

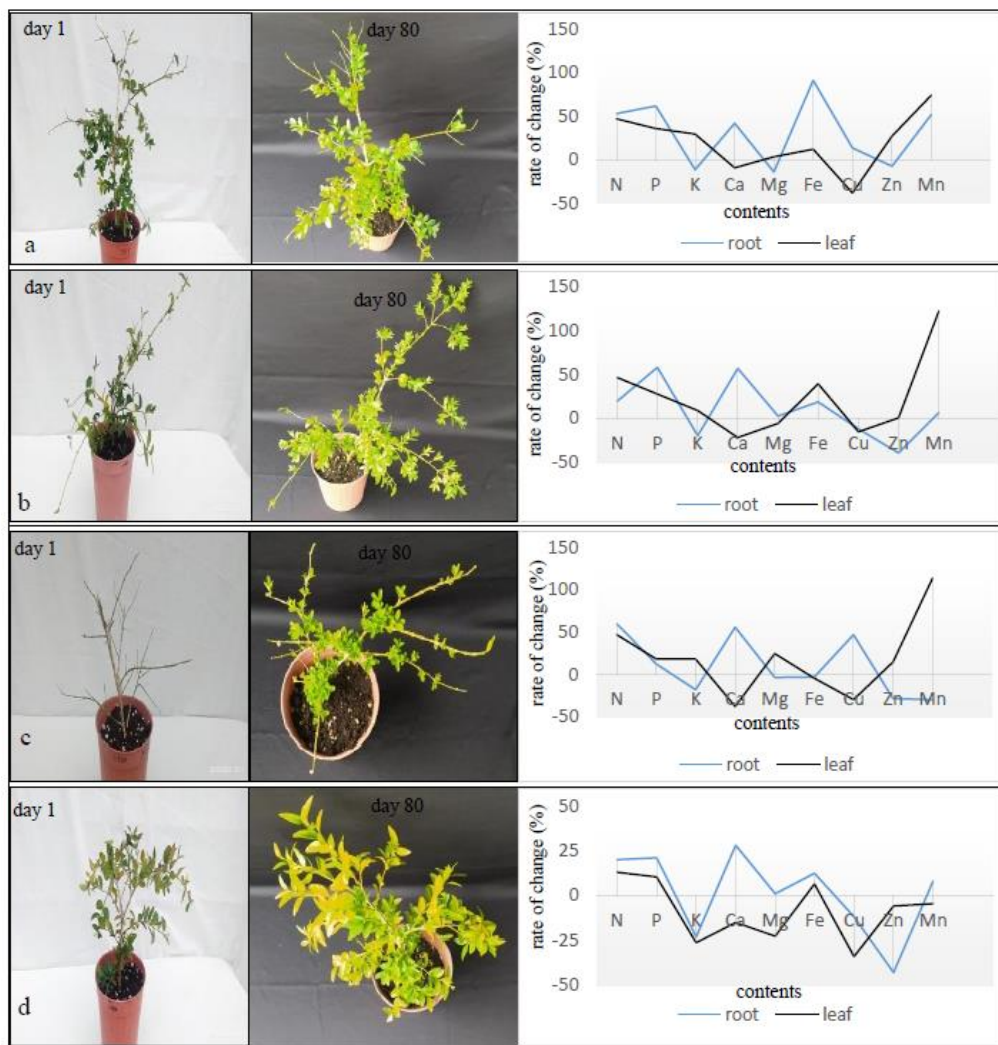
Effects of Mechanical Defoliation and Pinching Applications on Plant Growth and Root System Analysis with Machine Learning in Boxwoods

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DOI: 10.15376/biores.19.4.7450-7477

GRAPHICAL ABSTRACT



Effects of Mechanical Defoliation and Pinching Applications on Plant Growth and Root System Analysis with Machine Learning in Boxwoods

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The effects of mechanical defoliation and pinching (1 cm tip cutting) on *Buxus* plant growth, nutrient mobilization, and root architecture were determined. When 100% defoliation was applied, the highest increase rates of 80.3% in shoots and 88% in leaves were observed compared to the control group. In contrast, the overall effects of defoliation and pinching were negative, with 100% defoliation having the most negative effects. The chlorophyll content of the newly formed young leaves was also 50% lower with 100% defoliation. Leaves and root nutrient mobilization changed significantly, depending on the effects of defoliation and pinching. Apart from a very small increase in root length and number of forks, the effects of the treatments were negative, with 100% defoliation having the greatest negative effect on root development. Most affected was the number of crossings, which was 78% lower than in the control. In addition, machine learning (ML) algorithms were used in the study, including multilayer perceptron, J48, PART, and logistic regression. The input variables were evaluated to model and predict the root features. The performance values of the ML algorithms were noted in the following order: Logistic Regression > PART > J48 > MultilayerPerceptron. As the severity of defoliation increased, the losses of the plant also increased. However, boxwood has mechanisms to compensate for these losses even when it suffers complete defoliation.

DOI: 10.15376/biores.19.4.7450-7477

Keywords: *Buxus sempervirens*; Root morphology; Leaf loss; Pinching; Plant characteristics; Nutrient distribution; Machine learning

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INTRODUCTION

Boxwood, has attracted the attention of people throughout the history of civilization due to its slow growth, long life, hard and durable wood structure, leafy and evergreen nature and easy reproduction. Boxwoods have a wide range of uses due to these properties. However, boxwoods are generally used as ornamental plants today. The best-known and most cultivated species is *Buxus sempervirens* (Köhler 2014). Boxwoods are used as ornamental plants in single and mass plantings, hedges, potted plants, topiary, and cut greenery (Batdorf 2005; Van Trier *et al.* 2005; Köhler 2014). In addition to being ornamental plants, boxwoods have also been used in the production of musical instruments, writing tablets, combs, carved ornaments, paintings, and sculptures due to their hard and dense wood (Batdorf 2005; Mitchell *et al.* 2018). Due to these properties, they have been grown intensively for centuries (Larson 1999; Van Trier and Hermans 2007). One of the

methods used to increase shoot yield in boxwood cultivation is pinching. Pinching is the removal of apical dominance to promote lateral branching and flowering (Sasikumar 2015; Ehsanullah *et al.* 2021). However, in natural environments, due to effects such as animals and wind, and in nurseries, during plant transportation, plant branches can break either completely or at their tips. Similarly, defoliation by anthropogenic disturbances, insects, and grazing animals affects plant growth and vegetation dynamics (Wang *et al.* 2020). Under natural conditions, plants can often suffer foliar damage from human activities (cutting of branches, use of pesticides that cause defoliation, *etc.*), as well as insects and grazing animals (Eyles *et al.* 2013). In addition, climate change can lead to defoliation. Climate change will increase the fitness and abundance of some forest pests and affect plant growth and vegetation dynamics (Dale and Frank 2017; Kuosmanen *et al.* 2018). These examples illustrate the fact that multiple biotic and abiotic stressors can cascade and alter forest vitality. The future of boxwood, one of the most important forest and ornamental plants, has been seriously threatened in recent years due to diseases and damaging influences. The most important of these are the box tree moth (*C. perspectalis*) (Van der Straten and Muus 2010) and the box tree borer (*C. buxicola*), which have seriously damaged box trees both in the wild and in the landscape in numerous European, Caucasian, and Turkish countries (Malapi-Wight *et al.* 2014). Both the box tree moth and the box tree borer cause leaf loss in box trees. For this reason, the plant may lose its physiological balance and therefore die. Considering the biodiversity associated with boxwood, the destruction of natural boxwood stands in limited areas by pests can have serious ecological consequences. For this reason, the extinction of box trees can indirectly cause the disappearance of many living organisms (Mitchell *et al.* 2018).

