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DWARF PEACH TREES BY USING ABSCISIC ACID HORMONE, MALEIC HYDRAZIDE AND CYCOCEL AS GROWTH INHIBITORS IN PHLOEMIC STRESS CONDITION GRAFTED ON VIGOROUS ROOTSTOCK

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ABSTRACT

The study was undertaken to evaluate the dwarfing effects of phloemic stress (represented by bark ringing) and growth inhibitors [abscisic acid hormone (ABA), Maleic hydrazide (MH) and cycocel (CCC)] applied to a connecting bark strip of partially ringed trunk by using two-year-old peach trees. A 2 cm length of bark was removed from the trunk leaving a 2 mm width connecting bark band to which the aqueous chemical solutions (ABA, MH and CCC) were applied. Positive correlations were found between the bark regeneration and tree growth. Higher concentrations of ABA, MH and CCC retarded both bark regeneration and shoot growth. ABA and MH showed the greater effect than CCC. ABA 2000 showed the greater effects than ABA 1000 ppm which completely inhibited the bark regeneration even resulting in tree death like complete ringing. Root weight was also reduced in accordance with the decline in bark regeneration. Thus these results indicate that the tree growth can be controlled by chemically modifying bark regeneration of partially ringed trees in which a 2 mm connecting strip (97% ringing) was left. The use of chemicals will be greatly reduced compared with the whole tree spray for tree dwarfing.

Keywords: Dwarfing, ringing, growth inhibitor, shoot growth.

INTRODUCTION

Small, compact, dwarfed or size controlled fruit trees provide easier pruning, thinning, spraying and harvesting, high production of high-grade fruit and lower cost of production (Tukey, 1964). The primary factor limiting the use of size controlling rootstocks in stone fruit production is the lack of suitable rootstocks with a wide range of compatibility among cultivars (De Jong *et al.*, 2001). Dwarfing rootstocks or genetically dwarfed cultivars that produce high quality fruit are not yet available for peaches as they are for apple (Erez, 1984).

Dwarfing techniques other than utilizing dwarfing rootstocks are to be developed. The effect of bark ringing (cut once) was not long lasting because new bark phloem was regenerated after few years that permit normal phloem transport downward (Tukey, 1964; Hossain *et al*., 2005). Hossain *et al.* (2006) observed that partial ringing (cut once) without using growth inhibitors retained the tree dwarfing until four years in three-year-old peach trees. Johnson (1998) reported that complete ringing was almost fatal, even though plant might die within months to 2-3 years (Hossain *et al*., 2005 and 2006). Arakawa *et al.* (1997) reported that bark ringing reduced vegetative growth in apple. They also stated that flowering in apple was significantly increased by bark ringing.

Sole use of growth inhibitors as spraying is costly, laborous and much more polluted the environment than use to the bark band (bridge) of trunk by swabbing cotton. That is why partial ringing combined with growth inhibitors can be more effective than sole use of either partial ringing or growth inhibitors. ABA has been known as a natural growth inhibitor. It was reported that lignin content increased in one-year-old peach shoot sprayed with ABA and CCC resulting dwarf the trees (Khamis and Holubowicz, 1978). They also reported that foliar application of cycocel (CCC) at 1000 ppm led to growth inhibition. Ito *et al*. (2000) observed that foliar application of MH increased the number of laterally born flower buds on Japanese pear shoots. The present research was attempted to innovate a new technique to use growth inhibitors on bark band by avoiding the spray of whole tree. Therefore, we determined the influences of different concentrations of ABA, MH and CCC on tree growth and the relationship between bark thickness (regeneration) and shoot growth as well as root growth.

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MATERIALS AND METHODS

Experiment 1

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

Plant materials:
Two-year-old peach (*Prunus persica* Batsch cv. 'Hikawahakuho') trees grafted on wild peach seedling rootstocks were used in this experiment starting from April 2002 to December 2002. The trees were planted in mid April 2002. The trees were spaced at 0.60 m x 1.0 m.

Intercultural operations
Weeding and irrigation were done at 7 days intervals and insecticides were applied when necessary. Fertilizers were applied in mid May at the rate of N, P and K (10%, 10% and 10%) 10 g per tree respectively. Five shoots were maintained per tree to ensure proper growth.

