

Effect of shoot vigor on the development of transmitting tissue and pollen tube growth in pistils of tetraploid grape, cv. Pione

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Summary

Effects of shoot vigor on the development of transmitting tissue (TT) and pollen tube growth in pistils of Pione grapevines (a hybrid of *Vitis vinifera* L. and *V. labrusca* L., tetraploid) were investigated anatomically. Shoot vigor was reduced by root zone restriction and pre-bloom shoot tipping, and was increased by severe winter pruning and application of high concentration fertilizer. The TT development in long and short shoots of the vines without root restriction was also compared. In cross sections of the upper and middle ovaries, a thicker TT and more pollen tubes were found in root zone-restricted vines compared to unrestricted vines. Also, thicker TT and more pollen tubes were observed in less vigorous, short shoots of unrestricted vines. Tipping did not improve the TT development. However, spur pruning and application of high concentration fertilizer to root zone-restricted vines did not inhibit the TT development and pollen tube growth, though shoots grew vigorously. From these results, we conclude that promoted TT development in less vigorous shoots of Pione grapevines allows for more pollen tubes to penetrate into the ovary, resulting in the production of acceptable clusters with a sufficient number of seeded berries.

Key words: pistil, pollen tube, tetraploid grape, transmitting tissue.

Introduction

The transmitting tissue (TT) formed along the axis of plant styles provides an effective pathway for pollen tubes to penetrate into ovary tissue (KRONESTEDT *et al.* 1986, O'BRIEN 1994, WEBER 1994, HOWPAGE *et al.* 1998). OKAMOTO *et al.* (2001) reported that the TT development in pistils of most tetraploid grape cultivars is severely inhibited compared to that of diploid pistils. The insufficient TT development in tetraploid grape pistils may be one of the main reasons for the inhibition of pollen tube penetration into the ovule, which causes poor set of normally seeded berries (OKAMOTO *et al.* 1984). Our previous work on the developmental process of the TT in grape pistils revealed that the retardation of the TT development in tetraploid grapes occurs during 1-3 weeks before anthesis, though the whole florets grow actively (OKAMOTO *et al.* 2002).

It is generally known that flower clusters developed in vigorous shoots of tetraploid grapes, such as Kyoho and Pione (hybrids of *Vitis vinifera* L. and *V. labrusca* L., tetraploid), usually result in severe flower shatter or set of seedless small berries (OKAMOTO 1994). Such undesirable berry set can be improved significantly by root zone restriction (IMAI *et al.* 1987) and cane pruning, both of which decrease shoot vigor (YAMANE 1991). However, it is not yet clear whether the effect of these cultivation techniques originates from the improved TT development or not.

The current study was carried out to clarify effects of shoot vigor on the TT development and pollen tube growth in pistils of the tetraploid grape cv. Pione.

Material and Methods

Plant material: Fifty 1-year-old vines of Pione (*Vitis vinifera* x *V. labrusca*, tetraploid) were used for this research. In March 1995, 30 vines were individually planted in plastic containers (40 l) as root zone-restricted vines. The others were planted in an ordinary way as unrestricted vines in the experimental vineyard of Okayama University (Tsushima, Okayama). Both the root zone-restricted and unrestricted vines were trained to a bilateral cordon system on vertical trellis under a plastic-film cover. The number of shoots per vine was limited to 3-5 for the root zone-restricted vines and 4-7 for the unrestricted vines. In 1998, 16 vigorous shoots longer than 100 cm and 8 shoots shorter than 60 cm were chosen from unrestricted vines one week before anthesis. Half of the long shoots were tipped.

In the early spring of 1999, the root zone-restricted vines were divided into 3 groups. Vines in the first group were spur-pruned and had only two shoots per vine. Vines in the second and third group were cane-pruned and had 6 shoots per vine. Each vine in the first and second group was supplied with 3 l of a complete liquid fertilizer (Ohtsuka House #1 and #2), containing 60 ppm of N, twice a week. Vines of the third group were supplied with the fertilizer containing 120 ppm of N. Thus, with unrestricted vines, we had a total of 4 vine groups in 1999.

