

REJUVENATING OLDER APPLE TREES BY ROOT PRUNING COMBINED WITH ARBUSCULAR MYCORRHIZAL FUNGI

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ABSTRACT

Older apple trees often demonstrate physiologically unreasonable shoot distribution due to root system aging, which results in lower fruit yield and poor fruit quality. Therefore this study was conducted to test whether root pruning combined with arbuscular mycorrhizal fungi could restore growth potential of forty-year-old Red Fuji apple trees (*Malus × domestica* Borkh.) in a commercial orchard in 2013, by root pruning along both sides of rows, 80 cm from the trunk, to a depth of 30 cm and application of 100 ml arbuscular mycorrhizal inoculum per plant. Results showed that the percentage of root colonized by mycorrhizal fungi increased as root pruning was combined with arbuscular mycorrhizal fungi, however mycorrhizal colonization was not seen in the control roots and roots only by root pruning. For control tree total number of shoots decreased by 28.22% in 2015 than in 2013 and shoots mainly distributed in the outer canopy accounting for 58.10% of the total, which caused the lower light intensity inside the canopy, followed by lower fruit yield and poor fruit quality. Compared to control plant, shoot reduced by 33.96 and 38.51% in the outer canopy but increased by 97.99 and 123.69% in the inner canopy in 2015, as well as 390.20 and 478.43% in the vertical height of 1.5 to 2.5 m canopy, respectively treated by root pruning alone and combined with arbuscular mycorrhizal fungi. Root pruning alone and combined with arbuscular mycorrhizal fungi also raised the relative light intensity by 38.71 and 60.26% in the inner canopy in 2015, subsequent fruit yield by 315.79 and 373.68% respectively, in comparison to control plant. Shoot re-distribution improved fruit quality such as increase in firmness and soluble solid. Data indicated that the effect of root pruning combined with arbuscular mycorrhizal fungi on the rejuvenation of older apple trees was stronger than root pruning alone. It is therefore concluded that root pruning combined with arbuscular mycorrhizal fungi can think of as a measure to renew the older apple trees.

Key words: fruit tree, biological input, root regulation, canopy restructure

INTRODUCTION

Jiaodong Peninsula is one of the main apple producing areas in China, but now individual apple trees are more than 40 years old and start aging. These trees often contain a large number of dead or dying limbs inside the canopy and the tree size

gradually reduces, and thus fruits produced are generally small, diseased or hardness. Such a tree needs to be severe cuts by which it can be brought back into production. Generally, all the old or large limbs are removed with chainsaw and this severely

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pruned tree will produce large number of new and vigorous shoots.

Although severe crown pruning is an accepted method used for rejuvenation of the older or abandoned apple trees at present, supposed that even if the canopy structure is temporarily recovered, can the aged root synchronously regain inherent activity so as to meet demands of aerial parts in water or nutrients? The root/shoot-balance of a tree is characteristic for a certain cultivar/rootstock-combination at a specific location and certain phenological stage [Sachs 2005]. Physiologically, new shoot growth forced by crown pruning ought to promote new root formation to regain the balance, and previous researches have demonstrated that there was a strong positive correlation between the rate of shoot extension and rooting after crown cutting [Wilson 1999, Cameron et al. 2001]. Undoubtedly for an older apple tree root activity is generally low and its root can't supply sufficient water or nutrient to support new growth only by crown pruning. Therefore, man-made intervention must be fulfilled, only in this way can the shoot growth maintain vigorous.

Root pruning in fruit trees has a long history as a management practice for the reduction of excessive shoot growth, mainly in apple but also in other crops. In several long-term experiments the main objective of root pruning, the reduction of shoot growth, was only reached in some years but not in others, or was not achieved at all [Ferree 1992, Autio and Greene 1994, Miller 1995]. Also, little is known about how the varying effects are brought about, and what could be done to adjust root pruning-if possible to special situation for proper results [Saure 2007]. Normally, new roots produce at the cut end to substitute for the original old ones, ultimately providing absorption and anchorage plants. Based on this principle, taking root pruning to renew old root is the most important step for the rejuvenation of older apple trees.

