.A., Fernández-Fernández, F., Harrison, R.J., Schmidt, S., 2013. Contributions of roots and rootstocks to sustainable intensified crop production J. Exp. Bot. 64, 1209-1222.
Harrison, N., Harrison, R.J, Barber-Perez, N., Cascant-Lopez, E., Cobo-Medina, M., Lipska, M., Conde-Ruiz, R., Brain, P., Gregory, P.J., Fernandez-Fernandez, F., 2016. A new three-locus model for rootstock-induced dwarfing in apple revealed by genetic mapping of root bark percentage J. Exp. Bot. 67, 1871-1881.
Jones, O.P., 1971. Effects of rootstocks and interstocks on the xylem sap composition in apple trees. Effects on nitrogen, phosphorous and potassium content. Ann. Bot. 35, 825-836.
Jones, O.P., 1974. Xylem sap composition in apple trees. Effect of the graft union. Ann. Bot 38, 463-467.
Kamboj, J.S., Browning, G., Quinlan, J.D., Blake, P.S., Baker, D.A., 1997. Polar transport of [3H]-IAA in apical shoot segments of different apple rootstocks. J. Hort. Sci. 72, 773-780.
Kamboj, J.S., Browning, G., Blake, P.S,, Quinlan, J.D., Baker, D.A., 1999. GC-MS-SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. Plant Growth Regul. 28, 21-27.
Lliso, I., Forner, J.B., Talón, M., 2004. The dwarfing mechanism of citrus rootstocks F&A 418 and #23 is related to competition between vegetative and reproductive growth. Tree Physiol. 24, 225–232.
Lockard, R.G., Schneider, G.W., 1981. Stock and scion growth relationships and the dwarfing mechanism in apple. Hort. Rev. 2, 315-375.
Nardini, A., Gasco, A., Raimondo, F., Gortan, E., Lo Gullo, M.A., Caruso, T., Salleo, S., 2006. Is rootstock-induced dwarfing in olive an effect of reduced plant hydraulic efficiency? Tree Physiol. 26, 1137-1144.
Neilsen, G., Hampson, C., 2014. Honeycrisp apple leaf and fruit nutrient concentration is affected by rootstock during establishment. J. Amer. Pom. Soc. 68, 178-189.
Olien, WC, Lasko, AN., 1984. A comparison of the dwarfing character and water relations of five apple rootstocks. Acta Hort. 146, 151-158.
Olmstead, M.A., Lang, N.S., Lang, G.A., Ewers, F.W., Owens, S.A., 2006. Examining the vascular pathway of sweet cherries grafted onto dwarfing rootstocks. HortSci. 41, 674-679.
Osonubi, O., Oren, R., Werk, K.S., Schulze, E-D., Heilmeier, H. 1988. Performance of 2 *Picea abies* (L) Karst stands at different stages of decline. 4. Xylem sap concentrations of magnesium, calcium, potassium and nitrogen. Oecologia 77, 1-6.
Seleznyova, A.N., Tustin, D.S., Thorp, T.G., 2008. Apple dwarfing rootstocks and interstocks affect the type of growth units produced during the annual growth cycle: precocious transition to flowering affects the composition and vigour of annual shoots. Ann. Bot. 101, 679–687.
Schurr, U., Schulze E-D., 1995. The concentration of xylem sap constituents in root exudate, and in sap from intact, transpiring castor bean plants (*Ricinus communis* L.). Plant Cell Environ. 18, 409-420.
Sharp, R.E., LeNoble, M.E., Else, M.A., Thorne, E.T., Gherardi, F., 2000. Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. J. Exp. Bot. 51, 1575-1584.
Simons, R.K., 1986. Graft-union characteristics as related to dwarfing in apple (*Malus*

