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Full Paper

Determination of abscisic acid hormone (ABA), mineral content, and distribution pattern of ^{13}C photoassimilates in bark-ringed young peach trees

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Abstract: Abscisic acid (ABA), mineral content, and ^{13}C photoassimilates in young leaf, shoot and root of peach trees (*Prunus persica* Batsch cv. Hikawahakuho) as affected by phloemic stress (bark ring) were studied. The trees were treated as control (no phloem ringing), partial phloem ringing (PR) and complete ringing (CR). Phloem ringing was made by peeling out 2 cm length of bark (phloem) from the trunk leaving a connecting 2 mm thickness of phloem strip (a band) while complete ringing (CR) left no phloem strip. Free, bound and total ABA content in peach shoot and leaf were higher in CR and PR treated trees than in control trees. The N and Ca content in the root were higher in PR and CR than in control. ^{13}C photoassimilates were higher in leaf, shoot and upper trunk in PR and CR than in control. The results show that PR and CR increase ABA content in leaf, ^{13}C photoassimilate content in shoot and leaf, and mineral content in root. The increase in the hormone (ABA) and mineral content in the leaf and root seems to affect the overall plant nutrition that leads to small-sized peach trees.

Keywords: abscisic acid, mineral content, ^{13}C photoassimilates, dwarfing, partial ringing, complete ringing, peach trees

Introduction

Phloem ringing as represented by partial ringing is a horticultural practice used to manipulate tree growth, development, and fruit growth in a variety of fruit species. Small, compact, dwarfed or size-controlled fruit trees provide for easier pruning, thinning, spraying, harvesting, high production of high-grade fruit and lower cost of production [1]. The primary factor limiting the use of size-controlled rootstocks in stone fruit production is the lack of suitable rootstocks with a wide range of compatibility among cultivars [2]. Jose [3] found that girdling treatments cause lower vegetative growth in relation to control in mango trees.

Arakawa et al. [4] reported that trunk growth of apple trees is significantly increased above the girdling point and reduced below it. Onguso et al. [5] reported that the increase of trunk circumference above the girdle might be caused by swelling of the trunk due to the accumulation of carbohydrates. They also stated that girdling blocks the translocation of sucrose from leaf to root through the phloem bundles. The block decreases the starch content in the root and accumulates sucrose in the leaf. Rose and Smith [6] found that complete girdling of the stems kills the plants and partial girdling weakens the plants.

It has been reported that spraying one-year-old peach shoot with ABA increases their lignin content and dwarfs the plant [7]. A sudden increase in endogenous ABA has been demonstrated for several stress phenomena like salinity, relative humidity, osmotic root stress and wilting [8]. Furthermore, the fruits brought about an increase in leaf ABA levels in soybean within several hours [9]. Goldschmidt et al. [10] observed that accumulation of ABA indeed reflects the tissue response to senescence-inducing stimuli. Davies [11] stated that girdling results in diminished N, P and Ca level in the leaf on the girdled branch compared with ungirdled one. However, there is few available literature on ABA, mineral content and ^{13}C photoassimilates as affected by bark ringing. This study was undertaken to determine the ABA, mineral, and ^{13}C photoassimilate content in peach trees and to obtain a quantitative estimation of the changes which occur during senescence as affected by phloem restriction (girdling) on the trunk.

Materials and Methods

Experiment 1

Site

The experiment was carried out in an orchard in the Ehime University Farm located in southern Japan.

Plant material

Two-year-old peach trees (*Prunus persica* Batsch cv. 'Hikawahakuho') grafted on peach seedling stocks (wild form) were used in this experiment during April 2004. The seedling rootstocks were collected from the nursery (potted seedling) and transplanted in the main field on 13 April 2004. The rootstocks were planted by maintaining pit. The pits were spaced at 0.60 m x 1.0 m. The tree height was 1.0 m initially and the trunk circumference and diameter was about 6 cm and 2.3 cm respectively. Weeding was done by maintaining row as required. Compound fertilisers were applied after

transplanting at the rate of 10% (w/v) each of N, P_2O_5 and K_2O per tree. Irrigation was applied once a week by hose pipe. Insecticide was applied once a month.