Measurement of shoot growth and pistil sampling: Shoot length, basal stem diameter, and midrib length of the leaves in the fruit zone were recorded at anthesis. The maximum diameter of the basal part of the rachis was also measured. Thirty florets were collected from

8 clusters in each vine group at anthesis. The length and diameter of the ovaries were measured under a dissecting microscope. Other 30 blooming florets in each vine group were self-pollinated with pollen collected from mature vines. The pollinated pistils were sampled 3 d later. All sampled florets were fixed with a FAA solution.

Ten leaves around the clusters were sampled from each treated vine at anthesis. They were dried in an oven at 95 °C and then powdered. The total N content was determined by a C-N coder (Yanako MT-600).

Observation of TT development and pollen penetration: Fixed samples were dehydrated by EtOH-BuOH series, embedded into paraffin blocks, and sectioned into 14 µm thick cross sections using a microtome. The sectioned pistils, sampled at anthesis, were stained with alcian blue and Schiff's reagent to observe the TT development in the pistils. TT cells were easily distinguished from the surrounding cortical cells and vascular bundles because of the smaller size with wider intercellular spaces stained deep purple (Fig. 1). The size of the TT (the area occupied with TT cells) was measured using an arealine meter (TAMAYA PLANIX 5000). The sectioned pistils sampled 3 d after pollination were stained with an aniline blue solution (0.2 % in 0.1 N K₃PO₄) and utilized for pollen tube observation under a fluorescent microscope.

Berry setting and seed formation: Eight to 12 clusters from each vine group were bagged with a polyethylene net at full bloom. The percentage of berry set was determined three weeks later by dividing the number of set berries by the total number of florets per cluster. The number of seeds per berry was counted for 50 berries from all treated vines at veraison.

Results

Shoot growth and berry set: Tab. 1 shows the size of the shoots and clusters at anthesis for each treatment in 1997 and 1998. Shoot length, stem diameter, and leaf size, were significantly smaller in root zone-restricted vines than in the unrestricted ones. The rachis diameter and the length and diameter of ovaries were also smaller in root zone-restricted vines. In the 1998 test, about 40 % of the total shoots were longer than 100 cm in unrestricted vines one week before anthesis, while 30 % of them were short (40 and 60 cm). Pre-bloom shoot tipping of long shoots did not affect the ovary size significantly. Short shoots in unrestricted vines had smaller clusters and ovaries than long shoots. Figs 2 and 3 represent growth curves of shoots and ovaries in the 1999 test. Growth of both, shoot and ovary in the root zone-restricted vines was significantly stimulated by applying high concentration fertilizer and spur pruning. In vines supplied with the high concentration fertilizer, the leaf N content was 3.54 % (on a dry weight basis). In spur-pruned and control vines, on the other hand, it was 3.00 % and 2.73 %, respectively.

In the 1997 experiment, far more seeded berries were set in clusters of root zone-restricted vines than in clusters of unrestricted vines (Tab. 2). In the 1998 experiment, the total number of berries per cluster was largest in tipped shoots of

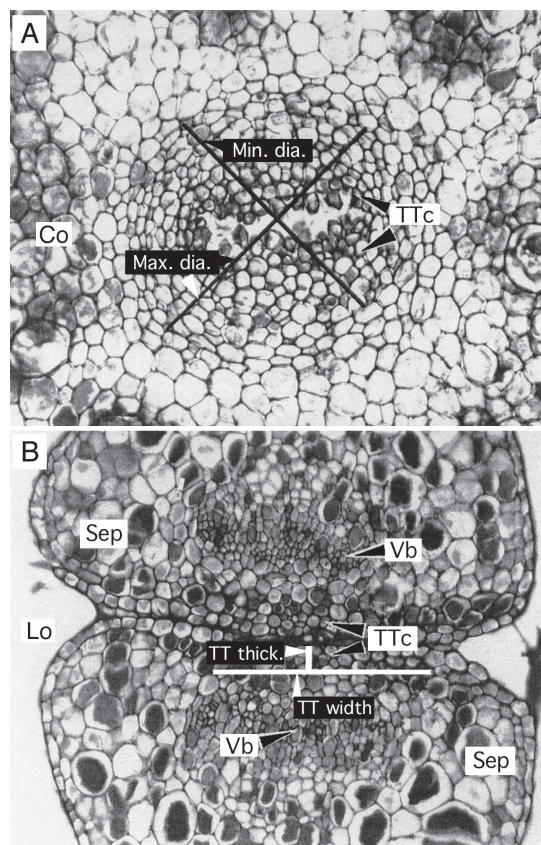


Fig. 1: Cross sections of a Pione pistil showing the transmitting tissue (TT) in the middle style (A) and the upper septa (B) (x100). Maximum and minimum diameters (black lines) of the TT in the middle style and the width and thickness (white lines) of upper septa were measured. Co, cortex; TTc, TT cells; Lo, locule; Sep, septum; Vb, vascular bundle.