Treatment setting
The treatments were control (unringed), partially ringed (PR) + water, PR + ABA 500 ppm, PR + 1000 ppm, PR + MH 1000 ppm, PR + MH 2500 ppm, PR + CCC 500 ppm and PR + CCC 1000 ppm. A partial ring was made by using a knife (thin razor blade type) on 6 June 2002. The partial ringing was consisted of removing a 2 cm length (vertically) bark (from trunk) leaving a 2 mm width (horizontal thickness) connecting bark band (strip) in the trunk, 10 cm above from the ground (Fig. 1). The aqueous solutions (ABA, MH and CCC) were applied to the bark strip (2 cm length and 2 mm width) swabbing 2-3 times by using cotton with the point of forceps (Fig. 1). They were applied at 15 days intervals and were continued until 3 months from 6 June 2002-15 August 2002).

Data collection
Shoot and regenerated bark growth were measured at 7 days intervals from 6 June 2002 – 22 August 2002. Regenerated bark growth was measured horizontally with vernier caliper. Total shoot length were measured in late November 2002 after tree growth was stopped completely. The percentage of flower bud and tree circumference were measured in late December 2002.

Experiment Design
The experimental design was completely randomized design. There were 5 replications and 8 treatments (including control) used in the experiment. A total of 40 trees used in the experiment. Mean seperation was done by Duncan`s multiple range test (DMRT). Standard errors were calculated for some data.

Experiment 2

Site: The experiment was carried out in the same location as mentioned in experiment 1.

Plant materials
Two-year-old peach (*Prunus persica* Batsch cv. 'Hikawahakuho') trees grafted on wild peach seedling rootstocks were used in this experiment starting from April 2003 to February 2004. The trees were planted in mid April 2003. The trees were spaced at 0.60 m x 1.0 m.

Intercultural operations
Weeding was done at 15 days intervals. Irrigation and insecticides were applied as needed. Fertilizers were applied in mid May at the rate of N, P and K (10%, 10% and 10%) 10 g per tree respectively. Heading back was done for all trees after one month of transplantation (in mid May). Five shoots were maintained per tree to ensure proper growth.

Treatment setting
The treatments were control (unringed), patially ringed (PR) + water, PR + ABA 1000ppm and PR + 2000ppm. A partial ringing was made by using a knife (thin razor blade type) in mid May 2003. The partial ringing was consisted of removing a 2 cm length (vertically) bark (from trunk) leaving a 2 mm width (horizontal thickness) connecting bark band (strip) in the trunk, 10 cm above from the ground. The aqueous solutions (ABA) were applied to the bark band (2 cm length and 2 mm width) swabbing 2-3 times by using cotton with the point of forceps. Growth inhibitors were applied at weekly intervals from the week of treatment setting and continued until 6 weeks (June 19 2003).

Data collection
Per Shoot growth and final regenerated bark growth were measured on 28 August 2003 after 3.5 months (15 weeks) of treatment setting. Regenerated bark growth was measured horizontally with vernier caliper. Total shoot length were measured in late December 2003 after tree growth was stopped completely. Percent flower bud and trunk circumference were measured in late December 2003. Flower bud was measured per 10 cm of shoot then it was converted into percent. Finally trees were uprooted and the root weights were determined in late February 2004.

Experiment Design
The experimental design was completely randomized design. There were 4 replications and a total of 16 trees used in the experiment. Mean seperations were done by Duncan`s multiple range test (DMRT). Standard errors were calculated in case of some data.

Measurement of leaf chlorophyll *in vivo:* The chlorophyll meter SPAD-502 (Minolta Co. Japan) was used for determination of chlorophyll in leaves. SPAD value was measured in late July 2003. Three leaves were selected from the middle part of shoot and a total of 15 leaves per tree were measured.