domestica Borkh.). Acta Hort. 160, 57-66.
Solari, L.I., DeJong, T.M., 2006. The effect of root pressurization on water relations, shoot growth, and leaf gas exchange of peach (*Prunus persica*) trees on rootstocks with differing growth potential and hydraulic conductance. J. Exp. Bot. 57, 1981-1989.
Sorce, C., Massai, R., Picciarelli, P., Lorenzi, R., 2002. Hormonal relationships in xylem sap of grafted and ungrafted *Prunus* rootstocks. Sci. Hort. 93, 333-342.
Sorce, C., Mariotti, L., Lorenzi, R., Massai, R., 2007. Hormonal factors involved in the control of vigour of grafted peach [*Prunus persica* (L.) Batsch] trees and hybrid rootstocks. Adv. Hort. Sci. 21, 68-74.
Soumelidou, K., Morris, D.A., Battey, N.H., Barnett, J.R., John, P., 1994a. Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. J. Hort Sci. 69, 719-725.
Soumelidou, K., Battey, N.H., John, P., Barnett, J.R., 1994b. The anatomy of the developing bud union and its relationship to dwarfing in apple. Ann. Bot. 74, 605-611.
Steudle, E., 2000. Water uptake by roots: effects of water deficit. J. Exp. Bot. 51, 1531-1542.
Sundberg, B., Uggla, C., Tuominen, H., 2000. Cambial growth and auxin gradients. In Cell and molecular biology of wood formation. Eds. R.A. Savidge, J.R. Barnett, R. Napier. Bios Scientific, Oxford, pp 169-188.
Tombesi, S., Johnson, R.S., Day, K.R., DeJong, T.M., 2010. Relationships between xylem vessel characteristics, calculated axial hydraulic conductance and size-controlling capacity of peach rootstocks. Ann. Bot. 105, 327–331.
Tubbs, F.R., 1973a. Research fields in the interaction of rootstocks and scions in woody perennials. Hort. Abstracts 43, 247-253.
Tubbs, F.R., 1973b. Research fields in the interaction of rootstocks and scions in woody perennials. Hort. Abstracts 43, 325-335.
van Hooijdonk, B.M., Woolley, D.J., Warrington, I.J., Tustin, D.S., 2010. Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot-signalling by auxin, gibberellin and cytokinin. J. Hort. Sci. Biotech. 85, 59-65.
van Hooijdonk, B.M., Woolley, D.J., Warrington, I.J., Tustin, D.S., 2011. Rootstocks modify scion architecture, endogenous hormones, and root growth of newly grafted 'Royal Gala' apple trees. J. Amer. Soc. Hort. Sci. 136, 93-102.
Warne, L.G.G., Raby, J., 1938. The water conductivity of the graft union in apple trees, with special reference to Malling rootstock No. 14. J. Pom. Hort. Sci. 14, 389-399.
Yamaguchi, S., 2008. Gibberellin metabolism and its regulation. Ann. Rev. Plant Biol. 59, 225-251.
Zhu, L.H., Li, X.Y., Welander, M., 2008. Overexpression of the Arabidopsis gai gene in apple significantly reduces plant size. Plant Cell Rep. 27, 289–296.
Zhang, H., An, H.S., Wang, Y., Zhang, X.Z., Han, Z.H., 2015. Low expression of PIN gene family members is involved in triggering the dwarfing effect in M9 interstem but not in M9 rootstock apple trees. Acta Physiol. Plant. 37, article number 104.

Table 1. The effect of the graft union on concentrations of cations and anions in xylem sap expressed from composite trees of M.9 (dwarfing) and MM.106 (semi-invigorating) Malus rootstocks grafted with Queen Cox scions at three different flow rates. Ion concentrations were quantified in xylem sap collected below the graft union (BGU) and above the graft union (AGU). The three data points used for comparative analysis between sampling positions and between rootstocks were obtained from each data set (analysis from a single tree replicate) at low, median and high flow rates, substituting log standardised flow rates into individual regression equations. P values of statistical significance of ion concentration with flow rates and difference between above and below the graft union are given, NS = not significant.

Ion	Rootstock	M.9					MM.106				
	Sap flow ($mm^3\ s^{-1}$)	18 (25^{th})	40 (median)	71 (75^{th})	Trend with flow	AGU/BGU difference	18	40	71	Trend with flow	AGU/BGU difference
	Sampling position	Sap concentration ($ng\ mm^3$)			*p*	*p*	Sap concentration ($ng\ mm^3$)			*p*	*p*
Ca^{++}	BGU	0.244 ±0.019	0.177 ±0.010	0.140 ±0.008	<0.0001	<0.0001	0.269 ±0.018	0.239 ±0.012	0.220 ±0.011	<0.0001	NS
	AGU	0.295 +0.020	0.252 +0.020	0.225 +0.010			0.288 +0.020	0.251 +0.020	0.227 +0.010		
Mg^{++}	BGU	0.138 ±0.016	0.102 ±0.010	0.083 ±0.008	<0.0001	<0.0001	0.158 ±0.016	0.141 ±0.012	0.130 ±0.011	<0.05	NS
	AGU	0.172 +0.017	0.146 +0.013	0.129 +0.011			0.171 +0.017	0.148 +0.013	0.134 +0.011		
Na^{+}	BGU	0.011 ±0.002	0.009 ±0.002	0.007 ±0.002	<0.01	<0.001	0.010 ±0.001	0.007 ±0.001	0.006 ±0.001	NS	NS
	AGU	0.007 +0.001	0.005 +0.001	0.004 +0.001			0.008 +0.001	0.006 +0.001	0.006 +0.001		
K^{+}	BGU	0.726 ±0.091	0.655 ±0.074	0.609 ±0.066	NS	NS	0.807 ±0.088	0.774 ±0.075	0.751 ±0.070	NS	<0.01