Studying the effects of biodegradation on young trees and plant responses can also provide a theoretical basis for the selection and management of species to restore vegetation (Wang *et al.* 2020). In addition to natural conditions, this damage can also occur under controlled conditions and when transplanting plants. For this reason, defoliation experiments are often used to simulate biodegradation in plant species (Wiley *et al.* 2017; Wyka *et al.* 2017). Leaf removal reduces the photosynthetic area of leaves and affects root viability and nutrient concentration of plants (Quentin *et al.* 2010; Barry *et al.* 2012; Eyles *et al.* 2013; Jacquet *et al.* 2014; Wiley *et al.* 2017). It has been reported that the photosynthetic capacity of the remaining leaves increases after defoliation, and plants rapidly produce new leaves (Korpita and Orians 2014; Qiu *et al.* 2016; Eyles *et al.* 2016). Plants can store and reactivate reserves to restore leaf area (Moot *et al.* 2021). Previous studies have shown that plants tend to allocate more biomass to the aerial parts after defoliation (Mukherjee *et al.* 2015; Wiley *et al.* 2017; Chen *et al.* 2017). To illustrate, physical leaf damage alters the pattern of resource allocation to different vegetative and reproductive organs (Marshall *et al.* 2005; Stevens *et al.* 2014; Wang *et al.* 2020). In general, species, even within the same genus, respond differently to defoliation, and marked differences in compensation time, biomass, and chemical content can occur. While the responses of *Eucalyptus globulus* and *Eucalyptus nitens* to defoliation were similar (Barry and Pinkard 2013), white oak (*Quercus alba*) and black oak (*Quercus velutina*) responded differently to defoliation (Rieske and Dillaway 2008). The plants preferentially distribute more carbon to the shoots to compensate for the reduced leaf area due to the loss of carbon (C) as a result of defoliation (Wang *et al.* 2020). It has been reported that the production of new leaves after defoliation leads to a decrease in root mass and increased root mortality in most plants (Kosola *et al.* 2001; Hikosaka 2005). Pinching is also used in addition to pruning in annual cut flower species such as African marigold (*Tagetes erecta*),

gypsophila (*Gypsophila oniculata*), and lisianthus (*Eustoma grandiflorum*) to stimulate the formation of new shoots (Cheong *et al.* 2002; De Pascale *et al.* 2005; Badge *et al.* 2014), and perennial ornamental plants such as Buxus (*Buxus* spp.), rose, and holly (Brum *et al.* 2007). However, it has been reported that a significant part of the N produced by the plant is consumed in flowers and reproductive organs (Zhang *et al.* 2021). Root growth of potted plants is a key element in the overall performance of the plant (Wraith and Wright 1998). A strong root structure has a positive effect on plant productivity by increasing plant water and nutrient uptake as well as resistance to diseases and pests. Under stress conditions, the underground parts of plants are most affected (Comas *et al.* 2013; Bucksch *et al.* 2014). Root architecture varies greatly in response to different nutrient deficiencies and shows the ability to adapt to ever-changing growth conditions through structural flexibility (Sun *et al.* 2017). Morphological changes in the root system are regulated by the plant's nutritional status and its interaction with the environment, which are detected by localized signals from the roots (Giehl *et al.* 2014; Razaq *et al.* 2017). However, it is not very easy to study the root structure, which is naturally located underground. This is the main reason why not many studies have based their results on the phenotypic characteristics of the root. In recent years, numerous advances have been made in the measurement of roots, in addition to the development of techniques such as software to analyze plant images that can describe root growth in a simpler, faster, more reproducible and descriptive way (Judd *et al.* 2015; Paez-Garcia *et al.* 2015). In addition, modeling techniques for the structure and activity of root features based on multivariate and machine learning methods have been investigated. However, further studies are still needed to determine the importance of root traits in influencing aboveground biomass (Moon *et al.* 2018; Awika *et al.* 2021; Tütüncü 2024).

Machine learning is used to predict the effects of many applications in agriculture, especially crop yields. It is one of the techniques used. Artificial neural networks, support vector machines, linear and logistic regression, decision trees and Naïve Bayes are some of the algorithms used for prediction. Difficulty in selecting the algorithms lies in not knowing which of the existing algorithms is suitable for the plant under study (Palanivel and Surianarayanan 2019). However, there is very little information on boxwoods regarding the effects of pruning on the roots, plant parts and nutrient content.

It is important to ensure the sustainable cultivation of crops. The effects of defoliation and pinching on plant development, nutrient content of roots and leaves in boxwood and architectural features of roots were evaluated by image analysis. In addition, the study attempted to model and predict the effects of applications on root architecture by using methods such as artificial neural network analysis and machine learning based on data mining.

EXPERIMENTAL

Materials

In this study, 3-year-old boxwoods taken from Samandağ'ı district of Hatay province (36°11'15.98" N 35°55'55.18" E, altitude 725-1250 m), Türkiye in 2020 and transferred to pots after rooting were used.

Experimental Design

Mechanical defoliation and pinching applications were carried out on March 15, 2023, before the shoot period. In the study, 30 plants were used for each group and all

leaves of 30 plants were counted for each group before leaf defoliation. In the first group the leaves were not removed, 25% of total leaves in the second group, 50% of the total leaves in the third group, and 100% of total leaves are in the fourth group were removed by hand. In the pinching application, the tips of the lateral and terminal branches of each of the 30 plants were measured with a ruler and cut off with pruners at 1 cm. The trees were regularly maintained (watering, weeding, *etc.*). No fertilizer was applied during the experiment. The average temperature in the greenhouse was 27.5 °C and the humidity was 65 °C in 2023. During the experimental period, the total sunshine duration was 1120 h.

Measuring Plant Growth Characteristics

Plant height (cm), plant height (cm), number of leaves, number of shoots, shoot length (mm), shoot diameter (mm), leaf width (cm), and leaf length (cm) were measured on June 30. While the plant height, leaf width and leaf length were measured with a ruler, the shoot length and shoot diameter were measured.

Chlorophyll Content

The effect of the applications on the leaves chlorophyll content was determined. For this purpose, measurements were made at the beginning of the vegetation period and at the end of the vegetation period.

First measurement: Chlorophyll content in plants to be included in each application, before applications to boxwoods measured on March 15 in old leaves.

Second measurement: Measurements were made on both old and newly formed leaves on June 30, the end of the first vegetation period after the applications. The measurements were taken on 10 leaves from each plant. The relative chlorophyll content in the leaf were measured using the SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). The upper four rows of leaves were used to measure the SPAD index.

Nutrient Content Analysis of Leaves and Roots

Old leaves and the youngest leaves that had completed their development (the 8th leaf from the growing tip) were removed. The plants with roots were removed from the pots on June 30. They were washed and dried with paper towels. Then the roots were cut from the healthy tip and the lateral parts of the root. The leaves and roots were dried at 65 °C for 48 h, and then three plants were randomly selected from each replicate of each application. The leaf and root samples taken from the plants were washed for chemical analysis, dried and ground at 65 °C until they reached a constant weight. Total nitrogen in the ground samples was determined using the modified Kjeldahl method (Kacar and Inal 2008). For the analysis of P, K, Ca, Mg, Fe, Mn, Zn and Cu, the plant samples were wet-burned (4:1, HNO₃:HClO₄) and read in the ICP-OES instrument (Soltanpour and Workman 1981).

Rooting Potential and Phenotypic Root Development Examinations

The root analysis program WinRhizo (Regent Instruments, Quebec, Canada) was used to study root architecture. The plants with roots were removed from the pots on June 30. The roots of the removed plants were carefully washed and cleaned. Then, the roots were transferred to the computer in three dimensions using the scanner of the device (Epson Expression 10000XL, Epson America Inc., Long Beach, CA, USA) and computerized. The following parameters of the root structure and the degree of rooting were examined using the WinRhizo program: WinRhizo software made it possible to determine the total root

length (cm), root surface area (cm²), root volume (cm³), average root diameter (mm), number of tips, number of forks, and number of crossings.