Treatment setting

Treatments were set on 13 June 2004. Partial ringing (PR) was done by using a small sharp knife to remove a 2-cm length of partial ring leaving a 2-mm width (thickness) of a connecting strip (bridge) in the trunk 20 cm above the ground (Figure 1). In case of complete ringing (CR) there was no connecting bridge or strip. There were 3 treatments (control, PR, and CR), each performed in quadruplicate. For each replication each individual tree was used. There were thus a total of 48 (4 x 3 x 4) trees used for four dates ((June 20, June 27, July 11 and August 10) for ABA and mineral content analysis.

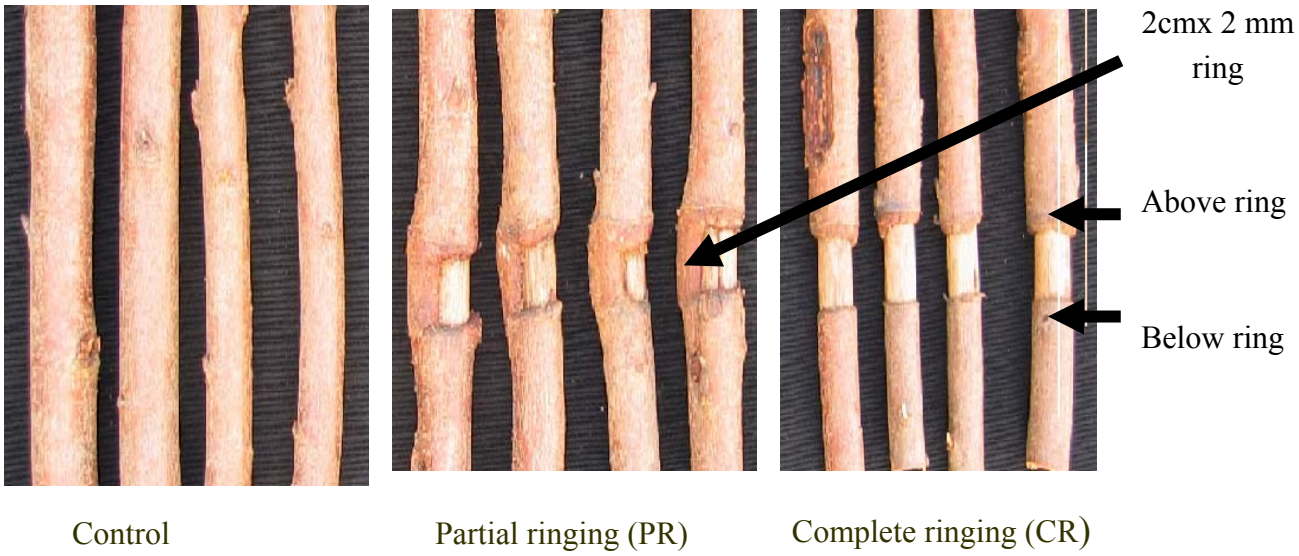

Figure 1. Photographs showing ringing structure and trunk circumference of peach trees as affected by partial and complete ringing (PR and CR)

Sample collection and preparation for ABA and mineral content analysis

Young leaf (at upper part of ringing), shoot and root samples were collected on the 1st, 2nd, 4th and 8th weeks after treatment (June 20, June 27, July 11 and August 10). Twelve trees were uprooted and washed on each date for ABA and mineral content analysis. Fresh leaves were separated, washed and kept in the freezer immediately after harvest and used for ABA analysis. Shoots and roots were used for mineral analysis.

Abscisic acid (ABA) analysis

The analysis was carried out according to the method of Most et al. [12]. A sample (1 g) was homogenised in 80% ethanol and filtered. They were then concentrated in vacuo to the aqueous phase using a rotary evaporator. The aqueous phase was mixed with insoluble polyvinyl pyrollidone (PVP) (500 mg/5ml H_2O) and filtered. The pH of the filtrate was adjusted to 8.5 using 5% NH_4OH followed by partitioning with CH_2Cl_2 (4 x 10 ml). The organic phase was discarded and the pH of the aqueous phase was adjusted to 3 using 1 M HCl. Partitioning was again repeated four times with CH_2Cl_2 as

explained above. The organic phase in turn was partitioned four times, each with 10 ml of a bicarbonate buffer (pH 10), and the alkaline aqueous phase was separated. The organic phase, containing free ABA was evaporated to dryness in vacuo.