	AGU	0.754 +0.082	0.709 +0.069	0.678 +0.063			0.961 +0.105	0.899 +0.087	0.857 +0.080		
$C_4H_4O_5^{--}$	BGU	0.120 ±0.069	0.094 ±0.054	0.0783 ±0.046	NS	NS	0.322 ±0.183	0.303 ±0.174	0.290 ±0.169	NS	<0.01
	AGU	0.248 +0.128	0.243 +0.127	0.240 +0.126			0.424 +0.241	0.430 +0.247	0.434 +0.253		
NO_3^-	BGU	0.199 ±0.076	0.571 ±0.222	1.223 ±0.497	<0.001	<0.01	0.124 ±0.048	0.290 ±0.113	0.540 ±0.219	<0.0001	NS
	AGU	0.103 +0.076	0.225 +0.210	0.400 +0.497			0.071 +0.048	0.196 +0.113	0.408 +0.219		
PO_4^{-3}	BGU	0.115 ±0.021	0.093 ±0.014	0.079 ±0.012	NS	NS	0.103 ±0.019	0.115 ±0.018	0.125 ±0.018	NS	NS
	AGU	0.111 +0.018	0.105 +0.014	0.101 +0.013			0.113 +0.020	0.119 +0.018	0.122 +0.018		
SO_{4--}	BGU	0.033 ±0.013	0.036 ±0.013	0.036 ±0.0145	NS	NS	0.052 ±0.020	0.056 ±0.022	0.059 ±0.024	NS	<0.05
	AGU	0.043 +0.015	0.039 +0.014	0.036 +0.013			0.038 +0.015	0.040 +0.016	0.042 +0.017		

Table 2. The relationship between ABA concentration and flow rate was modelled with an exponential decay function and the average exponential decay curve parameters are shown below the graft union (BGU) and above the graft union (AGU) of composite trees of M.9 (dwarfing) and MM.106 (semi-invigorating) *Malus* rootstocks grafted with Queen Cox scions.

Rootstock	BGU		AGU	
	Asymptote (a)	Slope (m)	Asymptote (a)	Slope (m)
M.9	14.9 ± 2.9 (n=6)	51.2 ± 19.0 (n=8)	15.0 ± 2.0* (n=7)	17.7 ± 7.4 (n=7)
MM.106	14.4 ± 4.0 (n=6)	15.2 ± 10.0 (n=8)	8.7 ± 1.0* (n=7)	47.3 ± 25.2 (n=7)

Note: the exponential decay curve parameters derived from $[ABA] = a + m.e^{-(flow/a)}$ where *a* is the asymptotic concentration (xx yy) at high flow rates, and *m* is the downwards slope at low flow rates. *Difference statistically significant ($p = 0.019$, t=2.9, d.f. = 9).

Table 3. Concentration of IAA in xylem sap collected from below the graft union (BGU) and above the graft union (AGU) in in M.9 (dwarfing) and MM.106 (semi-invigorating) composite *Malus* trees.

	Xylem [IAA] (pg mm^3)	
Rootstock	BGU	AGU
M.9	0.18	0.11
MM.106	0.11	0.20
Union		0.825
Rootstock		0.814
Union x rootstock		0.052[1]

Note: Results are means of 7-13 replicates with associated standard errors. [1]ANOVA denotes a significance of 0.05.

Table 4. Xylem sap zeatin [Z] and zeatin riboside [ZR] concentrations in sap collected from below the graft union (BGU) and above the graft union (AGU) in M.9 (dwarfing) and MM.106 (semi-invigorating) composite *Malus* trees.

	Xylem sap [Z] (pg mm^3)		Xylem sap [ZR] (pg mm^3)	
Rootstock	BGU	AGU	BGU	AGU
M.9	0.37	0.18	0.46	0.22
MM.106	0.42	0.23	0.86	0.22
Union	0.027*		0.003**	
Rootstock	0.557		0.722	
Union x rootstock	0.951		0.763	

Note: Results are means of 8-17 replicates with associated standard errors. *ANOVA denotes a significance of 0.01 and ** 0.001.

Table 5. The effect of the graft union on deliveries of cations and anions in xylem sap expressed from composite trees of M.9 (dwarfing) and MM.106 (semi-invigorating) Malus rootstocks grafted with Queen Cox scions at three different flow rates. Ion delivery rates below the graft union (BGU) and above the graft union (AGU-) were calculated by multiplying concentrations by sap flow rates. The three data points used for comparative analysis between sampling positions and between rootstocks were obtained from each data set (analysis from a single tree replicate) at low, median and high flow rates, substituting log standardised flow rates into individual regression equations. P values of statistical significance of ion delivery with flow rates and difference between above and below the graft union are given, NS = not significant.