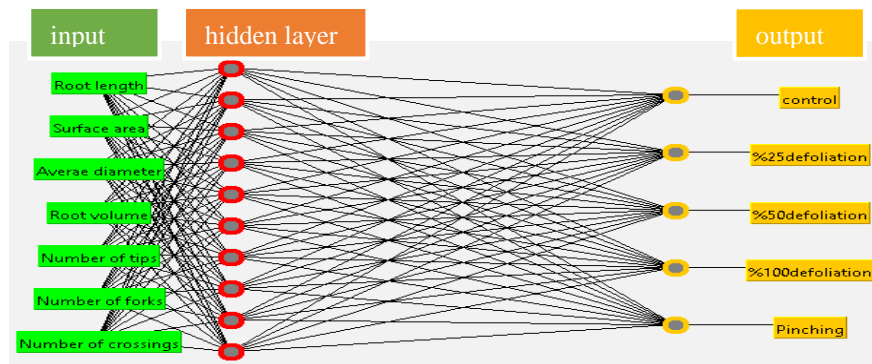


Fig. 1. The MLP structure with 7 inputs, 5 outputs, and 10 hidden neurons

Modeling Procedures and Classification Techniques

To model and predict the effects of treatments on root characteristics after defoliation and pinching of *B. sempervirens*, different data mining algorithms available in WEKA 3.9.6 (Machine Learning Group, University of Waikato) (Bouckeaert *et al.* 2016) were applied to the dataset. The results obtained were compared. A model was created by selecting the algorithm with the highest success rate among these algorithms. Four machine learning methods – Multilayer Perceptron, J48, PART and logistic regression - were used in the study. The input variables consisted of one species and seven different root characteristics are measured (root length, root surface area, root volume, average root diameter, number of tips, number of forks and number of crossings) The target variables (output) control, 25%, 50%, 100% defoliation and pinching included (Fig. 1).

Data Evaluation

The study was conducted using a completely randomized design, each containing a single seedling, and 30 replicates were evaluated for each treatment. Analysis of variance was performed using SPSS statistical software version 20.0 and differences between treatments were compared using Duncan's multiple comparison test (within 5% and 1% error limits).

RESULTS AND DISCUSSION

Characteristics of Plant Growth

The applications significantly influenced plant development. Pinching had the biggest effect, as it increased plant height (14.8%), plant width (4.8%), and shoot length (9.2%) compared to the control. The highest results were found in the number of shoots (80.3%) and number of leaves (88%) with 100% defoliation compared to the control. The highest results (18% and 11%) were found for the increase in shoot diameter at 25% and 50% defoliation. The applications for leaf width and leaf length showed similar effects, except for 100% defoliation. Plant height (28.6%), shoot length (42%), shoot diameter (11%), leaf length (18.2%), and leaf width (43.3%) were the applications that produced the lowest results at 100% defoliation. The lowest results were found for plant width (14.5%) and number of leaves (47.3%) compared to the control at 25% defoliation. The number of

shoots (88%) was lowest when pinching was applied compared to the control (Fig. 2).

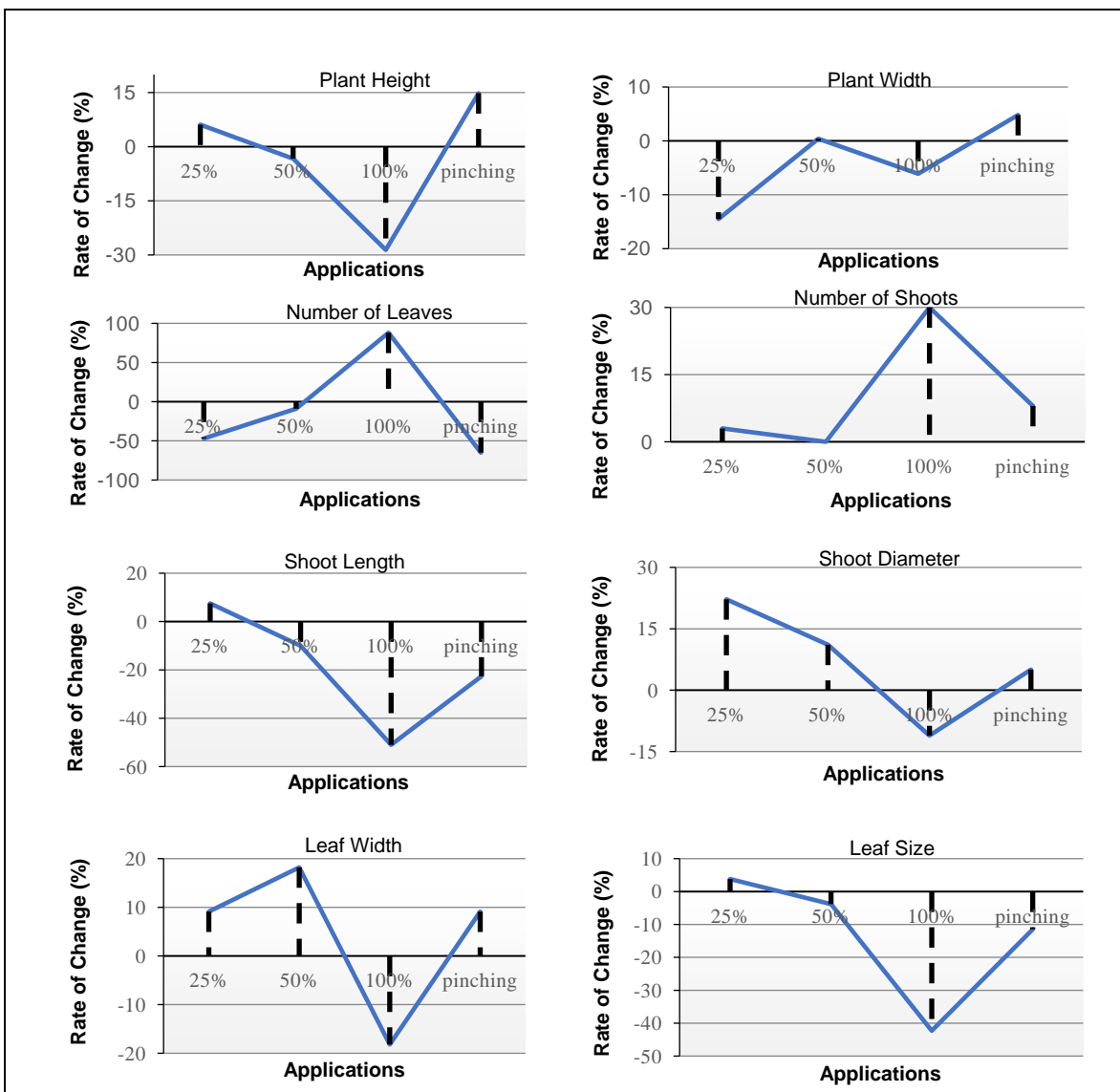


Fig. 2. Rates of change of the characteristics of the upper part of the plant compared to the control values of the analysis results after defoliation and pinching (25%, 50%, 100% defoliation and pinching).

Changes in the nutrient content of the roots and leaves

The applications had a significant effect on the amount of plant nutrients in the roots. According to the results of the application, the N, P, and Ca contents increased in all three applications. The K content and Zn content were lower in all treatments compared to the control. The Mg content was lower at 25% and 100% defoliation compared to the control, but a slight increase was observed at 50% and pinching. Again, Fe and Mn contents were lower at 100% defoliation compared to the control, while they increased in the other applications. While the Cu content was lower in the 50% and pinching treatments than in the control, an increase was observed in the 25% and 100% treatments. In all other treatments, the nutrient element contents increased compared to the control. The greatest decrease was observed for Zn. Among the applications, the greatest loss of nutrients was

found in 100% defoliation, while the least loss was observed in 25% defoliation (Figs. 3 and 4).