Root pruning produces inevitable damage to root and the length of time required to replace the root system mainly depends on species, mode, timing, soil condition, health-state of the tree, etc. [Retamales et al. 2004]. Often, 3 to 5 years is required to reach 100 percent of the original spread and surface area. That is to say less original absorbing root area must

still support a full canopy and in this way physiological responses can not compensate for the imbalanced ratio of crown to root before root replacement. It is here to present an alternative scenario in which poor growth of pruned root is compensated for by nutrient or water uptake via arbuscular mycorrhizal fungi (AMF) pathways. The promotion in uptake nutrient of host by AMF has been deeply explored and elucidated in previous studies [Smith and Read 2008, Smith et al. 2011]. Otherwise, damage to roots by pruning can provide an access point for the entry of fungus, under this condition AMF readily establish symbiosis with host. No research was seen about rejuvenation of older apple tree by combination of root pruning and AMF, therefore this study aimed to test the effect of root pruning and AMF on renewing the older apple trees.

MATERIALS AND METHODS

Field site

The experimental site was located in Yantai Institute, China Agricultural University, Yantai City, Shandong Province, China (119°34'–121°57'E, 36°16'–38°23'N). Forty-year-old Red Fuji apple trees (*Malus × domestica* Borkh.) growing in a commercial orchard were planted in south-north rows at a spacing of 4.0 m between rows and 3.0 m within the row. The selected mycorrhizal inoculum of *Diversispora versiformis* (*Glomus versiforme*) consisted of 15 isolated spores per milliliter and was provided by Qingdao Agricultural University, Shandong Province, China. The soil used in this study was cinnamon soil classified by FAO/UNESCO soil classification system, its main physical and chemical properties included bulk density 1.32 g cm⁻³, organic matter 30.85 g kg⁻¹, available nitrogen 38.65 mg kg⁻¹, available phosphorus 12.19 mg kg⁻¹, available potassium 57.83 mg kg⁻¹, pH 7.1 (1 : 2.5, soil: water suspension).

Experimental design

The experiment was conducted based on a completely randomized block design with three replicates in the embryo stage of apple trees in 2013. Each

block consisted of 10 trees arrayed in the same row. Three combinations were applied: Treatment 1 (control): non root pruning without AMF, Treatment 2: root pruning alone, Treatment 3: root pruning combined with AMF.

Roots were pruned along both sides of the rows, 80 cm from the trunk, to a depth of 30 cm, with a shovel in the stage of tree germination. Soil outward root incision was gently removed with the width of 20 cm and subsequently 100 ml per tree AMF (approximately 1500 spores) was uniformly sprinkled around the root cut, at last soil removed was again refilled. Watering and fertilization were fulfilled as necessary.

Research methods

Canopy shape was described by Wei et al. [2004]. Each canopy was divided into different layers and positions using 0.5 m × 0.5 m × 0.5 m cubes located by bamboo poles. Horizontal positions of the canopy were classified as inside, central and outside (<1 m, 1–2 m, >2 m from the trunk, respectively). The vertical extent of the canopy was divided into six levels (<1 m, 1–1.5 m, 1.5–2.0 m, 2.0–2.5 m, 2.5–3.0 m, >3 m from the base of the tree trunk, respectively). The amounts of shoots were tested in every cube in the duration of spring shoot stopping growth to leaves falling, respectively. The relative light intensity of canopy was measured in different levels and positions using TSE-1332A digital illuminometer in the typical sunny days during mid-August. Measurements were taken for four times each day: 8 am, 11 am, 2 pm and 5 pm, respectively, and the values were calculated using average values for the 4 times. Percentage colonization of roots by AMF was analyzed respectively using a gridline intersect method after staining the roots with trypan blue in the full growth of apple roots in July and September [Koske and Gemma 1989]. The yield per tree and fruit quality were measured in mid-October. Fruit quality was tested for the firmness with a GY-1 type fruit firmness meter, soluble solid content using a PR-gital-glucose meter and the titratable acid content by titration using 0.1 mol · L⁻¹ NaOH [Jung and Choi 2010].

Statistical analysis

The data were analysed using SAS 9.1 software and the means were compared using Duncan's multiple range test at $P < 0.05$. Figures and some calculations were provided by using Microsoft Excel 2010.