Ion	Rootstock	M.9					M.M.106				
	Sap flow ($mm^3\ s^{-1}$)	18 (25^{th})	40 (median)	71 (75^{th})	Trend with flow	AGU/BGU difference	18 (25^{th})	40 (median)	71 (75^{th})	Trend with flow	AGU/BGU difference
	Sampling position	Ion delivery rate ($nmol\ s^{-1}$)			*p*	*p*	Ion delivery rate ($nmol\ s^{-1}$)			*p*	*p*
Ca^{++}	BGU	4.39 + 0.27	7.03 + 0.44	9.91 + 0.62	<0.0001	<0.0001	4.82 + 0.23	9.51 + 0.46	15.59 + 0.76	<0.0001	0.103
	AGU	5.32 + 0.33	10.04 + 0.61	15.96 + 0.97			5.19 + 0.25	9.99 + 0.48	16.10 + 0.78		
Mg^{++}	BGU	2.48 + 0.31	4.08 + 0.51	5.86 + 0.73	<0.0001	0.0007	2.81 + 0.26	5.55 + 0.51	9.11 + 0.83	<0.0001	0.70
	AGU	3.35 + 0.42	6.04 + 0.75	9.29 + 1.15			2.85 + 0.27	5.45 + 0.51	8.73 + 0.81		
Na^{+}	BGU	12.32 + 1.70	24.27 + 3.35	39.79 + 5.49	<0.0001	<0.0001	14.60 + 0.98	31.11 + 2.11	53.97 + 3.65	<0.0001	0.0007
	AGU	13.57 + 1.86	28.22 + 3.87	48.13 + 6.61			17.29 + 1.17	35.79 + 2.42	60.83 + 4.12		

K^+	BGU	12.32 + 1.70	24.27 + 3.35	39.79 + 5.50	<0.0001	<0.0001	14.61 + 0.99	31.11 + 2.11	53.97 + 3.65	<0.0001	0.0007
	AGU	13.57 + 1.86	28.22 + 3.88	48.13 + 6.61			17.29 + 1.17	35.79 + 2.43	60.83 + 4.12		
$C_4H_4O_5^{--}$	BGU	3.42 + 1.14	5.75 + 1.92	8.40 + 2.81	<0.0001	<0.0001	5.72 + 0.75	11.98 + 1.58	20.54 + 2.72	<0.0001	<0.0001
	AGU	6.68 + 2.22	14.20 + 4.72	24.59 + 8.17			7.51 + 0.99	17.02 + 2.25	30.88 + 4.09		
NO_3^-	BGU	3.58 + 1.38	22.71 + 8.77	87.19 + 33.68	<0.0001	0.0019	2.49 + 0.84	11.45 + 3.86	34.79 + 11.72	<0.0001	0.031
	AGU	1.98 + 0.74	9.65 + 3.64	30.60 + 11.56			1.24 + 0.42	8.13 + 2.74	31.25 + 10.53		
PO_4^{---}	BGU	2.07 + 0.35	3.69 + 0.62	5.63 + 0.95	<0.0001	0.027	1.89 + 0.29	4.67 +0.71	9.02 + 1.38	<0.0001	0.73
	AGU	2.05 + 0.34	4.36 + 0.74	7.58 + 1.28			2.04 + 0.31	4.73 + 0.72	8.73 + 1.33		
SO_4^{--}	BGU	0.59 + 0.27	1.39 +0.63	2.57 + 1.17	<0.0001	0.20	0.94 + 0.16	2.19 + 0.37	4.07 + 0.68	<0.0001	<0.001
	AGU	0.77 + 0.34	1.55 + 0.70	2.58 + 1.16			0.68 + 0.12	1.59 + 0.27	2.93 + 0.49		

Table 6. Root dry weights and total leaf areas from composite M.9 (dwarfing) and MM.106 (semi-invigorating) *Malus* rootstocks grafted with 'Queen Cox' scions.

Rootstock	Root dry weight (g)	Total leaf area (m^2)
M.9	113.9	1.69
MM.106	294.9	2.23
t Stat	7.68	3.603
p	<0.001	0.002
d.f.	10	12

Note: Results are means of eight replicates with associated standard errors. T-Test was used assuming unequal variances.

Table 7. Comparison of cation and anion deliveries from rootstocks and into scions of composite trees of M.9 (dwarfing) and MM.106 (semi-invigorating) *Malus* rootstocks grafted with Queen Cox scions. Delivery rates were calculated by multiplying sap ion concentrations by sap flow rate and expressed as rate per unit mass (kg - root) of root or rate per unit canopy leaf area (m^2 - shoot). P values of statistical significance of root- or shoot-specific delivery rate with flow rates and difference between above and below the graft union are given, NS = not significant.

Ion	Rootstock	Delivery from the root, or to the shoot	Sap flow			*p values*		
			25 (18 mm^3 s^{-1})	Median (40 mm^3 s^{-1})	75 (71 mm^3 s^{-1})	Rate	Rootstock	Rate x rootstock
Ca^{++}	M.9	Root	43.4 + 3.3	69.4 + 5.3	98.0 + 7.5	<0.0001	0.0001	0.0027
	MM.106		16.6 + 1.1	32.8 + 2.2	53.8 + 3.6			
	M.9	Shoot	3.2 + 0.2	6.0 + 0.3	9.5 + 0.5	<0.0001	0.040	0.50
	MM.106		2.4 + 0.1	4.5 + 0.3	7.3 + 0.4			
Mg^{++}	M.9	Root	24.5 + 3.2	40.3 + 5.2	58.0 + 7.6	<0.0001	0.0002	0.0008
	MM.106		9.8 + 1.4	19.5 + 2.2	32.1 + 0.5			
	M.9	Shoot	1.8 + 0.2	3.5 + 0.3	5.5 + 3.6	<0.0001	0.056	0.55
	MM.106		1.4 + 0.1	2.7 + 0.2	4.3 + 0.4			
Na^{+}	M.9	Root	1.87 + 0.26	3.16 + 0.43	4.61 + 0.63	<0.0001	0.0001	0.68
	MM.106		0.61 + 0.07	0.96 + 0.11	1.34 + 0.16			
	M.9	Shoot	0.07 + <0.01	0.11 + 0.01	0.16 + 0.02	<0.0001	0.76	0.008
	MM.106		0.06 + <0.01	0.12 + 0.16	0.20 + 0.03			
K^{+}	M.9	Root	129 + 13	258 + 26	427 + 43	<0.0001	0.0001	0.42
	MM.106		50 + 4	107 + 10	186 + 17			
	M.9	Shoot	8.0 + 1.0	16.8 + 2.1	28.6 + 3.6	<0.0001	0.89	0.98