vines with unrestricted root zone, though more than half of them were seedless and small-sized (Tab. 2). Inflorescences in long and untipped shoots showed a severe flower shatter resulting in poor berry set, while those in short shoots of both, vines with unrestricted and restricted root zone showed normal berry set (Fig. 4). Neither high concentration fertilizer application nor severe winter pruning, tested in 1999, affected berry set and seed formation.

TT development in styles and ovaries: Tab. 3 shows the TT size in cross sections of various parts of styles and ovaries. In the 1998 test, the average diameter of the TT in the style base was smaller in root zone-restricted vines than in unrestricted vines, though no significant difference was found in the middle of the style. In ovaries, in the top and middle ovule position, the width of TT in vines with restricted root zone was smaller than in those with unrestricted root zone. However, TT thickness was significantly larger in restricted vines than in long shoots of unrestricted vines. Short shoots of unrestricted vines developed thicker TT in their ovaries compared to longer shoots. In the 1999 experiment, application of high concentration fertilizer to root zone-restricted vines affected the TT development insignificantly. However, spur pruning increased the TT diameter in the basal styles and its width in all parts of the ovary, though TT thickness in the top position of ovules decreased slightly.

Table 1

Effect of root zone restriction, shoot length, and shoot tipping on the shoot and cluster development of cv. Pione grapes at anthesis

Year and treatment	Length (cm)	Shoot Number of nodes	Basal diameter (mm)	Leaf ^z Midrib length (cm)	Cluster and pistils			
					Rachis Length (cm)	Rachis Diameter (mm)	Ovary Length (mm)	Ovary Diameter (mm)
1997								
Root zone restriction	102.9 b ^y	16.4	1.06 b	17.8	15.3	3.9 b	1.98 b	1.77 b
Unrestricted	135.9 a	17.9	1.18 a	18.2	16.2	4.6 a	2.28 a	2.00 a
1998								
Root zone restriction	62.5 c	11.9 b	0.96 c	15.8 c	14.5 c	4.2 bc	1.58 c	1.77 c
Unrestricted								
Long	120.5 a	14.8 a	1.36 a	18.6 a	18.1 a	6.3 a	2.29 a	2.21 a
Long + tipping	81.6 b	10.2 c	1.39 a	19.5 a	19.2 a	6.9 a	2.36 a	2.31 a
Short	76.8 b	13.0 ab	1.20 b	16.8 bc	16.7 b	4.8 b	2.14 b	1.94 b

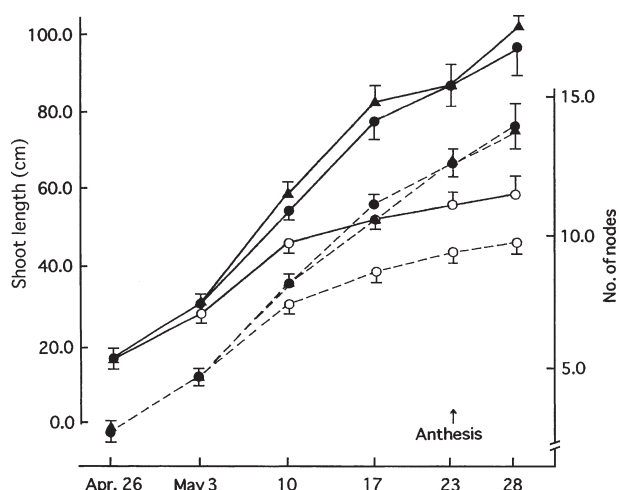
^z Leaves attached at the cluster position.^y Significance in each column and each year by Duncan's Multiple Range Test ($p < 0.05$), $n = 10$.

