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The Mutual Effects of Pinching and Promalin® on Root Architecture in Two Boxwood Species

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Boxwoods are constantly pinched to shape and form hedges, and Promalin® application is used to boost their shoot yield. Having good root development is crucial for the healthy development of plants; however, the effects of such applications on plant root development have generally been ignored in previous studies. Hence, this study evaluated the effects of applications on the root growth of two boxwood species (*Buxus sempervirens* L. and *Buxus balearica* Lam.) during the summer dormancy period. Pinching and Promalin® (0, 1 000, 2 000, and 4 000 ppm) were applied to one-year-old seedlings at three different times. Results showed that pinching had a negative effect on root development in both species and both years. Root development was lower in pinched plants than in control plants, which were not pinched. Promalin® at 2 000 ppm was found to be the most effective application on the root parameter. Root development was also higher in the second year than in the first year, depending on temperature, humidity, and the duration of sunshine. It is recommended to apply 2 000 ppm of Promalin® and not prune *B. sempervirens*. However, if pinching is to be performed, application of Promalin® will be useful to improve root development. For *B. balearica*, applying pinching and Promalin® together will help increase root development depending on the application time (in periods when the temperature is high).

Keywords: *Buxus sempervirens*, *Buxus balearica*, root properties, WinRhizo, pinching, Promalin®

INTRODUCTION

Two primary centers of diversity, the Caribbean-Latin America and Asia, are home to approximately 123 species of *Buxus* spp., and a small number is also found in Africa (WFO Plant List 2023). In Türkiye, *Buxus sempervirens* is distributed in the Black Sea, Marmara, and Mediterranean Regions, while *Buxus balearica* is distributed only in the Mediterranean region. Both species grow slowly. Boxwood species grow in the form of evergreen shrubs or trees. Due to their morphological properties, boxwoods are mainly used for a variety of decorative purposes, including ornamental plants, hedges, and potted plants (Köhler 2014; Sarı and Çelikel 2021). Commercial boxwood production requires higher productivity. Summer dormancy can be reduced in boxwoods by pruning or defoliation, shading, or applying hormones exogenously, which could shorten the production period and improve plant quality (Musselwhite et al. 2004). Pruning is a widely used technique to achieve better decorative appearance of boxwood trees and to increase shoot productivity. Similarly, Promalin® has been reported to significantly increase stem elongation and shoot yield (Musselwhite et al. 2004; Batdorf 2005; Calatayud et al. 2008; Söğüt 2016; Çelikel 2020; Çelikel and Demir 2020; Chauhan et al. 2020; Sarı and Çelikel 2021; Janowska and Andrzejak 2022; Tütüncü and Çelikel 2022). As the bulk of research suggests, studies on pruning and Promalin® applications focus on examining the development of above-ground organs in plants; however, there are few studies on the effects of these practices on root development.

Root growth in potted plants is a central element in plant performance (Ramireddy et al. 2018). Under stress conditions, subsoil parts are affected the most in plants (Zonta et al. 2006; Comas et al. 2013; Bucksch et al. 2014; Ramireddy et al. 2018). Root length and root density are positively linked with mineral element uptake, particularly for elements with low solubility (Marschner 2012). Water access is also influenced by root architecture, and under some circumstances, a correlation has been shown between root system size and tolerance to drought stress (Comas et al. 2013; Ramireddy et al. 2018). On the other hand, as Bayındır and Kandemir (2023) reported, the increase in total root length is an important indicator of the increase in the upper part development of the plant. Therefore, it is evident that plants with healthy root development will show better growth. The impacts of cultivation-related applications are readily visible and observable on the plant's upper organs, but their effects on the roots are typically more difficult to discern. This is because it is not very easy to examine the root structure, which is, by nature, under the ground. However, many advances have been made in root measurements in recent years, with the development of techniques such as plant image analysis software that can be easier, faster, more reproducible, and more descriptive of root growth (Judd et al. 2015; Paez-Garcia et al. 2015).

After sprouting in the spring, boxwoods exhibit summer dormancy in response to rising temperatures and abscisic acid (Musselwhite et al. 2004). As in fruit trees, Promalin® and pruning applications are done at the beginning of the applications to break the summer dormancy and increase the shoot yield in ornamental plants. Many studies have been conducted on the effectiveness of these practices in increasing shoot yield; however, how root development is affected by pruning, pinching, Promalin® applications, and breaking summer dormancy in boxwood remains unknown. Hence, this study examined how root development in boxwood is impacted by Promalin® treatments and pinching. This study also aimed to determine the optimal application technique to ensure that root development in boxwood is not adversely affected by periodic pinching treatments.