	MM.106		7.8 + 0.9	16.3 + 2.0	27.9 + 3.5			
$C_4H_4O_5^{--}$	M.9	Root	35 + 11	55 + 18	76 + 24	0.0005	0.68	0.08
	MM.106		21 + 7	46 + 15	79 + 26			
	M.9	Shoot	4.0 + 0.9	8.8 + 1.9	15.4 + 3.4	<0.0001	0.59	0.50
	MM.106		3.2 + 0.8	7.3 + 1.8	13.2 + 3.2			
NO_3^-	M.9	Root	35 + 14	225 + 87	864 + 336	<0.0001	0.04	0.04
	MM.106		9.6 + 3.7	44 + 17	134 + 52			
	M.9	Shoot	1.2 + 0.5	5.9 + 2.3	18.8 + 7.1	<0.0001	0.36	0.03
	MM.106		0.5 + 0.2	3.6 + 1.5	14.1 + 5.9			
PO_4^{---}	M.9	Root	20.5 + 3.4	36.6 + 6.1	55.8 + 9.2	0.0001	0.028	0.032
	MM.106		7.3 + 1.2	17.9 + 3.0	34.6 + 5.7			
	M.9	Shoot	1.27 + 0.17	2.71 + 0.36	4.70 + 0.63	<0.0001	0.18	0.28
	MM.106		0.87 + 0.11	2.02 + 0.27	3.74 + 0.51			
SO_4^{--}	M.9	Root	6.0 + 2.4	13.8 + 5.6	25.1 + 10	<0.0001	0.42	0.76
	MM.106		3.5 + 1.4	8.4 + 3.4	15.7 + 6.4			
	M.9	Shoot	0.47 + 0.15	0.95 + 0.30	1.59 + 0.51	<0.0001	0.46	0.03
	MM.106		0.28 + 0.09	0.68 + 0.23	1.28 + 0.45			
	MM.106		0.28 + 0.09	0.68 + 0.23	1.28 + 0.45			

Table 8. Comparison of ABA deliveries from rootstocks and into scions of composite trees of M.9 (dwarfing) and MM.106 (semi-invigorating) *Malus* rootstocks grafted with Queen Cox scions. Delivery rates were calculated by multiplying sap ion concentrations by sap flow rate and expressed as rate per unit mass (kg - root) of root or rate per unit canopy leaf area (m^2 - shoot).

Hormone	Rootstock	Delivery from the root, or to the shoot	Sap flow			*p values*		
			25 (18 mm^3 s^{-1})	Median (40 mm^3 s^{-1})	75 (71 mm^3 s^{-1})	Rate	Rootstock	Rate x rootstock
ABA	M.9	Root (ng kg^{-1} s^{-1})	3.87^{c} + 0.68	6.20^{d} + 1.09	8.74^{e} + 1.54	<0.0001	0.0003	0.1698
	MM.106		0.79^{a} + 0.13	1.45^{b} + 0.24	2.26^{c} + 0.37			
	M.9	Shoot (ng m^{-2} s^{-1})	0.16^{ABC} + 0.03	0.33^{DF} + 0.07	0.53^{E} + 0.11	<0.0001	0.122	0.1257
	MM.106		0.13^{A} + 0.02	0.21^{CD} + 0.05	0.30^{BEF} + 0.06			

Values followed by different letters (either upper or lower case) are significantly different from each other.

Table 9. The effect of rootstock vigour and TIBA on polar IAA transport intensity and velocity through 4-mm-long stem sections of three micro propagated Malus rootstocks with differing vigour control (7.33 dwarfing, Pi.80 semi-invigorating and M.7 invigorating).

		Control		TIBA (100 mmol m^{-3})	
Rootstock		ıtensity 3q h^{-1})	elocity nm h^{-1})	ıtensity 3q h^{-1})	elocity nm h^{-1})
7.33	Dwarfing	6.1	5.1	4.6 ± 0.2 (25%)*	5.6 ± 0.5
Pi. 80	Semi-invigorating	39.2	4.6	16.5 ± 1.5 (58%)	5.5 ± 0.6
M.7	Invigorating	61.0	8.4	7.8 ± 1.3 (87%)	6.4 ± 0.3
TIBA		***	*NS*		
Rootstock		***	*		
TIBA x Rootstock		***	*NS*		

Note: Data are means of four to ten replicates with standard error of the mean values. *Percentages shown represent the reduction in PAT intensity in the presence of 100 mmol m^{-3} TIBA relative to no TIBA. ANOVA *p* values, *denotes 0.05 and ***<0.001.