Fig. 2: Effect of high concentration fertilizer and severe pruning of root zone-restricted Pione grapevines on shoot growth in the one-month period before bloom 1999. ○ = Cane pruning and normal fertilizer application; ● = cane pruning and high concentration fertilizer; ▲ = spur pruning and normal fertilizer application. Solid lines, shoot length; dotted lines, number of nodes per shoot. Vertical bars indicate SE.

Tab. 4 shows the effect of root zone restriction and shoot length on the number of TT cell layers in the cross sections of the ovary in 1998. In both, top and middle ovules, the number of TT cell layers was smaller in long shoots of unrestricted vines than in shoots of restricted vines. In the position of top ovules, short shoots of unrestricted vines developed more TT cells than long shoots, though there was no difference in the middle ovules.

Pollen tube growth in pistils: Tab. 5 shows the number of the pollen tubes per pistil in various parts of the style and ovary 3 d after pollination. More pollen tubes were detected in most parts of the style and ovary in root zone-restricted vines than in unrestricted vines in the 1997 experiment. A similar trend was observed in 1998, though the difference was not so distinct. On the other hand, pre-

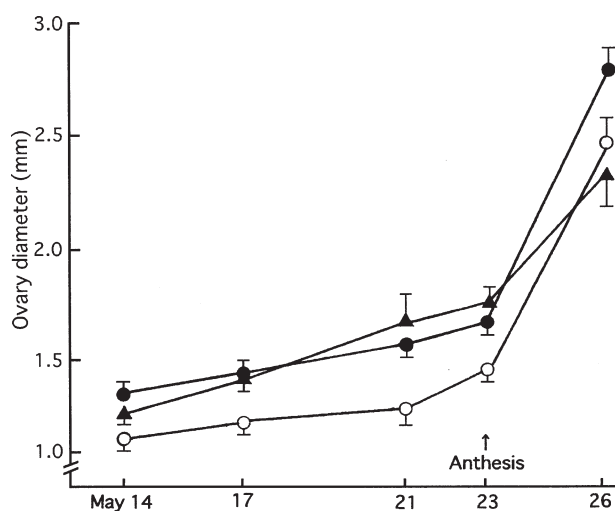


Fig. 3: Effect of high concentration fertilizer and severe pruning of root zone-restricted Pione grapevines on ovary growth in a 10-d period before bloom 1999. ○, ●, ▲: see Fig. 2.

bloom shoot tipping of long shoots of unrestricted vines decreased significantly the number of pollen tubes in the middle styles and the top locules. In short shoots of unrestricted vines, the number of pollen tubes in the upper part of the ovary was larger than in long shoots, though the difference was not obvious in other parts.

In 1999, application of high concentration fertilizer to root zone-restricted vines, decreased the number of pollen tubes in basal styles and in ovaries at the top, middle, and base positions of ovules. However, the effect of severe pruning on pollen tube growth was not distinct.

Discussion

The effectiveness of root zone restriction to control shoot vigor in tetraploid grapes has been established by

Table 2

Effect of root zone restriction, shoot length, fertilizer level, and pruning severity on berry set and seed formation in cv. Pione grapes^z

Year and treatment ^y	Total	No. of berries set per cluster				Mean seed number per berry
		0	No. of seed per berry			
			1	2	≥3	
1997						
Root zone restriction	74.1 a ^x	9.3	52.4 a	11.9	0.7	1.83 a
Unrestricted	26.1 b	9.0	15.9 b	11.7	0.0	0.70 b
1998						
Root zone restriction	44.7 b	0.0 c	13.4 b	21.6 a	8.9 a	1.83 a
Unrestricted						
Long	30.0 c	14.8 bc	13.5 b	1.7 b	0.0 b	0.54 b
Long + tipping	98.3 a	52.5 a	40.9 a	4.9 b	0.0 b	0.52 b
Short	61.5 a	25.3 b	31.8 ab	3.1 b	1.1 b	0.67 b
1999						
Root zone restriction						
Cane pruning	51.3	0.0 b	27.6 b	20.6 a	3.1	1.50
High fertilizer	57.0	2.9 b	46.7 a	6.4 b	1.0	1.12
Spur pruning	46.2	8.8 a	21.1 b	14.5 ab	1.8	1.45

^z 8-12 clusters per treatment were examined. Each cluster retained ca. 300 florets after cluster trimming at anthesis.