MATERIALS AND METHODS

The research was carried out at the Black Sea Agricultural Research Institute in Samsun, Türkiye.

Plant Material

Cuttings were collected from their natural environment in November 2019 and 2020 and transferred to the rooting medium on December 1. *B. sempervirens* cuttings were collected from the Çamlıhemşin district of Rize province. *B. balearica* cuttings were collected from the Habib-i Neccar Mountain Nature Protection Area in Hatay province (Table 1).

Table 1. Locations where plant materials were collected.

Species	Coordinates	Altitude (m)	Location
B. sempervirens	52°10’ 15.97”N 52°25’ 35.11”E	1000 – 1200	Rize Province Çamlıhemşin Meydan Village
B. balearica	36°12’ 34.00”N 36°10’ 58.14”E	180 – 200	Hatay center, Habib-I Neccar Mountain, behind the Saint Pierre Church

A humidity- and temperature-controlled (rooting medium) greenhouse was preferred to take root cuttings. The cuttings were trimmed to be about 10 cm in length and treated with fungicide (Captan 50 wp) and indole butyric acid (IBA) at 3 500 ppm (Langé 2014). Then, the cuttings were planted on rooting tables with a mixture of peat and perlite (3:1). Rooted cuttings were removed 150 d after planting. A sufficient number of young plants (720) were obtained for the experiment in both species, and the rooted cuttings were transferred to pots (2 L) containing a mixture of peat and perlite (3:1, v/v).

Climatic Conditions

The average temperature inside the greenhouse was 27.6ºC in 2019 and 32.0ºC in 2020. The average night temperature was measured at 19.2°C in 2019 and 20.7°C in 2020. The average air temperature was measured at 25.6°C in 2019 and 27.5°C in 2020. The 2nd year was observed to be drier than the 1st. The average relative humidity was 66% in 2019 and 58% in 2020; as the temperature increased, the relative humidity decreased. Also, the total sunshine duration was 1 010 hours in 2019 and 1 123 hours in 2020 during the experiment period, meaning that the 2nd year had more sunny days.

Experimental Design

Since summer dormancy is effective at the end of the spring development period, June and July were chosen for the applications. Root measurements were not made before the applications so as not to damage the plants. About 1 cm of the seedlings were cut and removed from the tip (top) region. Promalin® (Sumitomo Chemical, 18.8 g/L 6-Benzyladenine + 18.5 g/L Gibberellin A4/A7) was applied at 0, 1 000, 2 000, and 4 000 ppm by spraying the whole plant (Table 2). Promalin® solutions were put into a 2-L hand-held pressure spraying pump and sprayed in such a way that all parts of the plants were completely wet.

Spreader adhesive (Gübretaş, STARWET) was added to each application in order to boost the effect of Promalin®. A 70% shade net was used for shading in the greenhouse environment setup for the experiment. In order to determine the effect of different Promalin® doses on boxwood species at different treatment periods, the experiments were set up

Table 2. Pinching and Promalin® applications.

Application Times	Applications
	No pinching + 0 ppm
	No pinching + 1000 ppm
	No pinching + 2000 ppm
1st application time: end of spring growth period (June 15) 2nd application time: 3 wk after the 1st application (July 7) 3rd application time: 6 wk after the 1st application (July 28)	No pinching + 4000 ppm
	Pinching
	Pinching + 1000 ppm
	Pinching + 2000 ppm
	Pinching + 4000 ppm

in a potted random plot design with three replicates and five plants in each replicate. Three hundred sixty pots/plant per species (1 plant per pot) and a total of 720 pots/plant were used. When watering the plants, the drying of the pot surfaces was taken into account. The plants received standard cultural treatments, and no fertilizer was administered during the experiment. In the 2nd year, new plants were treated with the same applications as in the 1st year. The effects of time, pinching, and Promalin® applications on the root architecture were determined both separately and in combination as Promalin®+pinching, time+pinching, time+Promalin®, and time+Promalin®+pinching.

Measurements of Root Architecture

The WinRhizo root analysis program (Regent Instruments, Quebec, Canada, Instuments) was employed to examine the root architecture of *B. sempervirens* and *B. balearica* species. Within the scope of this study, rooted plants were removed from the pots 75 d after the applications (1st application on September 1, 2nd application on September 22, and 3rd application on November 13). The plant roots removed from the pots were carefully washed and cleared of the growing material. Three plants from each replicate were selected, and measurements were made on a total of 216 plants of both species. This study was repeated in the 2nd year. Afterwards, the roots were placed on the scanner (Epson Expression 10 000XL, Epson America Inc., Long Beach, CA, USA) of the device and transferred to the computer in three dimensions (Figs. 1, 2, and 3).