Figure legends

Fig 1. Typical Ca^{++} delivery (nmol m^{-2} s^{-1}) above graft union, plotted on log/log scales against flow in mm^3 s^{-1} for replicate 1 (M9 rootstock). The regression equation is y = 0.7315x-0.3421.

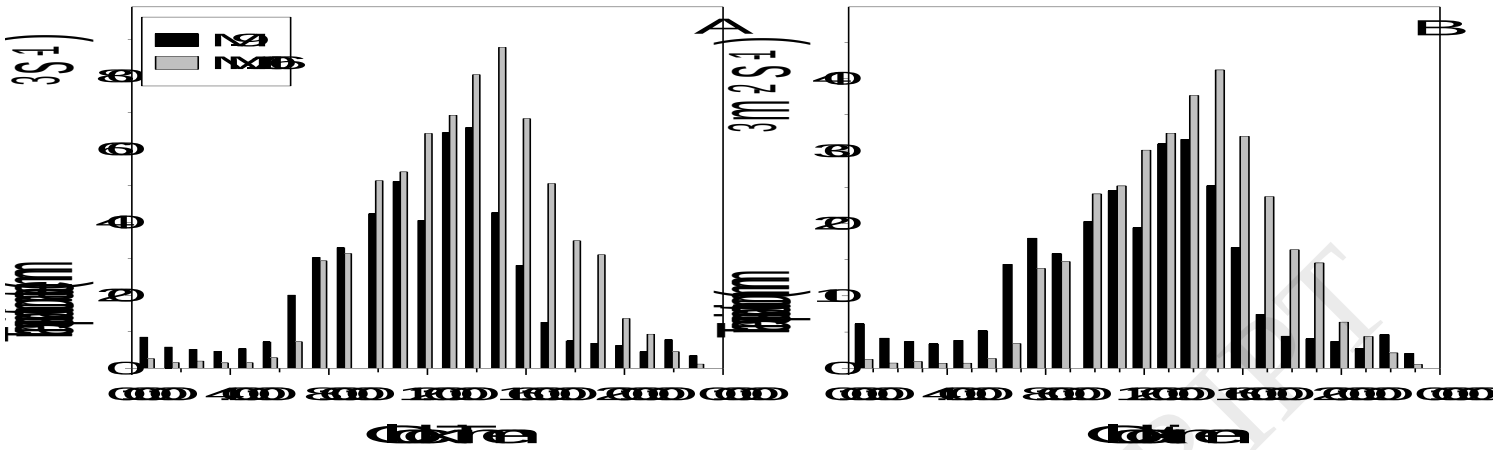
Fig 2. A & B Measurements of water lost from potted, intact transpiring trees in the 24 h preceding sap collection, expressed as (A) flow rates and (B) in terms of the leaf area of the canopies.

Fig 3. Osmolality of xylem sap samples collected at different flow rates from several positions within composite trees of A) M.9 (LSD = 1.45, $p < 0.05$, n = 6-8) and B) MM.106 (LSD = 0.92, $p < 0.05$, n = 8) rootstocks grafted with Queen Cox scions. Asterisk indicates a statistically significant difference across the graft union ($p < 0.05$). Sap was collected from above and below the graft union (AGU and BGU, respectively) at a range of sap flows that encompassed whole tree transpiration rates, and from osmotically exuding, detopped roots over a 24-h period. Results are means of eight replicates with associated standard errors.