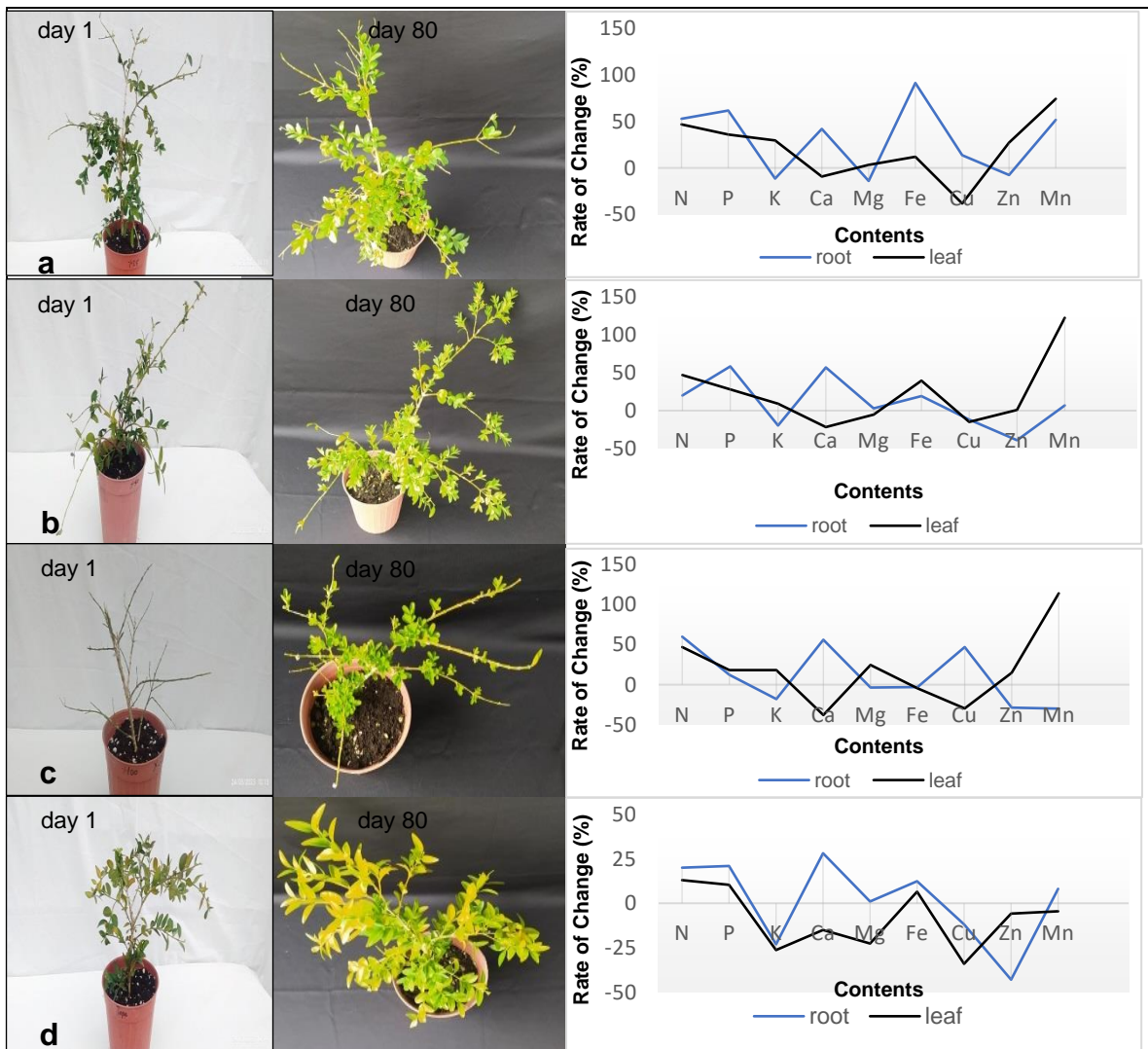


Fig. 3. The rate of change of nutrient content in the roots and leaves of the plants compared to the reference control of the analysis results after fertilizer application (a: %25 defoliation, b: %50 defoliation, c: %100 defoliation, d: pinching)

The applications had a significant effect on the amount of plant nutrients in the leaves. According to the results of the applications, N and P increased with all four applications, but least with the application of pinching. While K, Zn, and Mn levels increased in 25%, 50%, and 100% of the applications, respectively, compared to the control, it decreased in the pinching application. The increasing trend in nutrient content varied according to the severity of leaf loss. Ca and Cu levels were found to be lower in all treatments compared to the control. While Mg content decreased in 50% defoliation and pinching applications compared to the control, it increased in 100% defoliation. While Fe increased in 25%, 50% defoliation and pinching applications, it was also found to be lowest in 100% defoliation. Pinching was generally the application with the lowest nutrient content. The application with the lowest levels of N, K, Mg, Zn, and Mn compared to the control was only a pinching application. In contrast, the application with the highest levels

of P, K, and Zn showed 25% defoliation. Fe and Mn were highest at 50% defoliation compared to the control. While the Mg content was highest at 100% defoliation, the N content had the same effect as the other 25% and 50% defoliation. The highest value in terms of foliar nutrient content was found for Mn application, followed by the amount of N. The lowest value was recorded for Ca and Cu, while the highest decrease was recorded for pinching application (Figs. 3 and 4).