RESULTS

Root colonization rate affected by root pruning and AMF inoculation

Due to non AMF inoculation with root, the root colonization rate in control and root pruning alone were zero throughout experiment, however roots were colonized by root pruning combined with AMF. Table 1 indicates that root colonization rate was respectively 15.4 and 17.3% in July and September, 2013, and gradually increased as time progressed. These results imply that the root length colonization rate was strong correlated with AMF application and time period after root pruning.

The distribution of shoots

The shoot distribution in different layer of apple canopy was described in Table 2. For control treatment total numbers of shoot were 2055, 1789 and 1475 per plant respectively in the year 2013, 2014 and 2015, and shoots distributed outside the canopy accounted for 54.45, 57.63 and 58.10% of the total, respectively. In the horizontal plane, the shoot numbers inside, central and outside the control canopy respectively decreased by 27.8, 37.6 and 27.9% in the year 2015 than those in the year 2013, showing control treatment preferred to inhibit shoot growth in area located in central canopy. In the vertical plane, the drastic decline in shoot number centered on the space of 1.5 to 2.5 m height of control canopy where decreased shoot accounted for 46.2% of the overall decrement in the year 2015. Compared to control treatment, root pruning alone and combined with AMF appeared diverse shoot distribution that shoot reduced by 33.96 and 38.51% in the outer canopy but increased by 97.99 and 123.69% in the inner canopy in 2015, as well as 390.20 and 478.43% in the vertical height of 1.5 to 2.5 m canopy, respectively. Data also suggested that root pruning combined with AMF had a larger effect on sprouting than root pruning alone.

The relative light intensity

Tree structure, including tree shape, shoot distribution and leaf quantity, clearly influences the relative light intensity in different position within canopy. Table 3 demonstrated that the relative light intensity gradually decreased from tree top to bottom and also from tree outside to inside, regardless of control, root pruning alone and combined with AMF. In the

horizontal direction, the relative light intensity of control treatment declined year by year wherever layer position was, however root pruning alone and combined with AMF increased the relative light intensity, especially in the inner canopy where the relative light intensity increased by 38.71 and 60.26% in 2015 as compared to control plant. In the vertical direction, the relative light intensity of control cano-

Table 1. Root colonization rate after root pruning and AMF inoculation

Tr	Colonization rate (%)					
	2013		2014		2015	
	July	September	July	September	July	September
1	0f	0f	0f	0f	0f	0f
2	0f	0f	0f	0f	0f	0f
3	15.4e	17.3e	22.8d	41.7c	50.5b	68.4a

Explanation: Tr – treatment: 1 – no root pruning and no AMF application, 2 – root pruning alone, 3 – root pruning and AMF application; each value represents the mean of 3 replicates; the different lower-case letters stand for significant differences at 0.05 level between years

Table 2. Shoot number in different layers of apple tree canopy

Height (m)	Tr	Shoot number (unit·plant ⁻¹)								
		inside			central			outside		
		2013	2014	2015	2013	2014	2015	2013	2014	2015
1	1	0n	0j	0i	48m	43fg	38l	217b	206b	186b
	2	0n	0j	0i	49lm	58ef	60j	147de	138d	133ef
	3	0n	0j	0i	52kl	60ef	63j	145de	135de	126g
1.0–1.5	1	75h	73f	64f	123f	109bcd	94g	180c	165c	144d
	2	79g	86e	90d	127e	138b	146d	135f	119g	111h
	3	85ef	92e	93d	131d	145b	150c	131fg	114h	105i
1.5–2.0	1	38l	22i	19h	129de	85de	52k	263a	245a	207a
	2	63i	101d	109c	187b	205a	214b	148de	130f	136e
	3	86e	115c	137b	194a	218a	236a	144e	120g	130f
2.0–2.5	1	54j	41h	32g	81j	54ef	36l	221b	209b	172c
	2	83f	132b	141b	119g	137bc	141e	128g	113h	104i
	3	124b	155a	158a	126ef	140b	147cd	122h	107i	96j
2.5–3.0	1	30m	28i	22h	156c	139b	122f	149d	132ef	99j
	2	49k	55g	60f	111h	99cd	89h	67j	52k	47k
	3	61i	71f	79e	102i	89de	83i	67j	49k	42l
>3.0	1	148a	121c	112c	54k	43fg	27m	89i	74j	49k
	2	121c	106d	93d	35n	13g	10n	59k	40l	35m
	3	106d	92e	90d	12o	5g	4o	55k	31m	28n