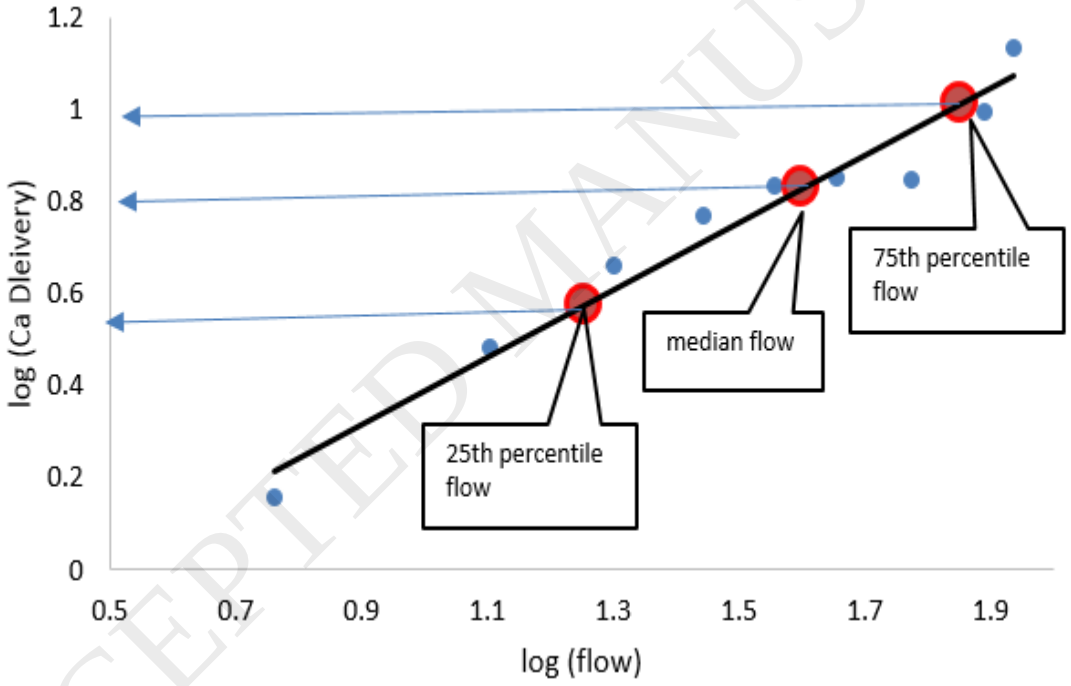
Fig 4. The change in xylem sap [ABA] at different flow rates. The relationship was modelled with an exponential decay function and the average exponential decay curves presented above and below the graft union of M.9 (dwarfing) and MM.106 (semi-invigorating) composite trees. Results are means of 5-6 replicates with associated standard errors.

Fig 5. ABA delivery rates in xylem sap samples collected at different flow rates and averaged across three flow rates (mm^3 s^{-1} - low [5-25], median [26-60] and high [>60] from above and below the union in composite trees of A) M.9 and B) MM.106 rootstocks grafted with 'Queen Cox' scions. Sap was collected from above and below the graft union (AGU and BGU, respectively) at a range of sap flows that encompassed whole-tree transpiration rates. Using a combined ANOVA derived from both rootstocks and all flows and positions,

Tukey comparisons based on rootstocks above and below the graft union, but within each flow rate, were used to test the significance of differences in ABA delivery. Results are means of 5-6 replicates with associated standard errors.