RESULTS AND DISCUSSION

Experiment 1

Figure 1 shows the ringing structure and position where aqueous solutions of the chemicals (growth inhibitors) were used. Shoot growth was lower in partial ringing (PR) + growth inhibitors treated trees than control (unringed) and PR + water treated trees (Fig. 2). A similar trend in shoot growth was observed in case of all treatments from 1-12 weeks. Similar trend of bark growth was found to shoot growth (Fig. 3). Total shoot length was lower in higher concentration of growth inhibitors (ABA 1000ppm, MH 2500ppm, CCC 1000ppm) treated trees than lower concentration (ABA 500ppm, MH 1000ppm, CCC 500ppm) (Table 1). The greater bark thickness (width) was observed in PR + water treated trees than PR + growth inhibitors treated trees (Table 1).

The higher inhibition of trees was found in lower concentration and the lower inhibition of trees was found in higher concentration of growth inhibitors. The percentage of flower bud was recorded higher in PR + growth inhibitors treated trees than control (unringed) and PR + water treated trees. Trunk circumference was higher above ring of PR + growth inhibitor treated trees than PR + water treated trees and was lower below ring in PR + growth inhibitor treated trees than PR + water treated. There was a little difference in case of trunk circumference among treatments (Table 1). Figure 4 shows relationship between shoot growth and regenerated bark thickness. A positive correlation was found between shoot growth and bark thickness. When bark thickness was higher then shoot growth was also higher. On the contrary, when bark thickness was lower then shoot growth was also lower. Plant architectures have been shown in Figure 5 as affected by PR and different concentrations of growth inhibitors. It was shown that shoot growth was influenced by bark thickness (width/regeneration) which affected by partial ringing and growth inhibitors.

Experiment 2

Shoot growth was lowest in partial ringing (PR) + ABA 2000ppm treated trees and was highest in un-ringed (control) trees (Table 2). Among all PR and ABA treatments, shoot length was lower in PR + ABA 2000 and ABA 1000 ppm treated trees than PR + water treated trees (Table 2). The highest final bark thickness was observed in PR + water treated trees and the lowest was in PR + ABA 1000ppm treated trees (Table 2). The bark

Table 1. Effect of growth inhibitors on shoot length, flower bud and trunk circumference of peach trees.

Treatments	Total shoot length (cm)	Flower bud (%)	Trunk circumference (cm)	
			Above ring	Below ring
Control (unringed)	525.4±8.8a	68.76±2.7a	6.0±0.24c	6.1±0.25a
Water	397.2±5.6b	65.52±5.9a	6.7±0.25a	6.3±0.25a
ABA 500ppm	290.0±5.3c	61.05±2.2a	6.1±0.26c	5.8±0.26b
ABA 1000ppm	245.2±8.0c	39.12±1.8b	6.2±0.25bc	5.7±0.25b
MH 1000ppm	261.1±6.2c	47.40±3.2b	6.1±0.28c	5.7±0.28b
MH 2500ppm	254.2±3.7c	44.84±1.4b	6.2±0.23bc	5.6±0.23b
CCC 500ppm	300.6±4.8c	53.44±5.1ab	6.3±0.27b	6.1±0.26a
CCC 1000ppm	275.5±5.4c	42.02±2.0b	6.4±0.26ab	5.9±0.26b

Means followed by the common letters in column are not significantly different at the 5%level by Duncan`s multiple range test (DMRT). Mean±SE (n = 5). In case of control trees, ring position were measured to take an idea about trunk circumference without ringing.

Table 2. Peach shoot growth, flower bud and root weight as affected by different treatments of PR and ABA.

Treatment	PSG (cm)	TSL/ tree (cm)	RBT		SPAD unit	Bud Flower (%)	TC		Root weight (g)
			Initial	Final			Above ring	below ring	
			(mm)				(cm)		
Control (Unringed)	47.4a	237.0a	---	---	41.8a	58.0b	5.4b	5.6a	111.7a
Water	34.2b	177.0b	2.0	10.9a	38.6a	63.0a	5.5b	5.2b	109.5a
ABA 1000ppm	25.5c	127.5c	2.0	5.3b	27.8b	64.8a	5.9a	5.5ab	77.4b
ABA 2000ppm	18.4d	92.0d	2.0	2.0c	20.0c	---	5.2b	4.8c	41.5c

Means followed by the common letters are not significantly differenence at the 5%level by Duncan`s multiple range test (DMRT). PR = Partial ringing, ABA = Abscsic acid, PSG = Per shoot growth, TSL = Total shoot length, RBT = Regenerated bark thickness, SPAD unit = From chlorophyll meter, TC = Trunk circumference.