For hydrolysis of bound ABA, the pH of the acidic aqueous phase was adjusted to 10.5 with 5% NH_4OH, then heated in a water bath at 60 ^oC for 45 min. The solution was left to cool at room temperature for 1 h and the pH was adjusted to 3 with 1 N HCl and partitioned four times with CH_2Cl_2. The organic phase was retained and evaporated to dryness in vacuo.

The methylation of ABA and the GC conditions were as follows. A prepared sample (1g) containing ABA was dissolved in 5 ml acetone/methanol (9/1) and the methylation with diazomethane was carried out using a method modified from Schlenk and Gellerman [13]. The test tubes were arranged with tube 1 containing acetone, tube 2: carbitol, KOH and NMSA (n-methyl-n-nitroso-p-toluenesulfonamide) (5 g/50 ml acetone), and tube 3: the sample dissolved in acetone/methanol. Nitrogen gas was passed through the sample for 3 min. The sample was evaporated overnight at room temperature with the help of a fan. The sample was taken up in 1ml acetone and 1 µl injected into a Gas Chromatograph (GC-8A, Shimadzu, Kyoto, Japan) equipped with an electron capture detector (^{63}Ni) and a glass column packed with Gaschrom Q (80-100 mesh) coated with 2% silicon OV-7. The injection/detector and column temperature were 240 ^oC and 230 ^oC respectively. The flow rate of the carrier gas (N_2) was 40 ml/min.

Mineral content analysis

One-gram of ground shoot or root samples was placed in a crucible and dry-ashed by heating in a muffle furnace for 5 h at 550^o C. One ml of 20% HCl was added to the residue which, after decantation of the acid solution, was rinsed with distilled water (2 x 1 ml). Finally 17 ml distilled water were added to the combined acid and rinses to make a 20 ml solution. This stock solution was used to measure K, Ca and Mg by an atomic absorption spectrophotometer (Shimadzu AA-6200, Kyoto, Japan). Phosphorus (as molybdate-reactive P) was estimated by a colorimetric method at 620 nm (Hitachi U-2001, Tokyo, Japan). For N analysis, 20 mg of leaf samples were taken and analysed by means of a CN coder (Sumitomo NC-80, Tokyo, Japan) [13].

Experiment II

Plant material

Potted (ϕ 30cm) two-year-old peach trees (*Prunus persica* Batsch. var. 'Hikawahakuho') grafted on wild peach rootstocks were used in this experiment in late July 2004. The treatments were the same as in Experiment1. There were a total of 9 trees used in the experiment.

^{13}C labelling experiment

The trees above the graft union were enclosed inside transparent polyethylene bags. $^{13}CO_2$ was generated by reacting 5.0 g of ^{13}C-rich BaCO3 (supplied by Nippon Chemical Industries Co. Ltd, Japan) with 50% lactic acid in a Petri dish inside the bag. A small fan was used to circulate the air in

the bag. The temperature in the bag during $^{13}CO_2$ treatment was maintained at 25-30^o C by intermittent misting over the bags.

Plant sample collection and $^{13}CO_2$ analysis

^{13}C labeling was carried out for 3 h from 9:00 to 12:00 a.m. on July 31, 2004. The trees were uprooted and washed, and shoots, leaves, trunks and roots were separately collected 24 h after $^{13}CO_2$ feeding. The samples were oven-dried (heated at 90^o C for 1 h to terminate enzyme activity and dried at 60^o C for 3 days), ground and used for analysis of $^{13}CO_2$. One milligram of each sample was used to determine the isotopic ratio between ^{12}C and ^{13}C in the sample by combustion using an infrared $^{13}CO_2$ analyser (JASCO EX-130S; Japan Spectroscopic Co. Ltd., Tokyo). The % excess of ^{13}C was calculated by subtracting the concentration of ^{13}C atoms in nature from the measured concentration in tissues fed with $^{13}CO_2$.

Design and statistical analysis

Treatments were set following a completely randomised design repeated in different trees. Mean separation was done by Duncan's multiple range test (DMRT) at 5 % level of significance.