Explanation: Tr – treatment: 1 – no root pruning and no AMF application, 2 – root pruning alone, 3 – root pruning and AMF application; each value represents the mean of 3 replicates; the different lower-case letters in the same column stand for significant differences at 0.05 level within a year

py was continuously decreased from the year 2013 to the year 2015, the extent to which depended on the layer position located in the canopy, particularly in the height of 1.5 to 2.5 m canopy where the relative light intensity was no more than 50%. Contrary to control treatment, root pruning alone and combined with AMF gradually increased the relative light intensity and the significant increase both appeared in the canopy height of 1.5 to 2.5 m, where root pruning alone and combined with AMF induced the relative light intensity to rise from mean value of 48.52 and 51.8% in 2013 to 51.65 and 59.18% in 2015, respectively.

The distribution of fruit yield

During the experimental 3 years, fruit yields horizontally distributed inside, central and outside the canopy of control treatment decreased from 6.6, 13.3 and 16.9 kg per plant in 2013 to 3.8, 10.0 and 14.8 kg

per plant in 2015, however root pruning alone and combined with AMF increased fruit yield distributed inside, central and outside of the canopy from 11.8, 15.1, 15.8, 20.0 kg and 18.0, 21.9 kg per plant in 2013 to 15.8, 18.0, 23.3, 25.6 kg and 25.1, 27.4 kg per plant in 2015, respectively. Data also suggested that root pruning alone and combined with AMF increased fruit yield inside the canopy by 315.79 and 373.68% in 2015 in comparison to control plants. Figure 1 showed that, for root pruning alone and combined with AMF, fruit was mainly scattered in the vertical height of 1.0 to 2.5 m canopy, especially with regard to the height of 2.0 to 2.5 m in which the highest yield located. Contrary to root pruning alone and combined with AMF, fruit yield of control treatment decreased despite vertical tiers of canopy and drastic decline happened in the height of 2.0 to 2.5 m canopy where fruit yield decreased by 24.7% in 2015 than that in 2013.

Table 3. The relative light intensity in different layers of apple tree canopy

Height (m)	Tr	Relative light intensity (%)								
		inside			central			outside		
		2013	2014	2015	2013	2014	2015	2013	2014	2015
<1	1	20.6o	20.0o	18.9m	22.3m	21.4o	21.1k	25.1p	24.2o	23.9n
	2	21.5no	25.4n	26.1l	24.4l	27.7m	28.1j	28.6n	31.4m	32.3l
	3	22.3n	27.6m	28.1k	26.0k	31.1k	32.0hi	31.7m	35.3l	35.7k
1.0–1.5	1	22.2n	20.4o	19.6m	24.1l	22.9n	21.4k	27.3o	25.9n	25.2m
	2	23.6m	28.8l	29.6j	26.7k	29.3l	29.9ij	31.3m	34.5l	34.9k
	3	25.1l	32.2k	32.9i	29.9j	34.5j	35.1gh	34.5l	39.6k	40.6j
1.5–2.0	1	34.5k	29.6l	27.9k	44.4h	33.5j	32.0hi	42.8k	41.1j	40.5j
	2	40.3j	47.2h	48.2f	48.7g	48.6g	49.2f	51.9i	56.2g	56.9g
	3	43.2h	52.4g	53.1e	42.8i	54.9f	55.5e	55.3h	60.9f	61.5f
2.0–2.5	1	41.7i	34.3j	32.7i	51.9f	39.8i	38.9g	47.8j	45.9i	45.1i
	2	46.1g	53.7f	34.6h	54.8e	56.5e	60.0d	56.5g	60.5f	61.0f
	3	48.6f	57.1e	57.9d	47.8g	61.3d	61.8d	60.3f	64.6e	65.3e
2.5–3.0	1	45.6g	42.1i	40.5g	57.4d	46.6h	46.1f	54.3h	52.6h	51.6h
	2	52.6e	58.5d	58.9d	60.6c	61.6d	62.0d	63.2e	67.1d	67.8d
	3	54.7d	62.3c	63.1c	54.3e	68.2c	69.0c	67.3d	70.8c	71.7c
>3.0	1	58.5c	54.7f	53.9e	71.6b	62.3d	61.8d	70.9c	68.3d	66.8d
	2	65.8b	70.2b	71.0b	74.9a	75.0b	75.7b	78.8b	81.2b	83.0b
	3	68.4a	74.7a	75.0a	70.9b	79.7a	80.3a	82.4a	85.2a	86.0a