Fig.1. Show the partial ringing structure (bark regeneration) after treatment.

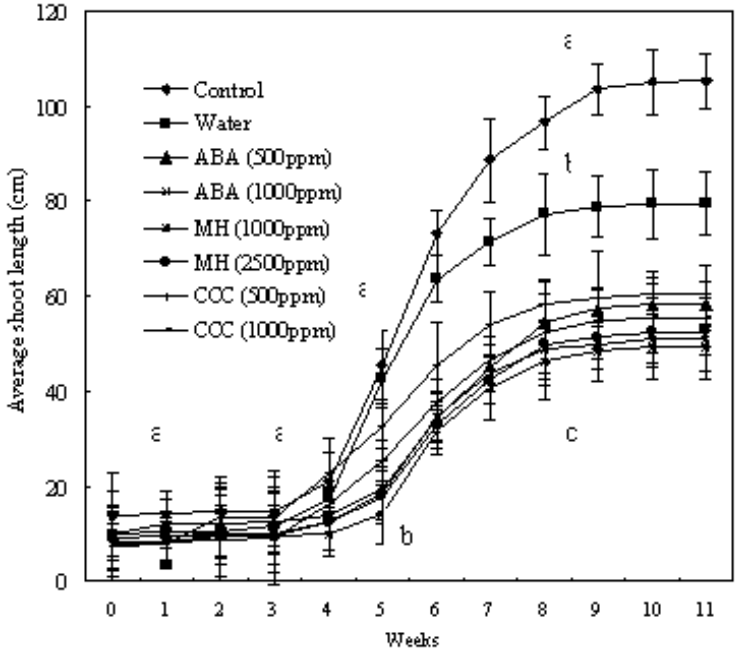


Fig. 2. Effect of partial ringing and growth inhibitors on new shoot growth in peach trees. Vertical bars indicate SE. Weeks are (0: June 6, 11:August 22). Means followed by the common letters in column are not significantly different at the 5%level by Duncan`s multiple range test (DMRT).

growth was higher in the lower concentrations (PR + ABA 1000ppm) compared with higher concentration (PR + ABA 2000 ppm). Trunk circumference was higher above ring and lower below ring in PR + ABA 1000 ppm than PR + water treated trees (Table 2). The minimum SPAD value was found in PR + ABA 2000 ppm treated trees and was a maximum in un-ringed trees (Table 2). The percent flower bud was greater in PR + growth inhibitors treated trees than PR + water and un-ringed trees (Table 2). Root weight was lower in PR + ABA 2000ppm than PR + ABA 1000 ppm and PR + water treated trees (Table 2). It seems that partial ringing and growth inhibitors, not only affected shoot growth but also root system. A positive correlation between bark width (thickness/regeneration) and shoot length was shown in Figure 6. The higher bark thickness the higher shoot length (Fig. 6) and root growth. The lower bark thickness the lower shoot length and root weight (Fig. 7). In Figure 8 photos show the shoot and root architecture as affected by PR and different concentrations of ABA. It was shown that shoot growth was influenced by bark thickness (width/regeneration) which affected by partial ringing and ABA.