Results and Discussion

Figure 1 shows the effect of partial and complete ringing on the trunk diameter after treatment. In control trees, no changes were observed above and below the ringing girdle. However, the trunk diameter increased above the ring and decreased below the ring in PR- and CR-treated trees compared to control (Table 1). Free ABA content was also higher in PR- and CR-treated trees than in control trees (Figure 2). In CR trees, free ABA content increased during the following 8 weeks, whereas in PR trees free ABA content increased more slowly up to the 4th week, then declined. For bound ABA content a similar trend was observed in both CR and PR trees. Total ABA content was also highest in CR followed by PR trees, both being more than in control trees (Figure 2). The percent nitrogen and calcium were lower in PR and CR trees than in the control for new shoot, old shoot and leaf, but it was higher in CR trees in the case of root (Figures 3-4). This may probably be due to the break in the connection between the upper and lower part of the ring. The percent phosphorous, potassium and magnesium content were found to be lower in the ringing treatments than in the control for shoot, leaf and root (Figures 5-7).

Table 1. Trunk circumference of peach trees as affected by PR and CR

Treatment	Trunk circumference (cm)			
	Initial	Above ring (A)	Below ring (B)	Ratio (A/B)
Control (no ringing)	6.2a	6.4b	6.5a	1.01b
PR (partial ringing)	6.1a	6.8a	6.6a	1.03b
CR(complete ringing)	6.1a	6.9a	6.3b	1.09a

Note: Means in column followed by the same letter are not statistically different at 5 % level of significance by Duncan's multiple range test (DMRT).

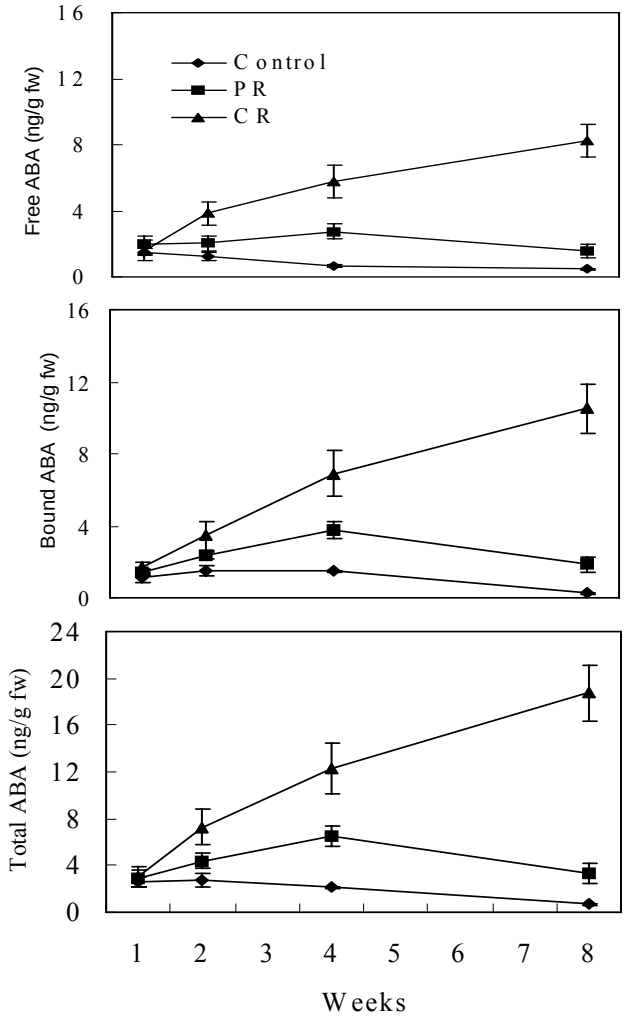

Figure 2. Free, bound and total ABA content in leaf of peach trees as affected by partial and complete ringing (PR and CR). Vertical bars indicate SE (n = 4).

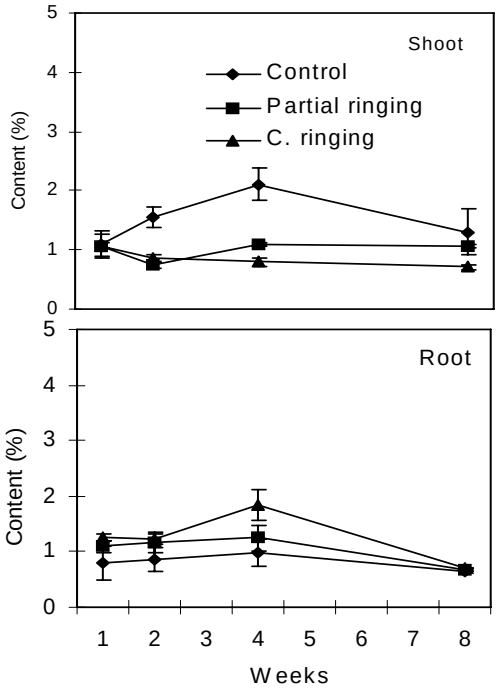

Figure 3. Nitrogen content in shoot and root of peach trees as affected by ringing. Vertical bars indicate SE (n = 4).