Explanation: Tr – treatment: 1 – no root pruning and no AMF application, 2 – root pruning alone, 3 – root pruning and AMF application; each value represents the mean of 3 replicates; the different lower-case letters in the same column stand for significant differences at 0.05 level within a year

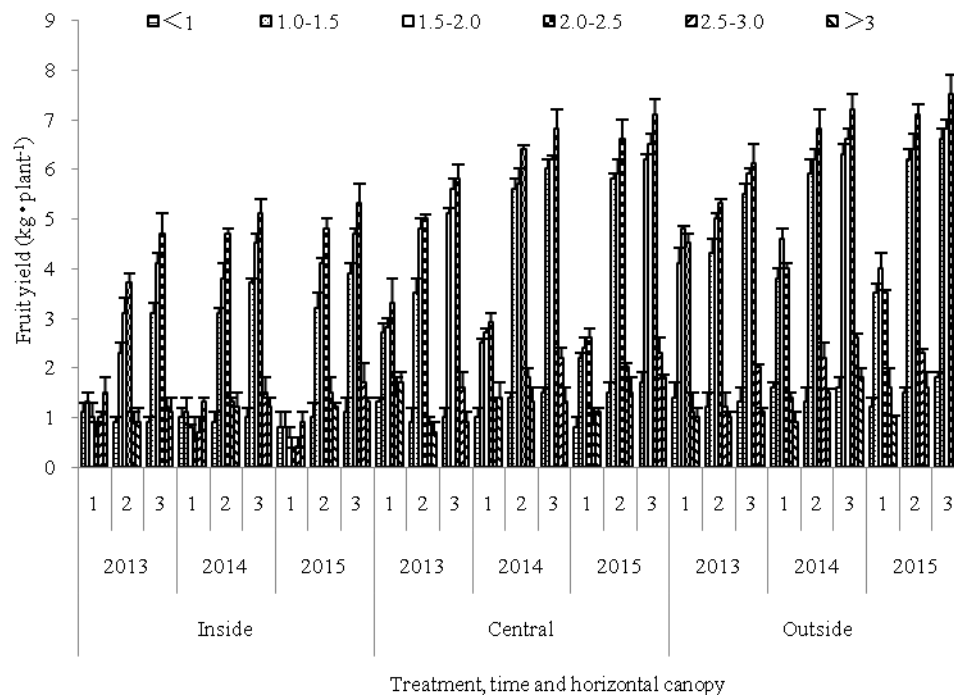


Fig. 1. The distribution of fruit yield in different layers of apple tree canopy; treatment: 1 – no root pruning and no AMF, 2 – root pruning alone, 3 – root pruning combined with AMF; each bar represents a mean \pm standard deviations; each value represents the mean of 3 replicates

The fruit quality

Differences of fruit quality within the canopy were shown in Table 4. The result indicated that firmness and soluble solid of control fruit decreased but titratable acidity increased year by year and the poorest fruit appeared in the height of 2 to 2.5 m canopy. Compared to control fruit, firmness and soluble solid of fruit treated by root pruning and root pruning combined with AMF obviously improved but titratable acid in fruit lightly decreased as time progressed. The best fruit also occurred in the canopy of 2 to 2.5 m height in which firmness, soluble solid and titratable acidity affected by root pruning and root pruning combined with AMF were 1.23, 1.25, 1.15, 1.16 and 0.80, 0.77 times control counterparts, respectively. Data statistically analysis illustrated that differences between root pruning alone and combined with AMF were sharp in fruit firmness and soluble

solid in 2013, not found in 2015 and no variation in titratable acidity was seen in the whole experiment.