^y For root zone-restriction, shoot length, and tipping, see Tab. 1.

^x Significance ($p < 0.05$) in each column and each year.

Table 3

Effect of root zone restriction, shoot vigor, fertilizer level, and pruning system of TT size^z in various parts of cv. Pione pistils

Year and treatment ^y	Style		Top locule	Ovary		Middle ovule
	Middle (10 ⁻³ mm)	Basal (10 ⁻³ mm)		Top ovule (10 ⁻³ mm)	Thickness (10 ⁻³ mm)	
1998						
Root zone restriction	196.7	162.5 b ^x	300.6	x 76.2 a	250.0 b x 18.1 a	193.0 b x 13.6 a
Unrestricted						
Long	200.5	175.7 a	310.1	x 42.8 b	307.0 a x 11.8 b	234.2 a x 9.7 b
Long + tipping	198.3	174.5 a	288.0	x 54.9 b	281.6 ab x 10.7 b	221.5 a x 10.6 b
Short	193.1	174.1 a	300.6	x 64.6 ab	303.8 a x 16.5 a	243.7 a x 12.1 ab
1999						
Root zone restriction						
Cane pruning	203.1	152.3 b	244.1 b	x 61.6	291.0 b x 17.8 a	285.8 b x 13.9
High fertilizer	211.6	165.8 ab	323.2 ab	x 56.8	351.3 a x 16.1 ab	310.9 ab x 13.1
Spur pruning	217.4	171.2 a	392.5 a	x 57.3	345.7 a x 15.2 b	346.5 a x 13.1

^z Presented as average diameters for styles and as width x thickness for ovaries.

^y See Tabs 1 and 2.

^x Significance ($p < 0.05$) in each column and each year. n=8.

IMAI (1987). OKAMOTO *et al.* (1989) have reported that young Pione vines grown in containers produce a larger number of seeded berries than vines whose roots extended freely. On the other hand, pre-bloom tipping of vigorous Kyoho shoots increased the number of seedless small berries but not of normal seeded ones (NAITO *et al.* 1980, OKAMOTO *et al.* 1980).

Our present data on shoot growth and berry set obtained in 1997 and 1998 are almost coincident with the previous reports. However, in 1999, application of high concentration fertilizer and spur pruning did not decrease the number of seeded berries per cluster, though they stimulated shoot growth significantly. It is highly interesting that the positive

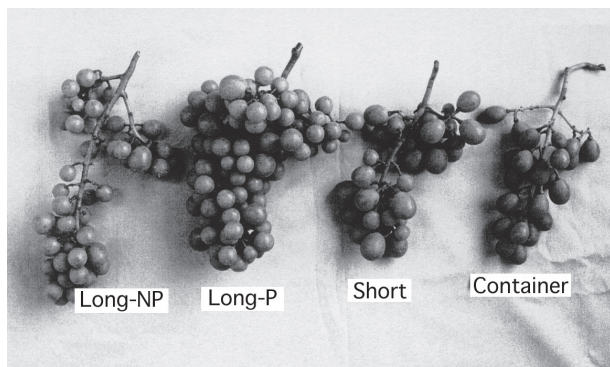


Fig. 4: Effect of prebloom shoot vigor, pre-bloom shoot tipping, and root zone restriction on berry set of Pione grapevines. From left to right: Poor berry set of a cluster produced at long shoots of vines with an unrestricted root zone, a cluster with many small seedless berries at tipped shoots, a normal cluster at short shoots of vines with an unrestricted root zone, and a normal cluster of root zone-restricted vines.

effect of root zone restriction on berry set of tetraploid grapevines could overcome the negative effect of treatments that accelerate shoot growth.