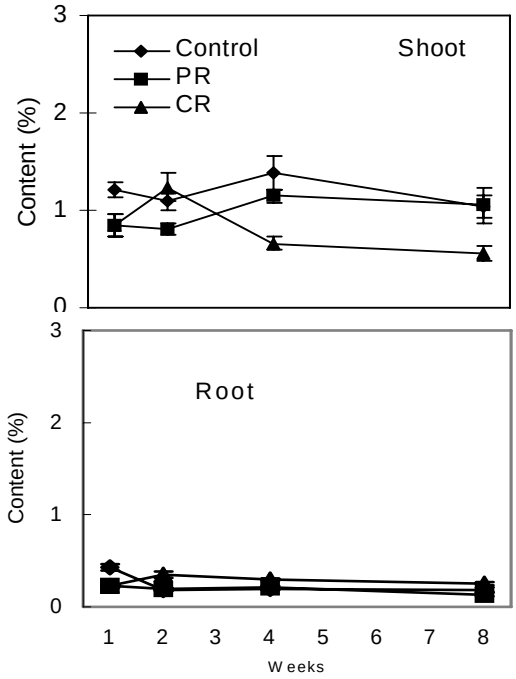

Figure 4. Calcium content in shoot and root of peach trees as affected by ringing. Vertical bars indicate SE (n = 4).

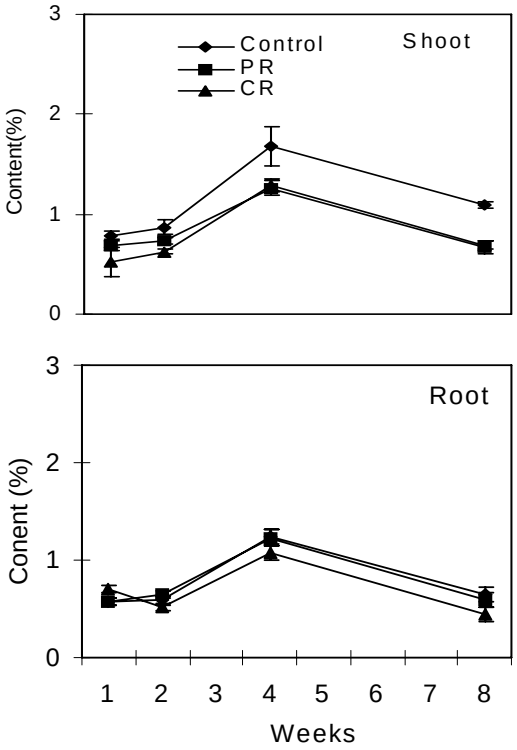

Figure 5. Phosphorus content in shoot and root of peach trees as affected by ringing. Vertical bars indicate SE (n = 4).

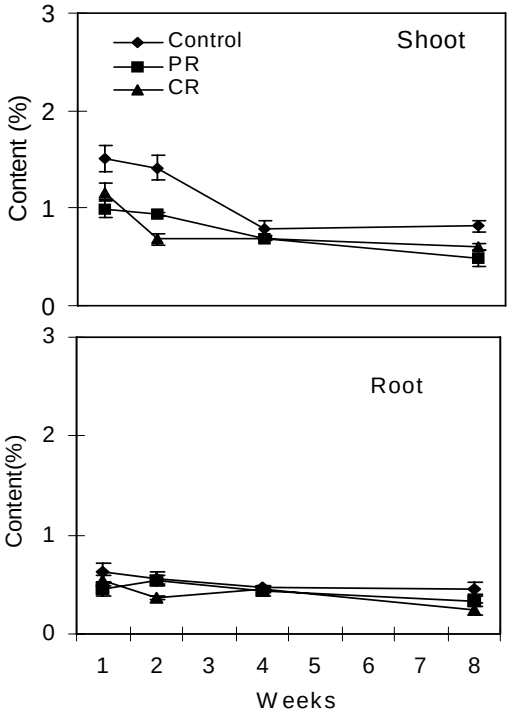

Figure 6. Potassium content in shoot and root of peach trees as affected by ringing. Vertical bars indicate SE (n = 4).