DISCUSSION

Root percent colonization is an important indicator suggesting plants become mycorrhizal [Yang et al. 2014a]. Results driven from this study implied that pruned root was nicely compatible with AMF and only in this way the function of arbuscular mycorrhizal symbiosis for older apple tree growths was well realized. Root pruning is recommended for root-balled trees to produce trees capable to rapid root regeneration and also advocated to inhibit shoot growth for restoring the root to shoot balance [Catherine et al. 1997, Saure 2007]. Actually, the acceptance of root pruning in reduction shoot growth in commercial fruit production is now still rather limited

Table 4. The fruit quality parameters in different layers of apple tree canopy

Height (m)	Tr	Firmness (kg·cm ⁻²)			Soluble solid (%)			Titratable acidity (%)		
		2013	2014	2015	2013	2014	2015	2013	2014	2015
<1	1	6.99f	6.83h	6.65d	10.35ef	10.33e	10.29g	0.30a	0.32a	0.34a
	2	7.39e	8.02c	8.08c	10.52de	10.94d	10.99f	0.30a	0.27b	0.26b
	3	7.46de	8.09g	8.12c	10.61de	10.99d	11.03f	0.29a	0.26b	0.25b
1.0–1.5	1	6.86g	6.79hi	6.60d	10.25fg	10.23e	10.19h	0.31a	0.32a	0.35a
	2	7.47de	8.07fg	8.11c	10.39ef	11.02d	11.06f	0.30a	0.28b	0.27b
	3	7.65c	8.11ef	8.15bc	10.58de	11.04d	11.34e	0.28a	0.27b	0.26b
1.5–2.0	1	6.82g	6.75ij	6.61d	10.22g	10.19e	10.08h	0.31a	0.34a	0.36a
	2	7.50de	8.19cd	8.26b	10.27ef	11.44c	11.47d	0.29a	0.27b	0.26b
	3	7.67c	8.23c	8.29ab	10.85d	11.60c	11.63d	0.28a	0.27b	0.26b
2.0–2.5	1	6.80g	6.71j	6.60d	10.20g	10.16e	10.06h	0.31a	0.35a	0.38a
	2	8.01b	8.36b	8.39a	11.40c	11.82bc	11.89c	0.28a	0.27b	0.28b
	3	8.20a	8.45a	8.52a	11.55c	12.00b	12.04b	0.27a	0.26b	0.27b
2.5–3.0	1	6.81g	6.73j	6.62d	10.31ef	10.23e	10.11h	0.33a	0.34a	0.36a
	2	7.91b	8.12ef	8.18b	11.33c	11.84b	11.86c	0.30a	0.29b	0.28b
	3	8.00b	8.23c	8.26b	12.27b	12.89a	12.98a	0.30a	0.29b	0.28b
>3.0	1	6.84g	6.79hi	6.64d	10.44ef	10.41e	10.37g	0.31a	0.32a	0.35a
	2	7.57cd	8.07fg	8.10c	12.57ab	12.96a	12.98a	0.30a	0.30ab	0.29b
	3	7.65c	8.15de	8.15bc	12.73a	13.02a	13.07a	0.30a	0.29b	0.27b

Explanation: Tr – treatment: 1 – no root pruning and no AMF application, 2 – root pruning alone, 3 – root pruning and AMF application; each value represents the mean of 3 replicates; the different lower-case letters in the same column stand for significant differences at 0.05 level within a year

regardless of previous reports that recorded the promising results. Therefore, this research is based on the situation that root pruning is only considered as an emergency measure for older apple trees associated with less shoot growth in the inner canopy, lower activity of root and succedent yield loss.

In this study, root pruning alone and combined with AMF inhibited shoot growth in the outer canopy but improved it in the inner canopy, enabling older apple tree to re-establish an optimal canopy structure. This certified that newly formed root system had a physiologically favorable growth potential, which was supported by the fact that root pruning removes part of the root tips, and in this way their apical dominance within the root, allowing for the formation of more side-roots and new root tips [Lloret and Casero 2002, Aloni et al. 2006]. Subsequently, the production of cytokinins could increase again that forced the new root system formation. The new root could pro-

vide more nutrients to satisfy the growth demand of aerial parts of the older apple tree. The re-distribution of shoot caused by root pruning alone and combined with AMF, in particular inside the canopy, depended not only on root regeneration but also on severe shoot pruning which removed apical dominances and released other buds under cut points from correlative inhibition. Also, root pruning alone and combined with AMF increased shoot number indicating vigor restoration of older apple tree. While control treatment had a decrease in shoot numbers across horizontal and vertical plane, because root system substantially degraded unable to absorb sufficient nutrition for over ground parts, consistent with findings presented by Saure [2007] that older trees had altered root/shoot balance at expense of the roots and in turn inhibiting the shoot sprouting.