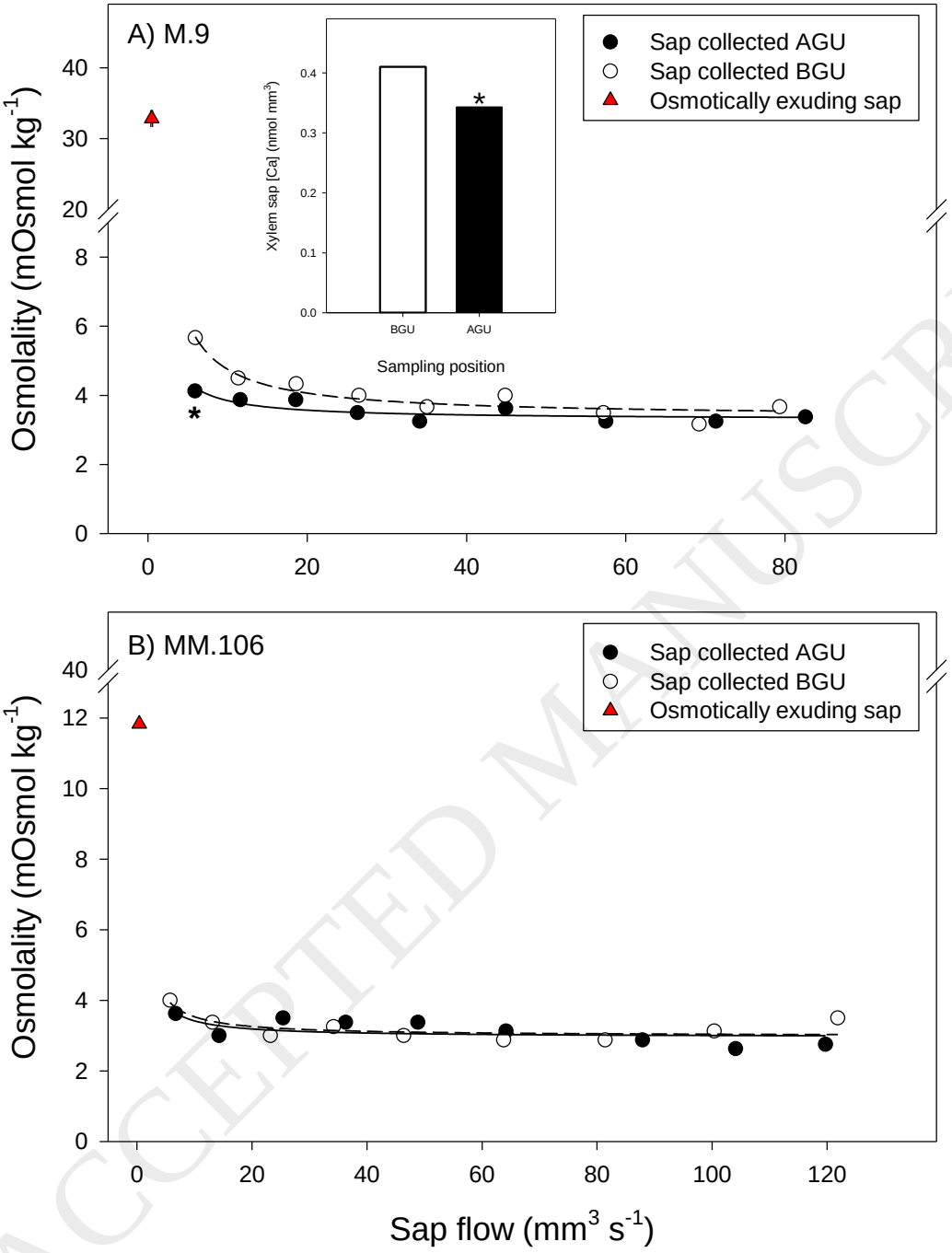


Else *et al*. Fig 1

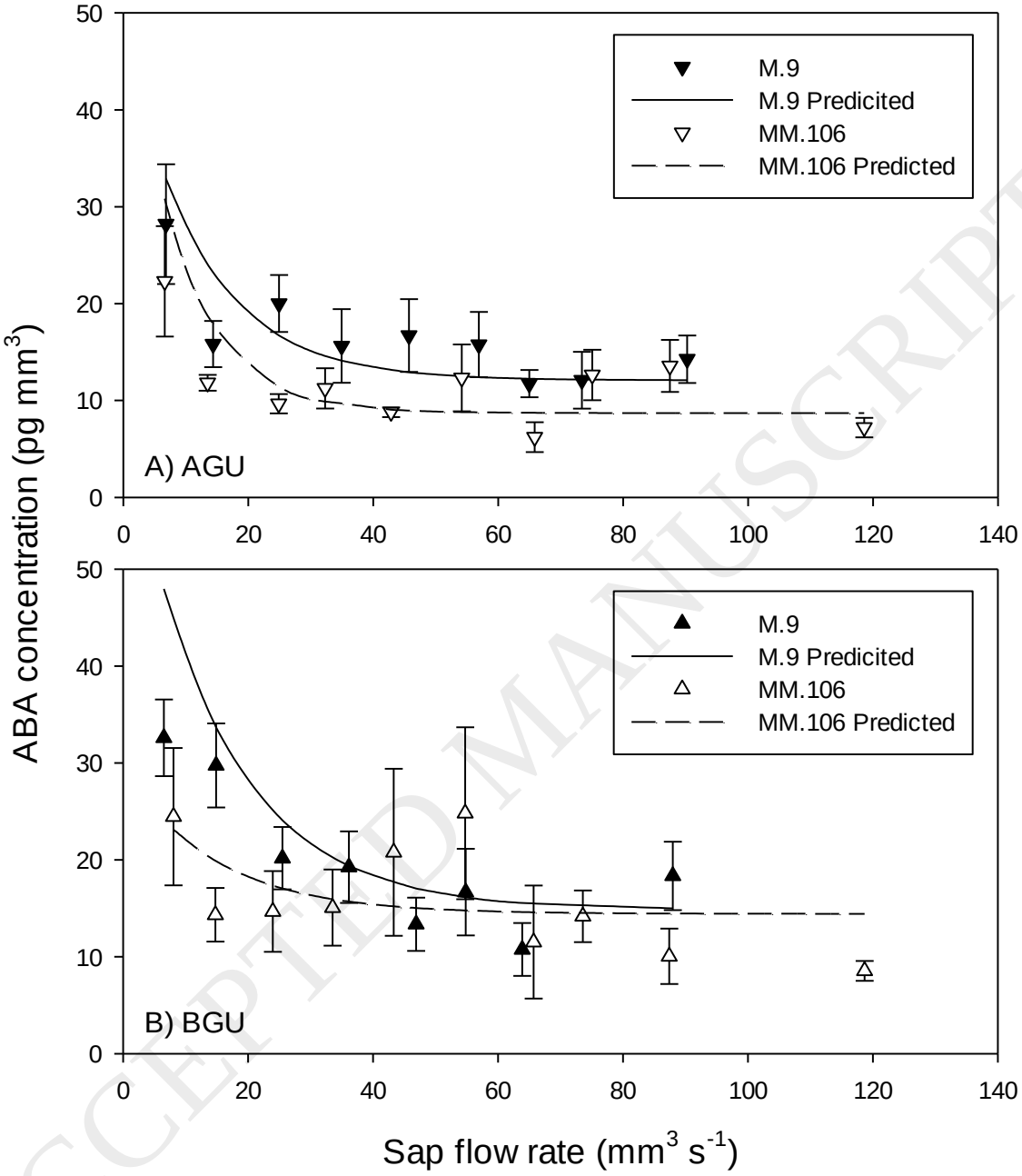


Else *et al*. Fig 2

Else *et al*. Fig 3



Else *et al*. Fig. 4



Else *et al*. Fig 5