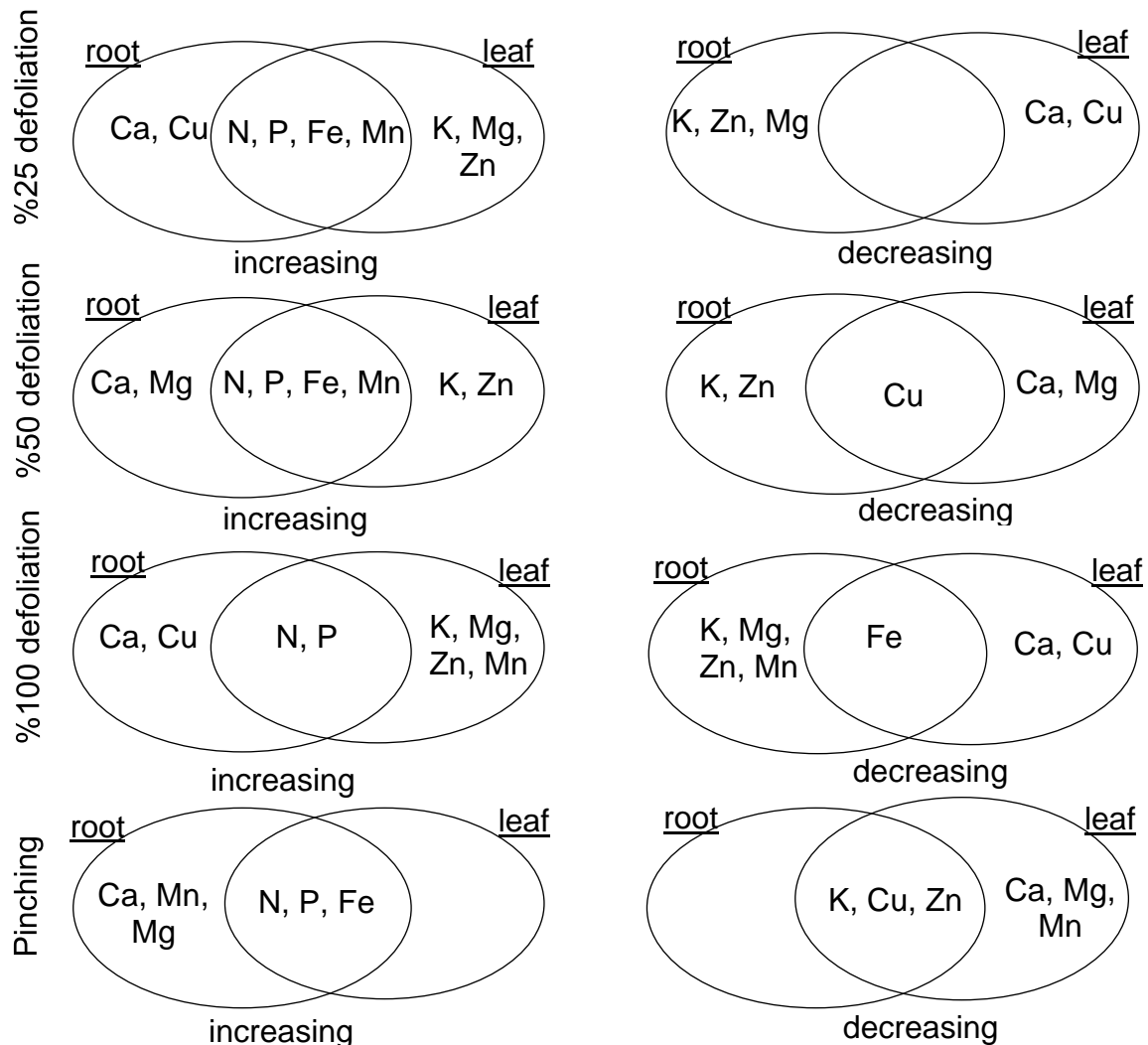


Fig. 4. Change in nutrient content in roots and leaves depending on defoliation and pinching application

Chlorophyll content

The effect of the applications on chlorophyll content was found to be statistically significant. The amount of chlorophyll in the plant leaves increased by 13% compared to the first measurement (15 March), from 78.6 CCI in the control to 89 CCI. It was found to increase by 13.4% from 77.7 CCI to 88.1 CCI at 25% defoliation. At 50% defoliation, it increased by 28% from 75 CCI to 96.2 CCI and at pinching application by 56% from 69.3 CCI to 108.1 CCI (Table 1). A small increase in the amount of chlorophyll was observed in the control plants.

The amount of chlorophyll in the newly formed leaves was compared with the amount of chlorophyll in the leaves at the first measurement. Compared to the first measurement (15 March), the amount of chlorophyll in newly formed leaves after 107 days (30 June) was 16% less in the control, 9% less in 25% defoliation, 14% less in 50% defoliation, and 50% less in 100% defoliation (Table 1).

Table 1. Effect of Defoliation and Pinching on the Chlorophyll Content of Leaves

Applications	First measurement (15 March) Old leaves	Second measurement (30 June)	
		Old leaves	Young leaves
Control	78.6	89.0	67.8
%25	77.7	88.1	71.1
%50	75.2	96.2	66.1
%100	75.2	0	50.1
Pinching	69.3	108.1	60.9

Morphological and architectural characteristics of the root

The architectural characteristics of roots were found to be significantly affected by applications. Root length was 7.7% higher in the 50% defoliation and 5.3% higher in the pinching application than in the control.

The number of forks was 2.3% higher in the pinching application than in the control. The effect of the applications on root root characteristics other than root length and number of forks was generally found to be lower than the control. At 25% defoliation, root length was 6.3% less, root surface area was 15.5% less, root volume was 13.6% less, number of tips was 5.3% less, number of forks was 7.7% less, and number of crossings was 22% less, while the root diameter value was the same as the control. It was found that at 50% defoliation the root surface area was 7.1%, the root volume 20.3%, the root diameter 33.3%, the number of tips 4.4%, the number of forks was 5.5%, and the number of crossings was 24.5%, which was significantly lower than the control. The lowest results were achieved with 100% defoliation. Accordingly, with 100% defoliation, root length was 18.8%, root surface area 31%, root volume 32.2%, root diameter 33.3%, number of tips 43.2%, number of forks was 33%, and number of crossings 78% lower than the control (Fig. 5).

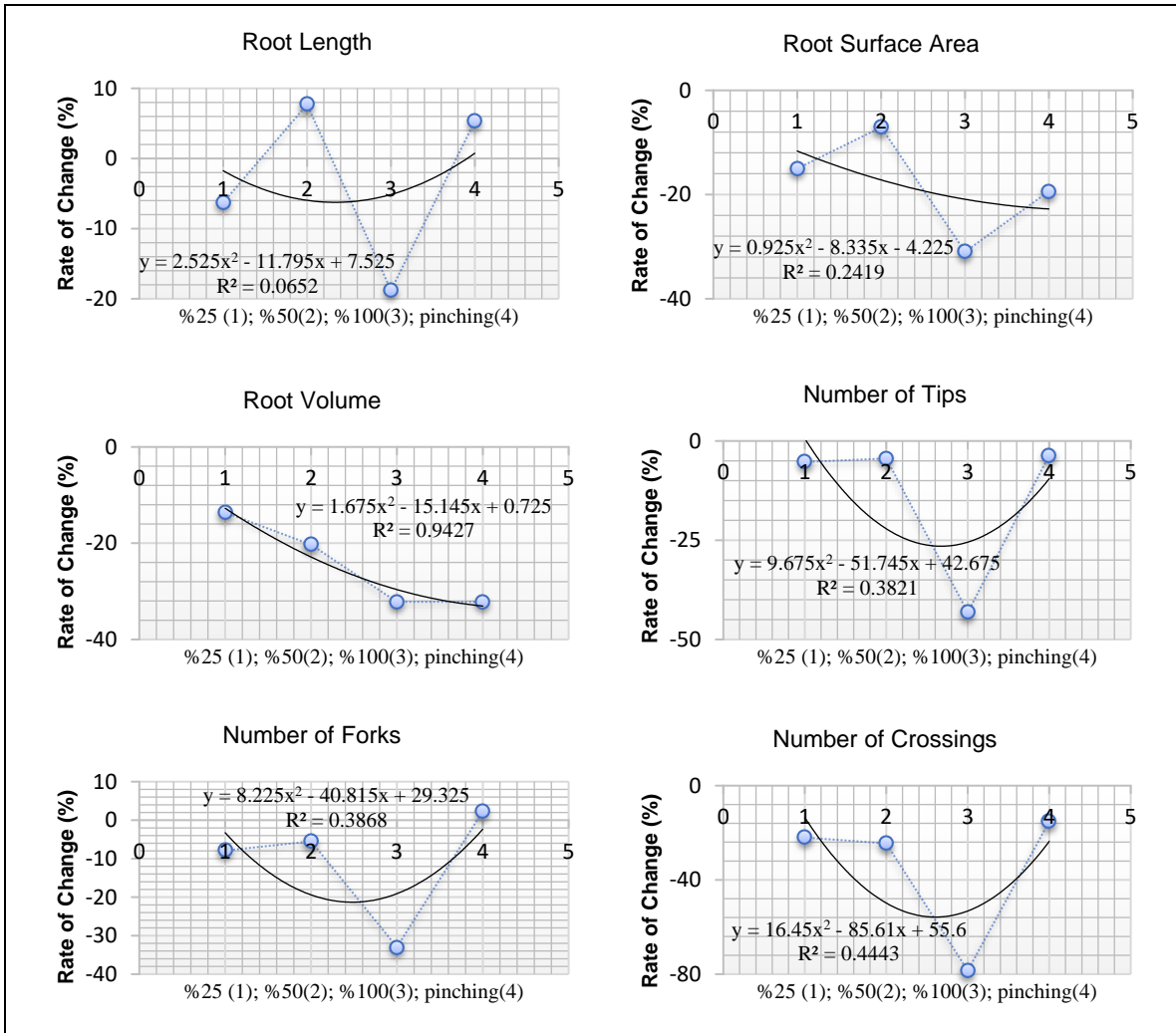


Fig. 5. The rate of change of the architectural root characteristics of the roots compared to the control values of the analysis results after defoliation and pinching (25%, 50%, 100% defoliation and pinching)

ML modeling analysis

The values classified by canopy clustering are divided into four groups. The values classified into 4 groups showed that 100% defoliation had the most effective (negative) effect on the architectural features of the roots, with 40% in the 2nd cluster. In cluster 0, pinching was 16% successful, in cluster 1, control was 16% successful, in cluster 3, defoliation was 25% to 12% successful and in cluster 4, defoliation was 50% to 16% successful. This grouping made it possible to verify the results of this study with artificial intelligence. A 100% defoliation is generally the most effective application achieved with classical methods. In addition, root length was determined as the most important result by using the select attribute result in the CfsSubsetEval algorithm and the BestFirst search method.