At the tree scale, light interception is affected by the intrinsic architectural pattern of a cultivar and

horticultural practices, such as training and pruning [Han et al. 2012, Da Silva et al. 2013]. Although severe shoot pruning was performed, weaken vigor of control plant gave preference to apical dominances outside shoot tips and resulted in more shoots appeared in the outer canopy that thus prevented light penetration inwards. Light intensity had strong relationship with fruit yield, the insufficient light into control canopy decreased flower bud formation followed by fruit yield, which has been also reported in other apple orchards [Jung and Choi 2010]. Root pruning and shoot pruning could more inhibit shoot growth avoiding renewal heading in older apple trees [Saure 2007]. Shoot re-allocation treated by root pruning alone and combined with AMF might prevent shading among the limbs to maximize the light intensity within the canopy. Adequate light distribution is also required at the fruiting set to ensure that dry matter is partitioned into fruits [Grappadelli et al. 1994]. This may be the cause of the increase in fruit yield treated by root pruning alone and combined with AMF in comparison to control treatment.

Fruit soluble solids and titratable acids were affected proportionally by the light penetration, as has been observed also in ‘Delicious’ apples. Root pruning alone and combined with AMF had greater soluble solids but lower titratable acids in fruits compared to control treatment. This indicated a reasonable light distribution in the canopy can ensure a sufficient supply of the metabolites required to produce high quality fruit [Feng et al. 2014]. Fruit firmness was positively related with the light intensity in this study, which also has been reported in the ‘Fuji’ apple trees [Jung and Choi 2010]. Root pruning alone and combined with AMF made fruits to expose to the sunlight to maintain their firmness because fruits stimulated protein synthesis related to the heat shock on the trees and delayed at maturation and softening [Ferguson et al. 1994]. However, fruit in control treatment had lower fruit quality due to worse light penetration coupled with weaken root absorption.

The majority of terrestrial plants species are capable of interacting with AMF in nature and the major advantage of AM is that they provide a very effective pathway by which nutrients are scavenged from large volumes of soil and rapidly deliver to cortical cells

within the roots, especially in phosphorous acquiring [Smith and Read 2008, Smith et al. 2011]. After root pruning there is a disadvantageous gap in root absorbing nutrients before restoring rooting potential, coincidentally AMF can offset the loss of roots based on their greater hypha network. Therefore, shoot number of root pruning combined with AMF was higher than that of root pruning alone, indicating that AMF played a major role in the enhancement of the tree vigor. The result had already verified by Yang and coauthors [2014b] who also had applied AMF to confirm if AMF could induce bioprotection for older apple tree in which conclusion was drawn that *Glomus versiforme* colonization of older apple plants markedly increased mineral nutrient concentrations in apple leaves, especially nitrogen and phosphorus nutrition. Only restoration of tree vigor can older apple trees freely regulate shoot number and light interception to form a favorable condition for their own growth.

CONCLUSIONS

Root pruning could better restore tree vigor of older apple trees obtained from this study to achieve rational canopy structure no matter AMF were reinforced. AMF was the first to compensate the loss of fruit yield and quality. So far, root pruning combined with AMF could regard as a measure for older apple tree to restore juvenile characteristics. However, the risks of negative side-effects are ignored and therefore whether root pruning combined with AMF has a bright future is debatable, and perhaps need additional experience.

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REFERENCES

- Aloni, R.E., Aloni, M.L., Ullrich, C.I. (2006). Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation,