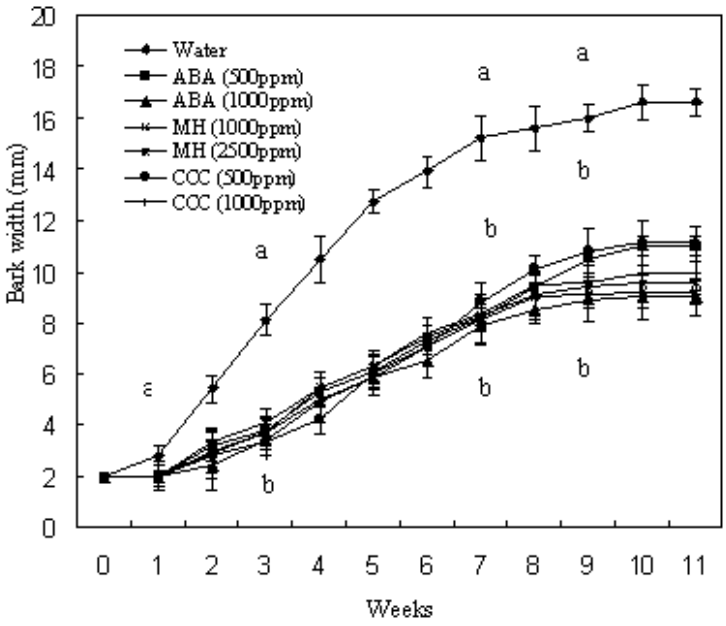


Fig. 3. Effect of growth inhibitors on bark width in peach trees. Vertical bats indicate SE (n=5). Weeks are (0: June 6; 11: August 22). Means followed by the common letters in column are not significantly different at the 5%level by Duncan`s multiple range test (DMRT).

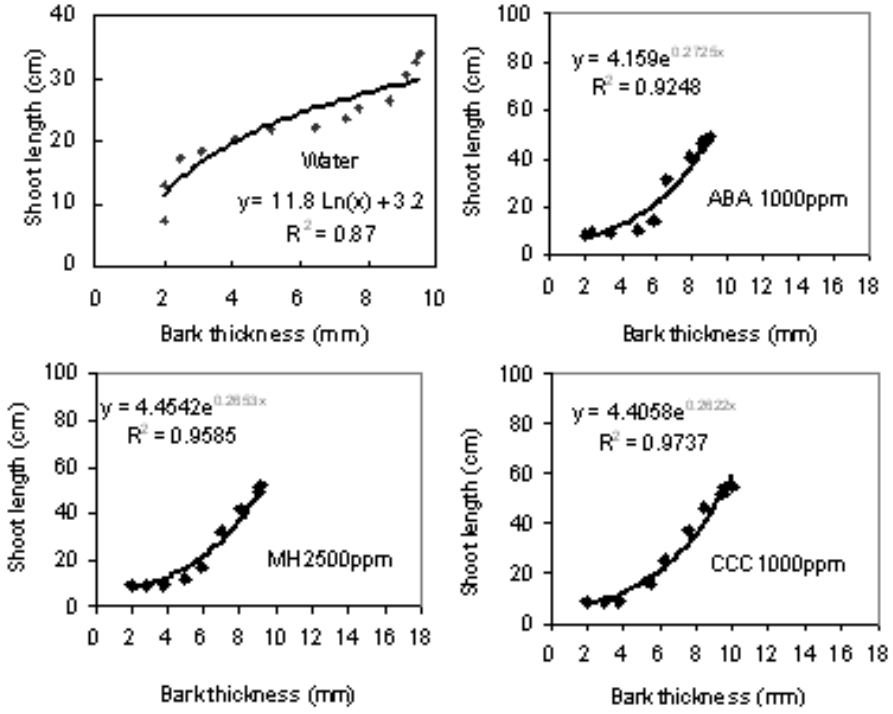
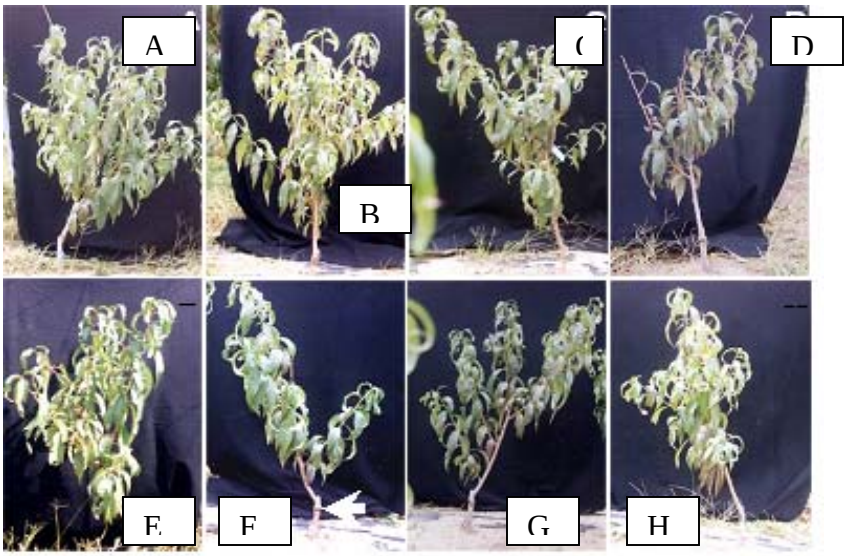


Fig. 4. Relationship between shoot growth and bark width (thickness) of peach trees in different concentrations of ABA, MH and CCC.