Artificial neural networks

The study considered 25 %, 50 %, and 100 % defoliation and pinching. After defining the total root length (cm), root surface area (cm²), root volume (cm³), average root diameter (mm), number of tips, number of forks, and number of crossings as output

variables, the output variable was predicted using the input variables. The decision tree for the decision is shown in Fig. 6.

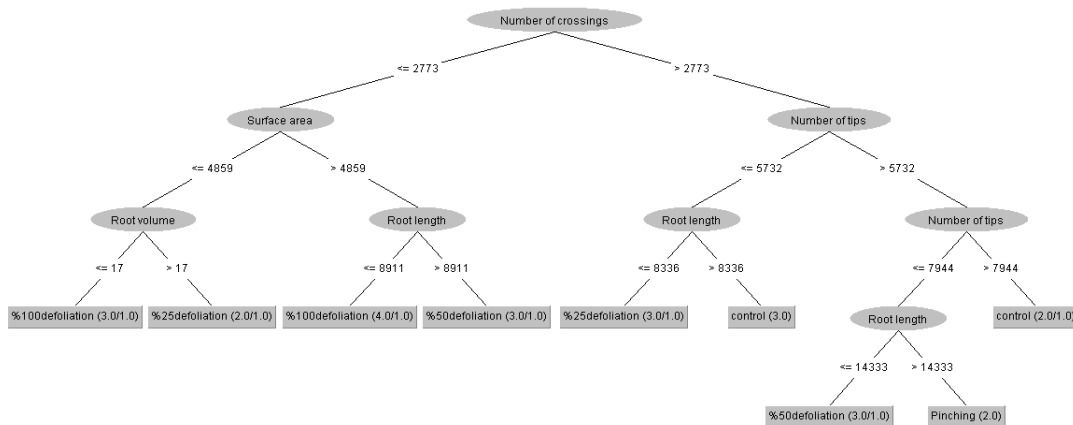


Fig. 6. Decision tree obtained by J.48 method

As shown in Fig. 6, to classify the applications of defoliation and pinching in terms of their impact on root architecture, it can be seen in the decision tree that the most important impact in the context of the study was on the number of crossings, and this is due to the impact of 100% defoliation. Indeed, the application and root trait reduced the number of crossings the most at 100% defoliation with 78% compared to the control. In this respect, ML made the correct prediction.

Choosing the most suitable model

Algorithms frequently mentioned in the literature (multilayer perceptron, J48, PART, and logistic regression) were used to select the most appropriate model. In this context, the most successful algorithm was selected based on the correct prediction rate. The selected algorithms were used to create models one after the other. As a result, it was decided to apply the logistic regression algorithm, which has the highest accuracy of 84%, to the data set. In selecting this algorithm, the accuracy value, duration and average absolute error were considered. Accordingly, the performance levels of the models created using the Multilayer Perceptron, J48, PART and Logistic Regression algorithms were compared and the resulting performance levels were noted in the following order: Logistic Regression > PART > J48 > Multilayer Perceptron (Table 2).

Table 2. Predictive Power of Machine Learning Models Depicts the Relationship Between the Variables Defoliation and Pinching and the Change in Root Architecture Characteristics of *B. sempervirens*

Classifiers	Accuracy (%)	Kappa	TP	FP	Precision	Recall	F-score
Logistic Regression	84%	0.80	0.84	0.04	0.85	0.84	0.84
PART	76%	0.70	0.76	0.06	0.80	0.76	0.74
J48	72%	0.65	0.72	0.07	0.76	0.72	0.71
Multilayer Perceptron	68%	0.60	0.68	0.08	0.74	0.68	0.65

DISCUSSION

Changes in the Growth Characteristics of Plants

In general, pruning is one of the most suitable techniques for effectively growing boxwood. However, the leaf losses that can occur during transportation and the harmful effects on the plants have important consequences for plant development. The applications were found to significantly affect the characteristics of the upper part of the plant and the architectural characteristics of the roots. In the study, the development of the upper parts of the plant gradually decreased with increasing defoliation, except for the number of leaves and the number of shoots, and reached a maximum at 100% defoliation. Among these results, the growth rate of 88% of leaves and 30% of shoots at 100% defoliation is one of the most important results of the study. While lower results were obtained in all other applications compared to the control, an increase in the number of leaves and shoots was observed at 100% defoliation. The increase in the number of leaves was a striking result. This finding showed that even if all the leaves of the boxwood were lost, the plant was prepared for this situation and was able to survive by adapting to the new conditions. Pinching is the removal of apical dominance to promote lateral branching and flowering (Sasikumar 2015; Ehsanullah *et al.* 2021). Shoot pruning can be an effective method to promote lateral shoot growth by freeing the shoot from apical dominance. In the study on *Rhododendron calendulaceum* Michx., it was found that pruned plants produced an average of 3.9 lateral shoots, while the unpruned plants produced an average of 1.7 lateral shoots (Malek *et al.* 1992). In this study, the increase in the number of shoots and leaves when pruning was applied was not as noticeable as when 100% defoliation was applied. In addition, plant height, plant width, shoot length, shoot diameter, leaf width, and leaf length were lower with 100% defoliation than with the other treatments. In fact, 100% defoliation removed the ABA in the leaves by dropping all the leaves and increased the activity of cytokinins and gibberellins in the plants, allowing the formation of new leaves and shoots. It is thought that the greatest hormone activity occurs at 100% leaf fall. Leaf fall and pruning of plants can also affect hormone levels and the activity of their organs. Edwards (1985) showed that after defoliation of apple trees, closed apical buds showed a threefold increase in gibberellin, a decrease in abscisic acid, and an increase in cytokinin compounds. This causes the buds to burst, allowing the newly growing leaves to utilise the available cytokines for growth. Similar to this study, Musselwhite's (2002) study on *B. sempervirens* 'Vardar Valley', *B. sinica* var. *insularis* 'Justin Brouwers', and *B. sempervirens* 'Suffruticosa' reported that pruning led to erratic results in terms of shoot yield, but leaf fall significantly increased the number of new shoots.

Changes in the Nutrient Content of Leaves and Roots

Research on the relationship between defoliation, pinching, and nutritional status of plants has been limited, particularly in boxwood. There is a great lack of information on this subject. Of the various plant parts analysed for nutrient content, the leaf is the most commonly used part. This is probably due to the ease of access and the fact that collecting the leaves generally does not endanger the life of the plants. The data of the roots are less than those of the above-ground parts. The nutrients in the leaves are often used to study the adaptation of plants to their environment and even predict the function of ecosystems (Guo *et al.* 2021; Wang *et al.* 2022). In general, the nutritional status of a plant can be better determined from the elemental concentrations of leaves than from other plant organs. Therefore, leaves are often used for plant analysis (Marschner 2012). In this study, the

changes in plant nutrients were investigated in both leaves and roots. Although defoliation of boxwood and application of needles significantly affected the nutrient content of roots and leaves, the N increase in roots and leaves showed a similar trend. Secondary leaves of deciduous *Quercus ilicifolia* were reported to have higher N uptake (May and Killingbeck 1995). While defoliation has a large negative impact on N and P uptake efficiency in older plants, only minor effects of defoliation before the main growth period of the plant were found (Zhang *et al.* 2021). When examining the results of the study, it was found that after defoliation and pinching application at the beginning of the growing season (March), the N and P content increased in both roots and leaves compared to the control.