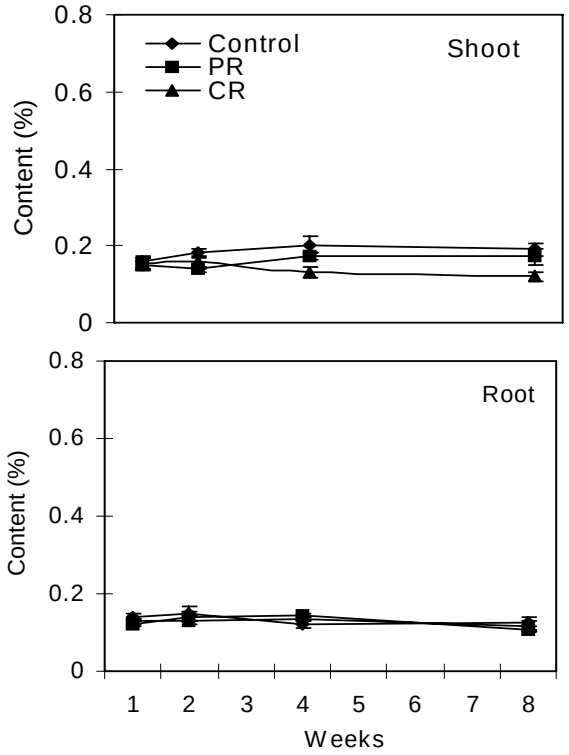

Figure 7. Magnesium content in shoot and root of peach trees as affected by ringing. Vertical bars indicate SE (n = 4).

The % excess of ^{13}C atoms was lower in the control than in CR and PR trees for shoot, upper part of trunk and leaf, but it was only slightly higher in CR and PR trees than in control trees for root (Figure 8). In the lower part of the trunk there was no difference between control and PR treatment. However it was lower in CR trees compared to control and PR trees. Apparently there was no % excess of ^{13}C atoms in CR trees for the lower trunk and root due to the disconnection between the upper and lower trunk (Figure 8). Figure 9 shows how the $^{13}CO_2$ feeding was carried out in potted two-year-old peach trees.

The results show that trunk growth (circumference) is higher above the ring than below it as a result of bark ringing. It is an effective dwarfing technique for young peach trees brought about by the stress phenomenon where bark (phloem) ringing produces more ABA as a result of blocking translocation of photosynthates from leaf to root. Arakawa et al. [4] reported similar results. They observed that trunk growth significantly increases above the girdling point and is reduced below it in apple trees. Onguso et al. [5] also reported a similar result. They stated that the trunk circumference is larger above the ring than below it. They also reported that the sugar and starch content are higher above the ringing than below. The ringing decreases the starch content in the root and accumulates sucrose in the leaf [14-15].

Mullins [16] reported that cytokinin stimulates root growth of young grapes. Antognozzi et al. [17] reported that the cytokinin activating compound, N_1-(2-chloro-4-pyridyl)-N_3-phenylurea (CPPU), increases the transverse diameter, the size and the fresh weight of olives. Park et al. [18] observed that stem growth in kinetin-treated persimmon trees is higher than in control trees. Cytokinin and other plant growth hormones stimulate cell division (cytokinesis) and influence the pathway of differentiation by stimulating RNA and protein synthesis [7]. They can accumulate in the leaf and cause the stomata to close, reducing transpiration and preventing further water loss. In this way they may affect the physiological processes in tree.

In the case of PR and CR trees, sucrose accumulates in the leaf and causes stomata closure resulting in an increase in ABA and a reduction in cytokinin in leaf, shoot and twig where it exhibits a growth inhibitory effect [8]. A sudden increase in endogenous ABA has also been demonstrated in several stress phenomena [8].

Hossain [19] has shown that the ABA content is higher in PR and CR trees than in control ones when the cytokinin level is lower. This could also be due to stress induced by PR or the cut-off in translocation of photosynthates from the leaf to the root brought about by CR.