- root apical dominance and root gravitropism. *Ann. Bot.*, 97, 883–893.
- Autio, W.R., Greene, D.W. (1994). Effects of growth retarding treatments on apple tree growth, fruit maturation and fruit abscission. *J. Hort. Sci.*, 69, 653–664.
- Cameron, R.W.F., Harrison-Murry, R.S., Ford, Y-Y., Judd, H.L. (2001). Ornamental shrubs: effects of stock plant management on the rooting and establishment of cuttings. *J. Hort. Sci. Biotechnol.*, 76, 489–496.
- Catherine, F.L., Peter, A., Catharine, W.T., Nigel, R. (1997). Root and shoot pruning in root-balled *Acer platanoides* L.: effect on establishment and shoot architecture. *Arboric. J.*, 21, 215–229.
- Da Silva, D., Han, L., Costes, E. (2013). Light interception efficiency of apple trees: a multiscale computation study based on MAppleT. *Ecol. Model.*, 290, 45–53.
- Feng, F., Li, M., Ma, F., Cheng, L. (2014). Effects of location within the tree canopy on carbohydrates, organic acids, amino acids, and phenolic compounds in the fruit peel and flesh from three apple (*Malus domestica*) cultivars. *Hortic. Res.*, doi: 10.1038/hortres.
- Ferguson, I.B., Lurie, S., Brown, J. (1994). Protein synthesis and breakdown during heat shock of cultured pear (*Pyrus communis* L.) cells. *Plant Physiol.*, 104, 1429–1437.
- Ferree, D.C. (1992). Time of root pruning influence vegetative growth, fruit size, biennial bearing, and yield of ‘Jonathan’ apple. *J. Am. Soc. Hort. Sci.*, 117, 198–202.
- Grappadelli, L.C., Lakso, A., Flore, J. (1994). Early season patterns of carbohydrate partitioning in exposed and shaded apple branches. *J. Am. Soc. Hort. Sci.*, 119, 596–603.
- Han, L., Costes, E., Boudon, F., Cokelaer, T., Pradal, C., Silva, D.D., Faivre, R. (2012). Investigating the influence of geometrical traits on light interception efficiency of apple trees: A modeling study with MAppleT. *IEEE 4th International Symposium on Plant Growth Modeling (etc.)*, 152–159.
- Jung, S.K., Choi, H.S. (2010). Light penetration, growth, and fruit productivity in ‘Fuji’ apple trees trained to four growing systems. *Sci. Hortic.* 125(4), 672–678.
- Koske, R.E., Gemma, J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizae. *Mycol. Res.*, 92, 486–505.
- Lloret, P.G., Casero, P.J. (2002). Lateral root initiation. In: Waisel, Y., Eshel, A., Kafkafi, U. (eds), *Plant roots – the hidden half*. Marcel Dekker Inc, New York/Basel.
- Miller, S.S. (1995). Root pruning and trunk scoring have limited effect on young bearing apple trees. *HortScience*, 30, 981–984.
- Retamales, J.B., Hipps, N.A., Atkinson, C.J. (2004). Effect of timing of root pruning and root IBA application on leaf mineral concentration and canopy light interception of ‘Cox’s Orange Pippin’ apple trees. *Acta Hort.*, 636, 51–57.
- Sachs, T. (2005). Auxin’s role as an example of the mechanisms of shoot/root relations. *Plant Soil*, 268(1), 13–19.
- Saure, M.C. (2007). Root pruning – a poorly understood management practice in fruit trees. *Internat. J. Fruit Sci.*, 7(2), 43–56.
- Smith, S.E., Jakobsen, I., Gronlund, M., Smith, F.A. (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: Interaction between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol.*, 156, 1050–1057.
- Smith, S.E., Read, D.J. (2008). *Mycorrhizal symbiosis*. Academic Press, New York.
- Wei, Q.P., Lu, R.Q., Zhang, X.C., Wang, X.W., Gao, Z.Q., Liu, J. (2004). Relationship between distribution of relative light intensity and yield and quality in different tree canopy shapes for ‘Fuji’ apple. *Acta Hortic. Sin.*, 31, 291–296 (in Chinese).
- Wilson, P.J. (1999). The growth and form of potted mother plants of *Eucalyptus globulus* Labill. ssp. *globulus* in relation to the rooting ability of stem cuttings. *J. Hort. Sci. Biotechnol.*, 74, 645–650.
- Yang, S.J., Zhang, Z.L., Xue, Y.X., Zhang, Z.F., Shi, S.Y. (2014a). Arbuscular mycorrhizal fungi increase salt tolerance of apple seedlings. *Bot. Stud.*, 55(1), 1–7.
- Yang, S.J., Zhang, Z.L., Zhang, Z.F., Shi, S.Y. (2014b). Arbuscular mycorrhizal *Glomus versiforme* induced bioprotection of apple tree against scar skin disease. *Acta Sci. Pol. Hortorum Cultus*, 13(5), 13–24.