With 100% defoliation, the plant tends to absorb more nutrients through the roots. In general, the content of N, P, Fe and Mn increased in both roots and leaves at 25% and 50% defoliation. While Ca, Mn and Mg only increased in the roots during pinching application, N, P, and Fe increased in both roots and leaves. With 100 % defoliation, only N and P increased. There was no common decrease in nutrient elements in roots and leaves at 25% defoliation, but Cu decreased at 50% defoliation, Fe decreased at 100% defoliation, and K, Cu, and Zn decreased with pinching application. The pinching application showed differences in the change of nutrient elements depending on leaf fall. This shows that the plant reacts differently to the various applications. Under normal conditions, the plant is in a physiological balance, and when it encounters a negative situation, it first tries to cope with this situation by increasing or decreasing photosynthesis to compensate for it. Among the nutrients, K, Mg, Ca, Fe, Cu and Mn are closely related to plant photosynthesis and have been reported to be particularly involved in the content of photosynthetic proteins and chlorophyll (Yruela 2013; Cui and Tcherkez 2021; Liu *et al.* 2021). In this study, it is assumed that boxwood ensures the mobilization of nutrients according to the place of use and demand to regulate the rate of photosynthesis. The study found that the plant generally tends to transfer K, Mg, Zn, and Mn contents from the root to the leaf during defoliation applications. The difference is that in 100% defoliation, unlike its transfer in other applications, it has been determined that Mn is transferred completely and directly from the root to the leaves. Accordingly, with 100% defoliation, the Mn in the root decreased by 30% compared to the control, was completely consumed and increased by 114% in the leaf. It is assumed that the reason for this transfer is the activating role of Mn in the plant, which forms the leaves from scratch during 100% defoliation. Mn acts as an activator for certain enzymes in plants (Wright *et al.* 2005; Andresen *et al.* 2018). Ca concentrations in the plant are generally relatively low, in contrast to other macronutrients such as potassium (Sanders *et al.* 1999; Hepler 2005). For maximum plant growth, boxwood requires high calcium storage in the leaves (53 to 65 meq/100 g tissue ODB) (Hefley 1979). Contrary to what the researcher reported, in this study, the average Ca content in the root increased by 68% compared to the leaf, while it decreased in the leaf. It has been reported that the calcium content in the leaf is particularly related to leaf fall and that early leaf fall can reduce the calcium content in the leaf (Kang *et al.* 2021). In addition, the increase in calcium content in the roots can be attributed to the stress the plant is subjected to by defoliation and pruning. When plants are exposed to environmental stresses such as drought, saline soils, pathogens, injury or nutrient deficiency, a rapid increase in cytosolic calcium concentration has been reported to occur as a result of Ca^{2+} import *via* plasma membrane ion channels (Steinhorst and Kudla 2013; Zhu 2016; Manishankar *et al.* 2018).

K did not show any increase in the root, on the contrary it decreased average by 12% compared to the leaf. K in the leaf increased significantly. It was found that Ca, in contrast to K, increased in the root and decreased in the leaf. In this study, the increase and

decrease of K and Ca were found to be related. This result is consistent with studies suggesting a correlation between cytosolic calcium and potassium uptake in different plant species (Johansen *et al.* 1968; Rains and Floyd 1970). K⁺ deficiency has been reported to trigger a rapid Ca²⁺ increase in Arabidopsis roots (Behera *et al.* 2017).

The Mg content was 113% higher in the root than in the leaves. However, the Mg content decreased in the root and increased in the leaf, especially at 25% and 100% defoliation. Mg and Ca concentrations in leaves are strongly correlated with photosynthesis and tree growth (Hochmal *et al.* 2015; Mendes and Marengo 2015). It has also been reported that excessive potassium accumulation reduces calcium and magnesium uptake (Behera *et al.* 2017; Tränkner *et al.* 2018; Barzegar *et al.* 2020). In the study by Hefley (1979) on *B. sempervirens* ‘Suffruticosa’ and *B. sempervirens* ‘Angustifolia’, it was observed that magnesium accumulation decreased with increasing potassium concentration. In this study, partly different results were obtained than assumed by the researchers. Only 50% defoliation supported the researchers’ results, and while Mg decreased in the leaf, K increased. At 25% defoliation, K and Mg increased in the leaf, and at 100% defoliation, both increased in the leaf. At pinching, Mg decreased in the leaf and increased in the root, while K decreased in both. According to Hefley (1979), calcium accumulation in leaves increased when calcium and magnesium concentrations increased and decreased with each increase in nitrogen. In this study, the Ca content increased in the root and decreased in the leaves. Mg, on the other hand, supports the researchers’ findings, but although it varied by application, it increased with N in both roots and leaves. In Hefley’s (1979) study on boxwood, it was emphasized that high N accumulation leads to insufficient calcium accumulation, which inhibits plant development. On the other hand, other studies have found that N fertilizer increases K, Ca, and Mg concentrations in *Bothriochloa ischaemum* tissues (Ai *et al.* 2017). However, the researchers’ findings relate to the results and evaluations of fertilizer applications. Different results were obtained in defoliation and pinching conducted in this study. The study found that increasing the N content did not reduce the Ca content; on the contrary, the Ca content in the root increased compared to the control. However, while the N content increased, the Ca content in the leaf showed no increase. The Cu content in the root was 53% higher than in the leaf. Again, Cu content in the root increased only at 25% and 50% defoliation, while it decreased in the other treatments. Previous studies have also reported that the accumulation of Cu is higher in the roots than in the upper parts of the plant. Copper was reported to accumulate in the root rather than the rice stem, especially in the root surface and epidermis instead of the xylem (Cui *et al.* 2019). In another study with cowpea, the root was found to store more copper than the shoot due to the high affinity of copper to the root wall (Kopittke *et al.* 2011). In this study, the Zn content in the root was average 162% higher than in the leaf, but since Zn tends to decrease compared to the control, no increase was observed in the root. On the other hand, it was observed that Zn tended to increase in the leaf except in the pinching application. Increasing Cu concentration in soil has been reported to decrease the amount of Zn in rice plant (Reddy *et al.* 2017). In this study, increasing Cu concentration by 25% in root and 100% in defoliation partially caused a decrease in Zn.

While the iron content in roots and leaves decreased with 100% defoliation, it increased in both roots and leaves with the other treatments. The iron in the roots was 76% higher than the iron in the leaves in all treatments. Depletion of Fe in the root apoplast pool causes permanent damage to the plant (Zheng *et al.* 2003). Root biomass increased in *Murraya exotica* L., *Spathiphyllum* ‘Sensation’ (Yeh *et al.* 2000) and *Medicago ciliaris* (M’Sehli *et al.* 2008) under Fe deficiency. In contrast, Troyer lemon and Taiwanica orange

plants were reported to be tolerant to Fe deficiency and to have less root biomass under Fe deficiency than under sufficient Fe conditions (Pestana *et al.* 2005). In this study, root traits decreased at 100% defoliation. This situation is similar to the other researchers' results, both in the root and in the leaf. This can be attributed to the decrease in Fe. In other treatments, the boxwood was able to partially tolerate the effects of the applications by increasing the Fe content in both roots and leaves. Some studies also reported that Fe deficiency accelerates Zn accumulation and leads to excessive Zn accumulation (Kobayashi *et al.* 2003). In the present study, a similar result was obtained only in the root. In the root, Fe increased and Zn decreased, except when pinching was applied, and in the leaf, Fe increased, except when 100% defoliation was applied, and Zn increased, except when pinching was applied.