The results show that growth inhibitors are effective as dwarfing components in peach trees when used together with ringing. Shoot growth was greater in control (unringed) and partial ringing + water treated trees compared with other treatments where growth inhibitors were used. Khamis and Holubowicz (1978) stated that foliar application of cycocel (CCC) at 1000 ppm led to growth inhibition and acceleration of leaf fall. It was found that ABA 2000ppm was toxic effect. When it was used weekly, tree growth were stopped after a certain



Tree structure after treatment

Fig. 5. Photos show the partial ringing and plant structure after treatment. A partial ring (arrow) of bark, 2cm in length was removed leaving a 2mm connecting strip. A: Control; B: PR + water; C: PR + ABA (500 ppm); D: PR + ABA (1000 ppm); E: PR + MH (1000 ppm); F: PR + MH (2500 ppm); G: PR + CCC (500 ppm); H: PR + CCC (1000 ppm). **ABA**: abscissic acid; **MH**: maleic hydrazide; **CCC**: cycocel.

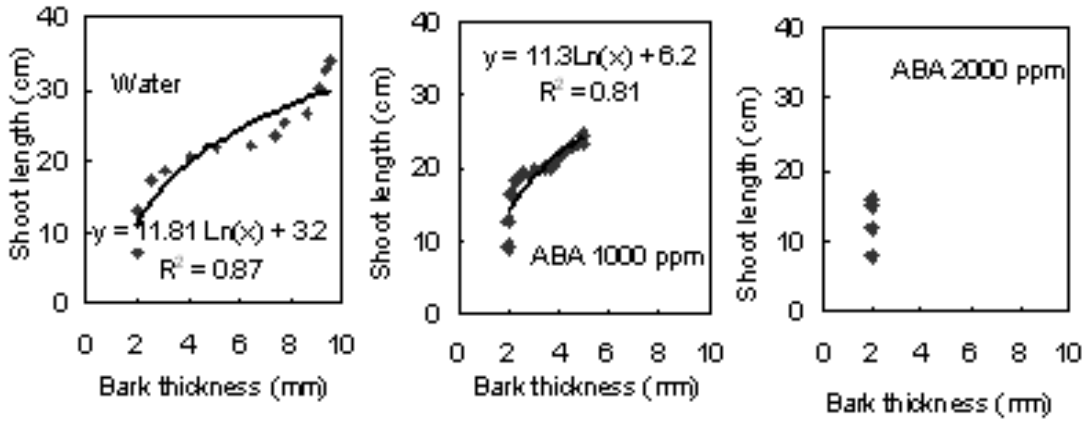


Fig. 6. Relationship between shoot length and bark thickness of peach trees in different concentration of growth inhibitor (ABA). In case of ABA 2000ppm there was no bark regeneration that is why after a certain period shoot growth was stopped and finally trees were died due to higher (toxic) effect.

period, withered and finally died. One possible explanation of the growth inhibition by growth inhibitor applied to bark is that growth inhibitors applied to the bark (cambial layer) might be translocated to the shoot through the phloem. Another explanation is indirect effect of the growth inhibitors through the growth inhibition of bark. It was observed that effectiveness of inhibitors on shoot and bark growth was higher in lower concentration and lower in higher concentration. It might be due to an increase of concentration of inhibitors. The inhibitors may inhibit shoot elongation by interfering with cell division as reported by Khamis and Holubowicz (1978). It was reported that when bark band (strip) of partial ringing in peach trees was cut (weekly and continuous) by razor blade which attached to mid portion of bark band, after few weeks peach trees were withered and finally died in case of continuous treated trees and for weekly treated trees after bark inhibition again bark started to regenerate (unpublished data).