Root zone restriction decreased the average diameter of TT in the style base and the width of TT in the ovaries. A similar effect was found in 1999, where TT width was larger in vines treated with high concentration fertilizer or severe winter pruning. The reduction of TT size in less vigorous shoots may be due to the inhibited growth of the whole pistil as shown in Tab. 1 and Fig. 3. However, the thickness of the TT in each part of the ovary was significantly larger in root zone-restricted vines. Pollen tubes pass through the

Table 4
Effect of root zone restriction and shoot length on the number of TT cells in ovaries of cv. Pione grapes^z

Treatment ^y	Position of ovules	
	Top	Middle
Root zone restriction Unrestricted	2.33 a	1.58 a
Long	1.96 b	1.29 b
Long + tipping	1.79 b	1.21 b
Short	2.46 a	1.29 b

^z Average number of TT cell layers in the cross section of ovaries (see Fig. 1). Twelve ovaries per treatment were examined.

^y See Tab. 1.

^x Significance ($p > 0.05$) in each column.

inside tissue of septa in ovaries, where the thickness of the TT is critically thin in tetraploid cultivars compared with diploid ones (OKAMOTO *et al.* 2001). Actually only a few TT cell layers were counted in the septum tissue in this experiment (Tab. 4). The increase of TT thickness and TT cell number in septa of root zone-restricted vines are thought to enable more pollen tubes to penetrate ovarian tissue (Tab. 5). The decrease of the number of pollen tubes in tipped shoots cannot be attributed to the TT development. Some nutritional and hormonal changes might take place in the pistils after shoot tipping as reported by OKAMOTO *et al.* (1980) and NAITO and KAWASHIMA (1980).

Table 5

Effect of root zone restriction, shoot length, shoot tipping, fertilizer level, and pruning system on pollen tube growth^z in cv. Pione pistils

Year and treatment ^y	Style		Locule Top	Ovary			Micro-pyle
	Middle	Base		Top	Ovule Middle	Base	
1997							
Root zone restriction	30.3 a ^x	9.4 a	5.6 a	2.3 a	1.42	1.04 a	0.83 a
Unrestricted	16.7 b	4.2 b	2.5 b	1.1 b	0.48	0.10 b	0.10 b
1998							
Root zone restriction	22.2 a	8.6 a	5.2 a	3.1 a	1.60 a	0.79 a	0.37 a
Unrestricted							
Long	19.0 a	6.9 ab	2.9 b	1.6 a	0.77 ab	0.33 ab	0.17 ab
Long + tipping	8.0 b	3.5 b	1.6 c	1.0 b	0.41 b	0.14 b	0.07 b
Short	14.2 ab	5.7 ab	4.3 a	2.6 a	0.88 ab	0.47 ab	0.06 b
1999							
Root zone restriction							
Root zone restriction	29.5	14.3 b	5.1	3.1 a	2.7 a	1.30 a	0.44
High fertilizer	30.5	14.2 b	4.6	1.4 b	0.5 b	0.38 b	0.38
Spur pruning	34.3	20.1 a	5.6	2.2 ab	1.4 ab	0.60 b	0.41

^z Presented as the average number of pollen tubes per pistil in each part of the pistil.

^y See Tabs 1 and 2.

^x Significance ($p < 0.05$) in each column and each year. $n = 24$.

No negative effect of promoting shoot vigor was found in TT thickness of the ovaries of root zone-restricted vines, which might be a main reason for the fact that the set of seeded berries was not affected. WANG *et al.* (1998) reported that the flower clusters and xylem sap of root zone-restricted Kyoho vines contained higher levels of sugars and lower levels of total N and amino acids at anthesis than those of unrestricted vines. KOMATSU and NAKAGAWA (1991), on the other hand, suggested that there are changes in endogenous GA, ABA and cytokinin levels in Kyoho florets due to shoot vigor and SADH treatment. It may be speculated that nutritional and hormonal conditions established by root zone restriction, though unknown yet, can maintain the normal TT development even if shoot growth was promoted by application of high concentration fertilizer or severe pruning. However, pollen tube growth in the ovary was somewhat inhibited by the high fertilization. The mechanism for this is still unknown but a very high content of nitrogenous compounds in florets may inhibit pollen tube growth similar to tipped shoots in unrestricted vines.

From these results, we conclude that the poor development of TT in the septum of Pione grapes is significantly improved in less vigorous short shoots. The insufficient TT development in vigorous shoots cannot be improved by pre-bloom shoot tipping. On the contrary, the positive effect of root zone restriction on the TT development does not disappear even if shoot growth is accelerated by some cultural conditions. The factors that normally promote TT development should be determined by further research on tetraploid grape pistils.

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