The following parameters of root structure and rooting levels were examined and determined using the WinRhizo program: total root length (cm), root surface area (cm^2), root volume (cm^3), average root diameter (mm), number of tips, number of forks, and number of crossings.

Data Analysis

The statistical analysis of the data obtained was performed using the SPSS package program. Differences between applications were compared with a 3-way ANOVA test

Fig. 1. 3D scan images of boxwood (*Buxus sempervirens*) roots (a: control, b: Promalin® 1 000 ppm, c: Promalin® 2 000 ppm, d: Promalin® 4 000 ppm, e: Pinching, f: Pinching + Promalin® 1 000 ppm, g: Pinching + Promalin® 2 000 ppm, h: Pinching + Promalin® 4 000 ppm).

Fig. 2. 3D scan images of boxwood (*Buxus balearica*) roots (a: control, b: Promalin® 1 000 ppm, c: Promalin® 2 000 ppm, d: Promalin® 4 000 ppm, e: Pinching, f: Pinching + Promalin® 1 000 ppm, g: Pinching + Promalin® 2 000 ppm, h: Pinching + Promalin® 4 000 ppm).

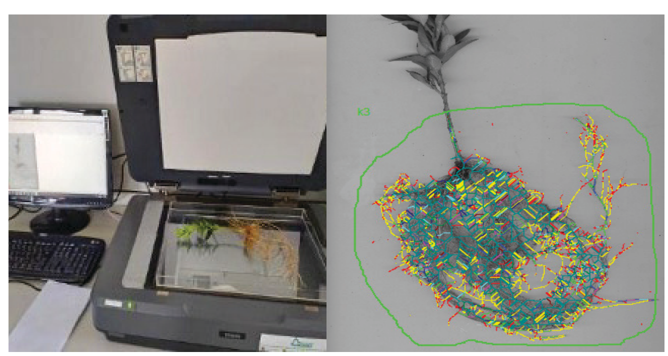

Fig. 3. Measurement of boxwood roots with 3D scanning WinRhizo program.

within 5% and 1% error limits. Differences in treatment levels were further evaluated for significance with Tukey post hoc comparisons.

RESULTS

As the analysis of variance showed, the effects of the treatments on boxwoods and the interactions between the treatments differed considerably in both species (Table 3).

Root Length

In the 1st yr, the root length of *B. sempervirens* was found to be 13% higher in unpruned plants ($P < 0.05$) than in pruned plants. Furthermore, interactions had no effect on root length. For the 2nd year, the combined effect of pinching and Promalin® (2 000 ppm) ($P < 0.01$) was found to increase the total root length by 88% compared to the control. Pinching was the application that reduced the total root length (13%) the most ($P < 0.05$) (Tables 3, 4, 5, and 6; Fig. 4).

In *B. balearica*, the combined effect of pruning and Promalin® (2 000 ppm) was the most effective application compared to the control in both years, increasing the root length by 27% ($P < 0.05$) and 18% ($P < 0.05$), respectively. The lowest result (5%) was determined at the 3rd time of the 2nd yr ($P < 0.05$) (Tables 3, 4, 5, and 6; Fig. 4).

Root Surface Area

Promalin® treatment in the 2nd yr increased the root surface area of *B. sempervirens* species by a maximum of 135% ($P < 0.01$). This was followed by the combined effect of pinching and Promalin® (2 000 ppm) ($P < 0.01$), which resulted in an increase of 110% in the 2nd yr. Also, pinching ($P < 0.01$) application was found to cause the highest decrease at 27% (Tables 3, 4, 5 and 6; Fig. 4).

No increase in root surface area was detected in *B. balearica* in the 1st year. In addition, the combined effect of pinching and time (at the time of first application) was determined to be the application that reduced the root surface area by 45% the most ($P < 0.01$). The highest root surface area increase was determined to be 48% in 2 000 ppm Promalin® application ($P < 0.01$), which was followed by the combined effect of pinching and Promalin® (2 000 ppm) with an increase of 41% ($P < 0.01$). The lowest value in the 2nd yr was determined as 7% ($P < 0.01$) the other 2nd time (Tables 3, 4, 5 and 6; Fig. 4).

Table 3. The significance levels of the effects of pinching and Promalin® applications at different times on root architectural features in *B. semperviren* and *B. balearica*.