In our result we have found CCC 1000 ppm was more effective than CCC 500ppm. Smith (1994) observed that higher (CCC 500 ppm) concentration was more effective than lower (250 ppm) concentration. He also explained that cytokinin and other plant growth hormones stimulate cell division (cytokinesis) and influence the path of

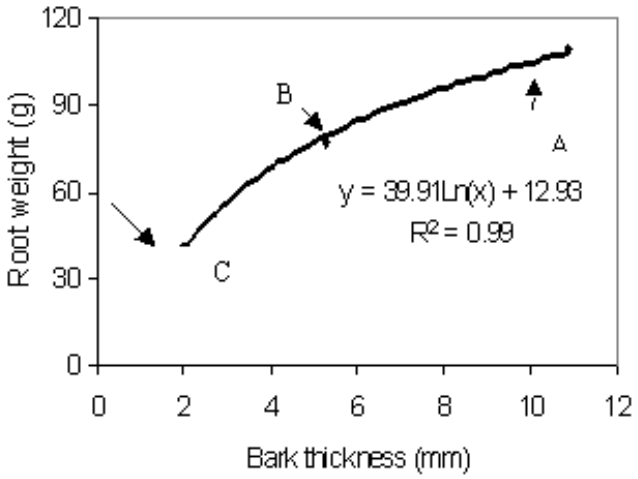


Fig. 7. Relationship between root weight and bark thickness of peach trees in different concentration of growth inhibitor (ABA). A= Partial ringing (PR), B = PR + ABA 1000 ppm, C = PR + ABA 2000 ppm.

Fig. 8. Photos show plant and root structure after treatment. A partial ring (arrow) of bark, 2 cm in length was removed leaving a 2mm connecting strip. A: Control, B: PR + water; C: PR + ABA 1000 ppm; D: PR + ABA 2000ppm, ABA: abscissic acid.

differentiation by stimulating RNA and protein synthesis. When growth inhibitors are used, they may interfere with cell division, elongation and differentiation. They may accumulate in leaves and cause the stomata to close, reducing transpiration and preventing further water loss. This way they may affect the plant physiological process (Smith, 1994). Kamuro (1995) reported that maleic hydrazide (MH) was a growth regulator that was used as an inhibitor of sucker development in tobacco. We have observed that percent flower bud was higher in PR + water and PR + growth inhibitors than control (unringed) trees. Ito et al. (2000) observed that foliar application of MH increased the number of laterally born flower buds on Japanese pear shoots. In our study Hossain *et al.* (2006) also found the similar result to our result. Trunk circumference was greater above ring and lower below ring in PR + growth inhibitors trees than PR + water trees. It might be due to more carbohydrate deposition above the ring by the suppression of bark band. Onguso *et al.* (2004) also found similar result to our results. Root growth was inhibited by ringing and ringing plus growth inhibitors. Ringing caused a significant decrease in gibberellin level in the root system (Wallerstein *et al.*, 1974). Onguso *et al.* (2004) stated that ringing blocks the translocation of sucrose from leaves to the root zone through phloem bundles. The block decreases starch content in root system and accumulation of sucrose in the leaves. It might be that the reduced level of gibberellins that prevent the hydrolysis of starch. A positive correlation was found between regenerated bark width and new shoot growth. From positive correlation we can understand the degree of effectiveness of PR + water and PR + growth inhibitors.

CONCLUSION

This study has shown that it is possible to make peach tree greatly dwarfed by using PR and PR + ABA, MH and CCC applied to the bark strips of partially ringed trees. PR + growth inhibitors are more effective than PR + water (sole use of PR). In our research technique, we used 97% ringing + growth inhibitors (ABA, MH and CCC) by swabbing method with cotton to the bark band (strip) surface only. We have found that they had dwarfing effect on vigorous peach trees grafted on vigorous rootstocks. However, the method might greatly reduce the use of amount (volume) of chemicals compared with the whole tree spray for tree dwarfing. Furthermore, this method is applicable for all fruit species even if they have no adequate dwarfing rootstocks.

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