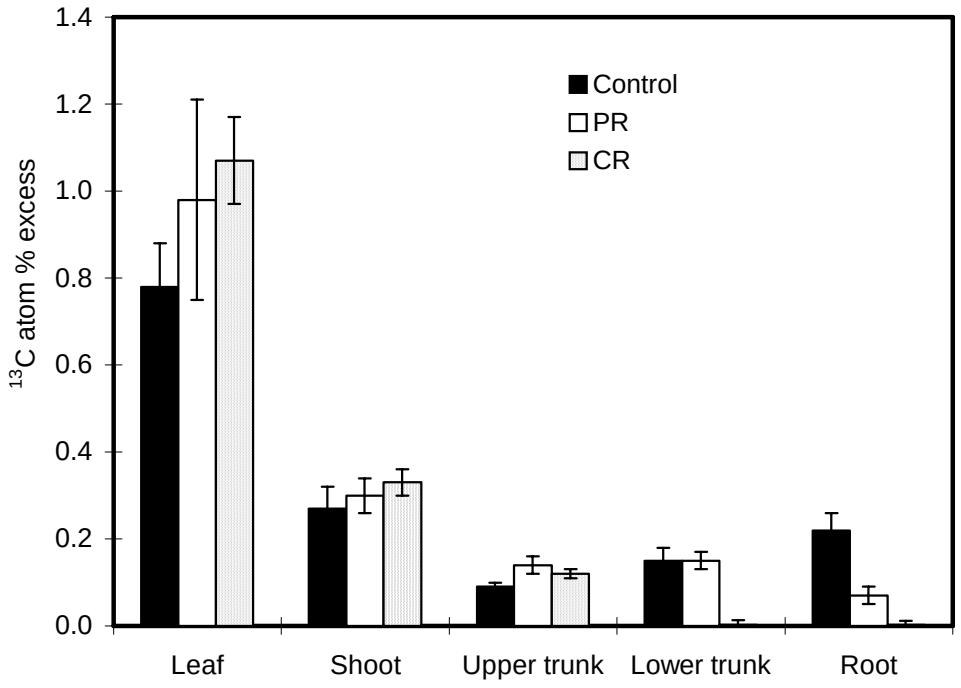

Figure 8. Transport of ^{13}C atoms in different parts of peach trees after $^{13}CO_2$ feeding. Vertical bars indicate SE (n = 3).

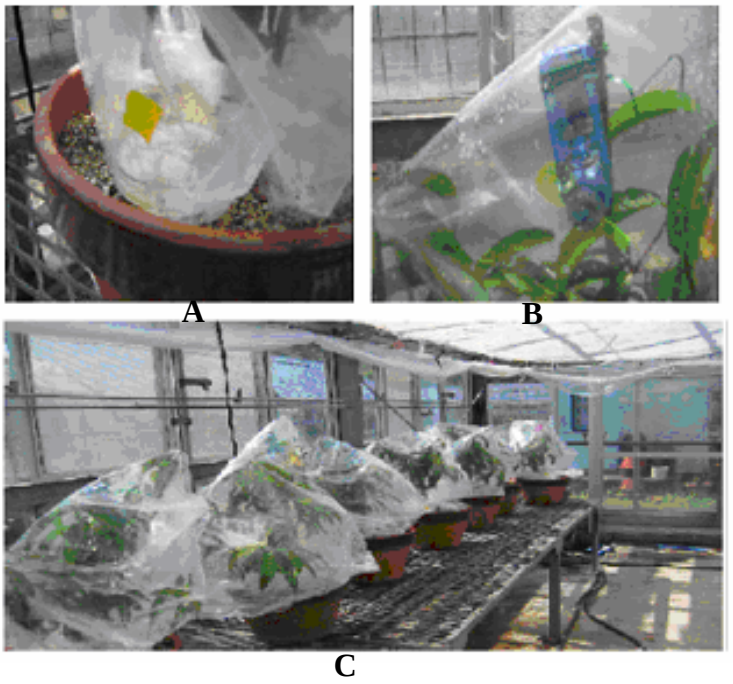

Figure 9. Photograph showing $^{13}CO_2$ feeding in potted two-year-old peach trees from 9-12 pm on July 31, 2003. A = Petri dish containing ^{13}C-rich $BaCO_3$ with 50% lactic acid, B = Small fan to circulate air in the bag, C= Potted peach trees covered with transplant polythene bags.

Conclusions

From our results it can be concluded that peach trees with PR produce more ABA hormones, nitrogen, calcium, and ^{13}C photoassimilates than the unringed (control) trees by causing nutrient and water stresses which can inhibit the trunk growth. As a result, dwarfing is exhibited by the whole tree. This probably can also be effective for other fruit species.

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