		B. sempervirens							*B. balearica*						
Plant Root Properties	Year	Time	Pinching	Promalin®	Promalin® x Pinching	Time x Pinching	Time x Promalin®	Time x Promalin® x Pinching	Time	Pinching	Promalin®	Promalin® x Pinching	Time x Pinching	Time x Promalin®	Time x Promalin® x Pinching
Root length (cm)	1	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns
	2	**	ns	**	**	ns	ns	ns	*	ns	ns	*	ns	ns	*
Root surface area (cm^2)	1	ns	**	**	ns	**	ns	*	**	ns	ns	ns	**	ns	**
	2	**	ns	*	**	ns	ns	ns	**	ns	**	**	ns	ns	*
Root diameter (mm)	1	*	*	*	ns	*	ns	ns	**	ns	ns	ns	ns	ns	ns
	2	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Root volume (cm^3)	1	*	*	*	ns	**	ns	ns	**	**	ns	ns	**	ns	ns
	2	*	ns	**	**	ns	ns	ns	**	ns	**	**	ns	ns	*
Number of tips	1	**	ns	ns	ns	ns	ns	ns	*	ns	**	**	**	**	*
	2	**	ns	**	*	*	ns	ns	*	ns	ns	*	*	ns	ns
Number of forks	1	**	**	**	ns	ns	ns	ns	**	*	**	**	**	**	**
	2	**	ns	**	**	*	ns	ns	**	ns	*	**	*	ns	ns
Number of crossings	1	**	**	*	ns	ns	ns	ns	ns	ns	*	**	**	**	**
	2	**	ns	*	ns	ns	ns	ns	*	ns	ns	ns	*	ns	ns

ns: not significant, * Significant at $P < 0.05$, ** Significant at $P < 0.01$

Root Diameter

In *B. sempervirens*, the application that increased the root diameter the most in the 1st and 2nd yrs (10% and 50%) was the application of 2 000 ppm Promalin® ($P < 0.05$). Time application at the 3rd time as well as the combined effect of pinching and time (time one) was determined as the applications that reduced the root diameter the most with 14% ($P < 0.01$). In *B. balearica*, the root diameter decreased by 9% ($P < 0.01$) in the 1st year of application (time one). The 2nd-yr applications were found to have no effect (Tables 3, 4, 5, and 6; Fig. 4).

Root Volume

In *B. sempervirens*, Promalin® application in the 1st ($P < 0.05$) and 2nd ($P < 0.01$) yrs increased root volume the most (14% and 200%). Likewise, the combined application of pinching and Promalin® (2 000 ppm) in the 2nd year had the same effect with 200% ($P < 0.01$) as the use of Promalin® alone. The application that reduced the root volume the most with 42% was determined when pinching and time were used together (time three) ($P < 0.01$). However, no increase was detected in the root volume of *B. balearica* in the 1st year. The root volume, on the contrary, was found to decrease. The application that reduced the root volume the most was the use of pinching and time (time one) that reduced the root volume by 73%. ($P < 0.01$). In the 2nd yr, the effect of pinching and Promalin® combination (2 000 ppm) was the application that increased the root volume the most with 67% ($P < 0.01$), which was followed by the administration of Promalin® that increased the root volume by 63% ($P < 0.01$) (Tables 3, 4, 5, and 6; Fig. 4).

Number of Tips

In the 1st year, no increase was detected in the number of root tips of *B. sempervirens*. However, effective applications were found to cause a decrease, with the highest decrease being pinching with 29% ($P < 0.01$). In the 2nd year, Promalin® performed as the best application, increasing the number of tips in the root by 77% ($P < 0.01$), and the combined effect of pinching and Promalin® (2 000 ppm) resulted in an increase of 66% ($P < 0.05$).

In *B. balearica*, on the other hand, the highest increase (270%) was found when time (time one), pinching, and Promalin® (2 000 ppm) were applied together ($P < 0.05$) in the 1st year. This was followed by the combination of pinching and time (time one), resulting in an increase of 92% ($P < 0.01$). The application of Promalin®, however, decreased the number of tips significantly to 18% ($P < 0.01$) at the second time. In the 2nd year, the number of root tips increased by 24% with Promalin® application ($P < 0.01$). It was followed by the combined effect of pinching and Promalin® (2 000 ppm), increasing the number of

Table 4. Average values of root properties obtained as a result of Promalin® application in 2019 and 2020.

Species	Year	Promalin® (ppm)	Root Length (cm)	Root Surface Area (cm^2)	Root Volume (m^3)	Root Diameter (mm)	Number of Tips	Number of Forks	Number of Crossings
B. sempervirens	2019	Cont.	3 217	2 068 b	11.0 b	2.0 b	2 401	8 640 ab	144 ab
		1 000	2 812	1 648 c	8.0 c	1.9 b	1 972	6 330 c	109 c
		2 000	3 247	2 186 a	12.5 a	2.2 a	2 482	9 527 a	150 a
		4 000	2 872	1 968 b	10.5 b	2.0 b	2 283	7 401 b	126 b
	2020	Cont.	4 182 c	1 806 c	7.0 c	1.2 c	2 181 c	6 024 c	614 c
		1 000	5 436 b	2 530 b	10.0 b	1.2 c	2 972 b	8 634 b	886 b
		2 000	7 514 a	4 251 a	21.0 a	1.8 a	3 852 a	1 3051 a	1 169 a
		4 000	4 492 c	2 451 b	10.0 b	1.5 b	2 875 b	8 145 b	842 b
B. balearica	2019	Cont.	3 454	2 673	21.0	2.7	373 c	1 457 b	222 ab
		1 000	3 677	2 566	19.0	2.5	379 c	1 443 b	232 ab
		2 000	3 825	2 364	21.0	2.7	481 a	1 652 a	277 a
		4 000	3 123	2 534	20.0	2.6	411 b	890 c	159 b
	2020	Cont.	4 708	2 106 b	8.0 b	1.4	2 317 b	6 880 b	678
		1 000	4 569	2 036 b	8.0 b	1.3	2 222 c	6 221 c	510
		2 000	6 102	3 127 a	13.0 a	1.7	2 876 a	9 271 a	713
		4 000	4 891	1 971 c	7.0 b	1.3	2 457 b	6 133 c	575

There is a significant difference between the means with different letters within the error limits of $P < 0.05$ or $P < 0.01$.

Table 5. Average values of root properties obtained as a result of pinching application in 2019 and 2020.

Species	Year	Pinching	Root Length (cm)	Root Surface Area (cm²)	Root Volume (cm³)	Root Diameter (mm)	Number of Tips	Number of Forks	Number of Crossings
B. sempervirens	2019	No	3 252 a	2 204 a	12.0 a	2.1 a	2 575 a	8 950 a	148 a
		Yes	2 822 b	1 732 b	9.0 b	2.0 b	1 994 b	6 999 b	116 b
	2020	No	5 488	2 873	12.0	1.5	3 039	9 428	940
		Yes	5 324	2 646	11.0	1.4	2 901	8 499	816
B. balearica	2019	No	3 435	2 604	16.8 a	3.0	407	1 517 a	234
		Yes	3 605	2 464	14.9 b	2.3	415	1 205 b	210
	2020	No	5 105	2 337	9.0	1.4	2 496	7 361	645
		Yes	5 030	2 284	9.0	1.4	2 440	6 891	593

There is a significant difference between the means with different letters within the error limits of $P < 0.05$ or $P < 0.01$.

Table 6. Average values of root properties obtained as a result of the effect of different application periods in 2019 and 2020.

Species	Year	Application Dates	Root Length (cm)	Root Surface Area (cm²)	Root Volume (cm³)	Root Diameter (mm)	Number of Tips	Number of Forks	Number of Crossings
B. sempervirens	2019	June 15	2 857	1 886	10.2 b	2.1 b	1 693 c	5 950 c	92 b
		July 07	3 247	1 864	8.9 c	1.9 c	2 334 b	7 893 b	145 a
		July 28	3 005	2 153	12.3 a	2.2 a	2 827a	10 080 a	159 a
	2020	June 15	7 033 a	3 512 a	14.0 a	1.5	4 088 a	12 834 a	1 323 a
		July 07	5 073 b	2 644 b	12.0 b	1.5	2 498 b	7 942 b	723 b
		July 28	4 112 c	2 124 c	9.0 c	1.4	2 324 b	6 114 c	587 c
B. balearica	2019	June 15	3 209	3 566 a	22.0 a	4.3 a	477 a	1 617 a	234
		July 07	3 811	2 024 b	10.0 b	1.6 b	384 c	1 460 b	245
		July 28	3 539	2 011 b	10.5 b	1.9 b	303 b	1 005 c	187
	2020	June 15	4 487 b	1 868 b	6.0 b	1.2	1 977 b	5 770 b	497 b
		July 07	5 198 ab	2 543 a	11.0 a	1.6	2 674 a	7 404 a	614 b
		July 28	5 517 a	2 518 a	10.0 a	1.4	2 752 a	8 204 a	746 a

There is a significant difference between the means with different letters within the error limits of $P < 0.05$ or $P < 0.01$.

root tips by 19% ($P < 0.05$). In addition, combined application of pinching and time (time two) resulted in the highest decrease, with its percentage being 10% ($P < 0.05$) (Tables 3, 4, 5, and 6; Fig. 4).

Number of forks

As for the root branching, the combined effect of pinching and Promalin® (2 000 ppm) was found to be the application that increased the number of root branches of *B. sempervirens* the most (135%) in the 2nd yr ($P < 0.01$). It was followed by the application of 2 000 ppm Promalin®, resulting in an increase of 117% ($P < 0.01$) in the 2nd year. However, pruning was found to be the application with an increase of 54%, the lowest result, in the 3rd application time ($P < 0.01$) (Tables 3, 4, 5 and 6; Fig. 4).

In *B. balearica*, on the other hand, the combined effect of time (time one), pinching, and Promalin® (2 000 ppm) increased the number of branches in the root the most by 155% in the 1st yr ($P < 0.01$). Pinching, however, was the application that reduced the root branching the most, with its percentage being 26% ($P < 0.05$). Promalin® application in the 2nd yr increased the number of root branches by 35%, making it the best practice ($P < 0.05$). In contrast, the number of root branches was observed to decrease to 26% ($P < 0.01$) at the 3rd time compared to the other times (Tables 3, 4, 5, and 6; Fig. 4).

Number of crossings

In *B. sempervirens*, Promalin® (2 000 ppm) was found to increase the number of root crossings by 4% and 90% in the 1st and 2nd yrs, respectively ($P < 0.05$). The highest decrease

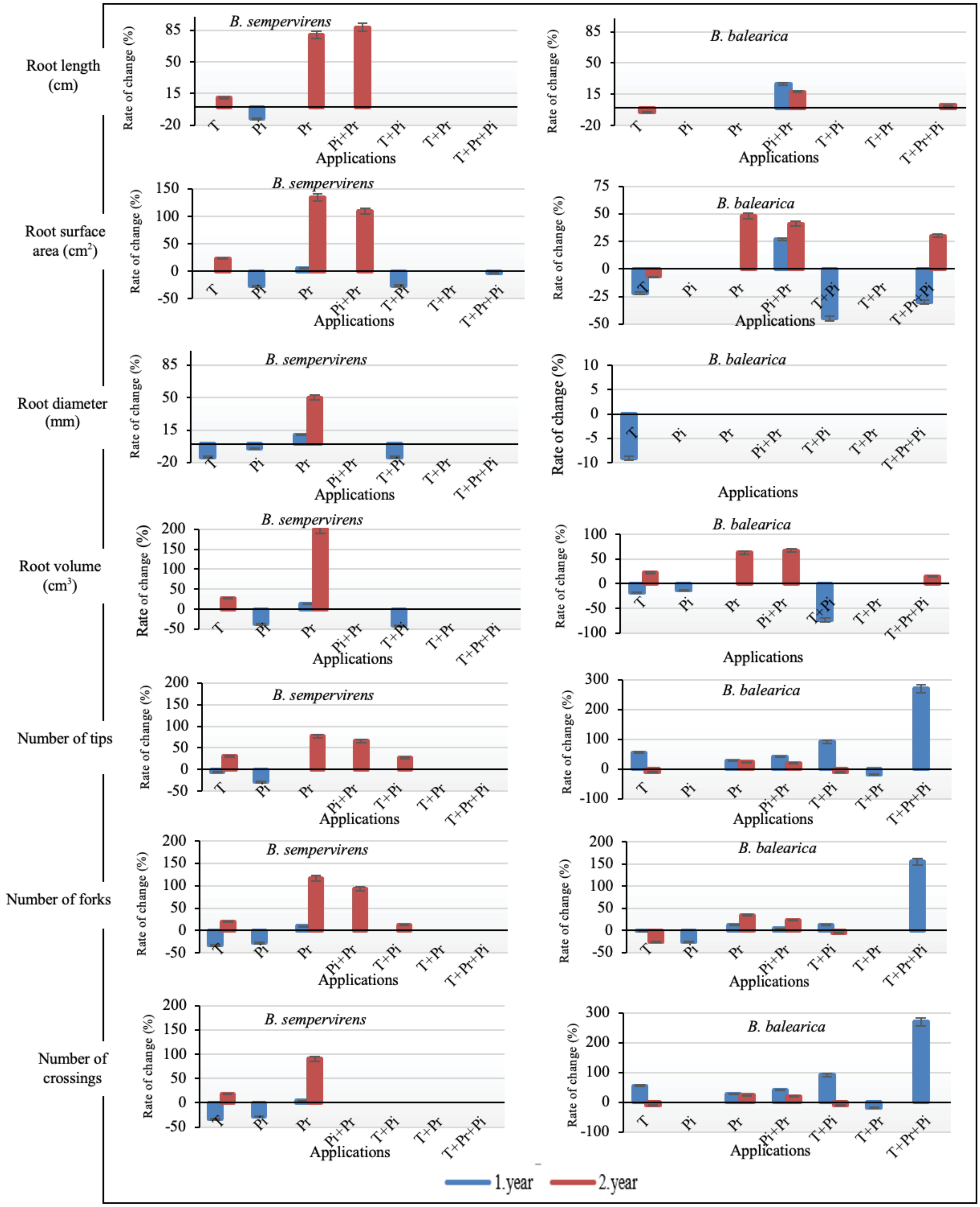

Fig. 4. Change rates of the effects of applications on root architectural features compared to the control (Time: T, Pinching: Pi, Promalin®: Pr, Promalin®+Pinching:Pi+Pr, Time+Pinching: T+Pi, Time+Promalin®: T+Pr, Time+Promalin®+Pinching: T+Pr+Pi).

(34%) was detected at the third time ($P < 0.01$). Interactions were shown to have no effect on the number of root crossings in both years. In *B. balearica*, on the other hand, the combined effect of time (time one), pinching, and Promalin® (2 000 ppm) was the application that increased the number of root crossings the most (342%) in the 1st yr ($P < 0.01$). It was followed by the combined effect of pinching and time (time one), resulting in an increase of 74% ($P < 0.01$). In the 2nd year, the highest increase (8%) was observed in the application of pinching and time ($P < 0.05$). Conversely, the highest decrease (46%) was determined in the application of time ($P < 0.05$) (Tables 3, 4, 5, and 6; Fig. 4).

DISCUSSION

Effects of Promalin® Applications

Promalin® applications had a positive effect on root development in both pinched and unpinched treatments of plants. The application of Promalin® combined with pinching was found to be more effective on root length than the application of Promalin® alone. Emongor et al. (2008) reported that Promalin® increased the leaf chlorophyll content by promoting the vegetative growth of plants, resulting in high photosynthesis and carbohydrate assimilation. Promalin® application in *B. sempervirens* (Sabatinos 1966) and gibberellic acid application in *Euonymus alatus* (Poston 2007) boosted the root length significantly.

In this study, the application of Promalin® alone was found to be more effective on root surface area compared to other applications. Plant growth regulators can increase root absorption capacity by improving root morphology (Sun et al. 2022). In addition, Lovelli et al. (2012) and Suchoff et al. (2017) stated that water and nutrient uptake became easier, and yield increased accordingly with the increase in root surface area in plants.

Promalin® treatment in the 2nd year had similar effects on root volume whether it was administered alone or together with pinching. It follows that the plant tends to increase its water absorption potential in the 2nd year. Researchers have reported that root volume, as well as root length, are important indicators of plants' water and nutrient absorption potential (Hımmelbauer et al. 2004; Zonta et al. 2006).

While Promalin® application increased the root diameter of *B. sempervirens*, it had no significant effect on *B. balearica*. Hodge et al. (2009) reported that plant roots can develop at different diameters with the use of different applications and cultivars. In this study, the highest results were obtained from both species with 2 000-ppm Promalin® application in both years. However, root diameter values are known to affect the absorption ability of the plant. Plants with smaller root diameters have higher absorption abilities (Huang and Eissenstat 2000; Boldrin et al. 2017; Sarıbaş et al. 2019). Therefore, it can be concluded that the low diameters obtained by 1 000 ppm applications for both species and years were more effective than other doses in increasing the absorption capabilities of the plant.

For the number of root tips, number of forks, and number of crossings, Promalin® (2 000 ppm) was the most effective application in *B. sempervirens*, whereas the combinations of time, pruning, and Promalin® (2 000 ppm) were found to be the most effective application in *B. balearica*. In addition, the number of root tips may increase or decrease at different rates depending on Promalin® applications, as do other root properties. Pecket (1960) reported that root growth was stimulated, and branching increased in response to gibberellin application. Zou et al. (2017) also determined that the number of tips in the root varies between 295 – 2 119, the number of crossings between 252 – 765, and the number of forks between 1 097 – 2 562 in trifoliate orange. Hayat et al. (2020) found that the number of forks of the 'Red Fuji' cultivar grafted on 'M.9', 'M.26', 'Chistock-1', and 'Baleng' rootstocks varied between 17 870 – 134 317.

Applications with Promalin® composition are especially effective in increasing root growth; however, when it comes to the effect of temperature in this study, particularly on the results in the 2nd year, morphological and physiological responses to water stress are decisive and guide adaptation to drought since the plant root is the most important organ for water and nutrient absorption (Shan et al. 2015; Liu et al. 2016). Zou et al. (2017) reported that plant roots are the first part of plants to encounter drought stress, and therefore root modification is vital for plants to adapt to drought.

Effects of Pinching

The 2-year study revealed that pinching also had a reducing effect on both *B. sempervirens* and *B. balearica*. As for *B. sempervirens*, pinching application was found to affect root architectural features and reduce all root features in the 1st year; however, it was not effective in the 2nd year. For *B. balearica*, pinching had a negative effect on the root volume and number of forks in the 1st year, but had no effect in the 2nd year. When pinching treatment was applied alone, it was found to be lower than the control in *B. sempervirens* in terms of root length (13%), root surface area (27%), root diameter (5%), root volume (38%), number of tips (29%), number of forks (28%), and number of crossings (28%). In *B. balearica*, pinching alone reduced root volume (13%) and the number of forks (26%).

Although the results differed between the years in this study, it was observed that pinching may cause an overall decrease in root development depending on the severity of the pinching. In Fare et al.'s (1988) study of *B. microphylla koreana*, it was reported that root development was greater in unpruned plants. In this study, it was concluded that pinching from 1 cm did not show sufficient effects.

The study on *B. sempervirens* by Boldrin et al. (2017) found that the root diameter ranged from 0.7 to 2.3 mm. In this study, it varied between 1.2 – 2.2 mm in *B. sempervirens* and 1.3 – 2.7 mm in *B. balearica*. These results suggest that root diameters could vary for both boxwood species depending on the effects of the applications. This situation can be explained as follows: the root diameters were reduced to compensate for plant losses, and a new arrangement was made in order to adapt to the current conditions as soon as possible. This situation shows that boxwood has the ability to adjust its position according to the current situation in order to restore root development. However, further studies are required to achieve more conclusive results.

Effects of Application Times

In the 1st year, the first application time was found to perform better in terms of the root surface area, root diameter, and root volume, whereas the 3rd time was better in terms of the number of tips, number of forks, and number of crossings. Overall, the 1st application time was better in the 2nd year. Similarly, in *B. balearica*, improved outcomes were obtained compared to the 1st application time in the 1st year and the 3rd application time in the 2nd year. The outcomes varied depending on when the application was made in both species.

When the 1st and 2nd yrs (27.6ºC in 2019 and 32ºC in 2020) are evaluated, it is evident that the 2nd year values are higher due to the effect of temperature (Table 6). Goldberg et al. (1976) reported that while deeper rooting was observed over the length of the drying cycles, in the upper soil layers, outcropping root development was observed under high humidity conditions. Jacobs et al. (2009) also found higher root growth to be one of the most effective drought avoidance mechanisms in plants. In this study, root growth increased in the 2nd year due to the effect of temperature. In contrast, it can be argued that root diameters decrease in the 2nd year due to heat stress. Low diameter values indicate a high absorption capacity and suggest that the plants reduce their root diameters under stress (Toprak et al. 2016). An increase in the number of tips formed in the roots, tips, and crossing densities resulted in a significant increase in the plants' nutrient uptake capacity (Craine 2006). This study has found that the application times had an impact to some extent; however, the effects of the application times varied between the years more evidently.

Application times for both types varied over the years. In both species, root development in plants that were not pruned generally yielded better results in terms of all characteristics. However, since root diameter is an indicator of plant absorption ability, it is desired to be small. The plant has tried to compensate for the losses caused by pruning by reducing the diameter of its roots. For this reason, pruning has been found to be more effective in reducing the diameter of the root. Promalin® application positively affected the root characteristics of both species. In addition, when pruning and Promalin® are applied together, the study revealed that Promalin® is able to compensate for the negative effects of pruning, with the most effective dose being 2 000 ppm. It has also been revealed that the optimal time to achieve better application results depends on the temperature values of the year in which the application will be made. As a result, the priority in plant development has always been root development. It has been reported that plants with successful root development also exhibit successful above-ground development (Adams and Moore 1983).

CONCLUSION

The root architecture results have shown that the third application time in the first year and the first application time in the second year were more effective in *Buxus sempervirens*. In *Buxus balearica*, the first application time in the first year and the second and third application times in the second year were more effective. The effects of application times differed between years in both species. Pinching had a negative effect on root development in both species and in both years. In this study, root development was lower in pinched plants than in those not pinched. The most effective application for root development was determined to be 2 000 ppm of Promalin®. When the applications were evaluated in terms of root development, the application of Promalin® alone was the most effective for *B. sempervirens*. However, the effects of applications varied in terms of root length, root surface area, root volume, and root diameter in *B. balearica*, whereas time, pinching, and Promalin® were more effective on the number of tips, number of forks, and number of crossings. Therefore, it is highly recommended to apply 2 000 ppm of Promalin® and not prune *B. sempervirens*. However, if pinching is required, Promalin® application would be beneficial to boost root development.

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