Chlorophyll Content

The results show that the chlorophyll content in the new leaves was low compared to the first measurement, while it increased in the old leaves in applications other than 100% defoliation. In addition, chlorophyll content was lowest in the newly emerged leaves after 100% defoliation compared to the other treatments (50.1 CCI). The highest increase in old leaves was seen when pinching was applied (56%). The effectiveness of mineral nutrients is crucial for the synthesis of chlorophyll and carotenoids. It has been reported that N is the most important element in chlorophyll biosynthesis (Waraich *et al.* 2015) and that N fertilization increases the formation of active photosynthetic pigments in leaves (Cooke *et al.* 2005; Razaq *et al.* 2017). In addition, there is evidence that P fertilization increases total chlorophyll content and plant growth in many woody plants, while protein and chlorophyll content decreases under P deficiency (Dutt *et al.* 2013; Razaq *et al.* 2017). In this study, N and P in the leaves increased in all treatments. Accordingly, chlorophyll content also increased.

Chlorophyll molecules consist of the elements C, H, O, N, and Mg. Mg has been reported to have a direct effect on chlorophyll biosynthesis, while Fe has an indirect effect (Suzuki and Shioi 2002; Albus *et al.* 2012). In this study, Mg content increased at 25% and 100% defoliation. Fe was only lower at 100% defoliation than in the control. It is hypothesized that the decrease in Fe, which is an important element for chlorophyll, causes the chlorophyll content in the new leaves to be low at 100% defoliation. The highest chlorophyll content (108.1 CCI) in old leaves was found in the pinching application. It was found that N and P as well as Fe increased in the leaves during pinching application. Ca and Mg decreased. It is assumed that the reason for the increase in chlorophyll in the pinching application is that Fe is used more in chlorophyll production than in other applications. In other applications, it is assumed that the use of Fe in new leaves formation due to defoliation reduces the use of Fe in chlorophyll production. This result does not support the studies reporting that Fe is indirectly effective in chlorophyll biosynthesis. Furthermore, contrary to the researchers, Mg was found to have an indirect effect. These results reinforce the assumption that Fe is the most important element involved in chlorophyll biosynthesis in boxwood. In addition, excess Zn reduces Mn uptake in plants (Tang *et al.* 2016). High doses of zinc have been shown to impair chlorophyll synthesis and prevent the plant from benefiting from iron, even when sufficient iron is present and magnesium is replaced in the center of chlorophyll (Qian *et al.* 2009). In this study, Fe and Zn showed no antagonistic effect except in pinching application.

Changes in the Architectural Characteristics of the Roots

Pruning or pinching has been reported to reduce root development while increasing lateral branching (Fare *et al.* 1988; Eissenstat and Duncan 1992). According to the results of this study, the applications generally had a negative effect on the development of the architectural features of the roots. The application with the lowest root architectural characteristics was determined to be 100% defoliation. In 100% defoliation, root length was 18.8%, root surface area was 31%, root volume was 32.2%, root diameter was 50%, number of tips was 43.2%, number of forks was 33% and number of crossings was 78.5% less than the control. Similarly, the study conducted on *B. microphylla* var. *Koreana* Nak. reported that root development was higher in boxwoods that were not pruned than those that were pruned (Fare *et al.* 1988). In addition, plants that have been pruned, pinched, or defoliated have been found to reduce the available carbon reserves for root development (Eissenstat and Yanai 2002; Wang *et al.* 2020). For example, Valencia orange trees were found to experience at least 20% root loss 4 weeks after pruning the tip of the main shoot (Eissenstat and Duncan 1992). In apples, leaves removal 6 weeks before natural leaf fall was found to result in high root mortality within 2 weeks (Head 1969). In contrast, researchers reported that pinching significantly increased root length in *Nerium oleander* L. species compared to those without pinching (Bañon Arias *et al.* 2001). In the study on Indian rhododendron (*Melastoma malabathricum*), Japanese rose (*Hibiscus rosa-sinensis*) and Christina tree (*Syzygium campanulatum*), the root lengths of the species ranged from 313 cm to 1664 cm (Dorairaj *et al.* 2020). In this study, it ranged between 8270 cm (100% defoliation) and 10979 cm (50% defoliation). In the apple cultivar ‘Red Fuji’ grafted on rootstocks ‘M.9’, ‘M.26’, ‘Chistock-1’, and ‘Baleng’, root surface area values were found to be between 451 and 2130 cm² (Hayat *et al.* 2020). In this study, root surface area varied between 5843 cm² (100% defoliation) and 8459 cm² (control). One of the most important characteristics to consider in root traits is root diameter. The results showed that the plant responded by reducing root diameter to compensate for the losses suffered and to increase nutrient and water uptake. In previous studies conducted on different plant species (*Pinus nigra*, *Cedrus libani*, *Quercus cerris*, *Pinus halepensis*, *Quercus coccifera*, *Ceraton silgua*, and *Pistacia lentiscus*), a high root diameter is an indicator of the plant’s high capacity to bind to the environment in which it grows, while low diameter values indicate a high uptake capacity. In fact, low diameter values have been reported to indicate that the root diameters of plants have decreased under stress (Toprak *et al.* 2016). Also in this study, The reduction in root diameters started with pinching and defoliation, while there was no change in root diameters in control plants. This is consistent with studies reporting that pruning and defoliation reduce root diameter. For this reason, it has been reported that a high proportion of fine roots is a desirable trait for plants (Pregitzer 2002; King *et al.* 2002; Stokes *et al.* 2009). In the study on *B. sempervirens*, root diameter values between 0.7 mm and 2.3 mm were found. In this study, root diameter values ranged from 2 to 3 mm and showed a tendency to decrease as the severity of defoliation and pinching application increased (Boldrin *et al.* 2017).

In the study on Indian rhododendron (*Melastoma malabathricum*), Japanese rose (*Hibiscus rosa-sinensis*), and Christina tree (*Syzygium campanulatum*), the root volume values of the species ranged from 1.3 cm³ to 25.6 cm³ (Dorairaj *et al.* 2020). It was found that root volume values ranged from 7.36 cm³ to 22.04 cm³ for the cultivar ‘Red Fuji’ grafted on rootstocks ‘M.9’, ‘M.26’, ‘Chistock-1’, and ‘Baleng’ (Hayat *et al.* 2020). In this study, root volume decreased with the application of defoliation and pinching and varied between 40 cm³ (100% defoliation and pinching) and 59 cm³ (control).

In the study on *Malus prunifolia* and *Malus royii*, the number of root tips was reported to vary between 428 and 731 (Wen *et al.* 2018). In the trifoliolate orange study, the number of root tips ranged between 295 and 2119 (Zou *et al.* 2017). In this study, number of root tips ranged between 3188 (100% defoliation) and 5617 (control). In the number of forks values in the ‘Red Fuji’ grafted on rootstocks ‘M.9’, ‘M.26’, ‘Chistock-1’, and ‘Baleng’, values between 17870 and 134317 were recorded (Hayat *et al.* 2020). In the trifoliolate orange (*Poncirus trifoliata*) study, the number of forks ranged between 1097 and 2562 (Zou *et al.* 2017). In this study, the number of forks were between 12253 (100% defoliation) and 18703 (control). In the study conducted by Zou *et al.* (2017) on trifoliolate orange, the number of root crossings was found to range between 252 and 765 as a result of different applications. In this study, the number of crossings was between 1083 (100% defoliation) and 5026 (control). As the number of root tips, forks, and crossings formed increases, the capacity of the plants to take up nutrients increases significantly (Craine 2006). However, in this study, the number of tips, forks, and crossing density was found to decrease with increasing severity of defoliation compared to the control, peaking at 100% defoliation. This result confirms once again that the priority of the plant is the development of the aerial parts.

The roots of plants can increase or decrease due to leaf loss, depending on the amount of carbon (C) in the plant (Wang *et al.* 2020). It has been reported that the production of new leaves after defoliation causes a decrease in root mass and an increase in root mortality in most plants (Kosola *et al.* 2001; Hikosaka 2005). In this study, plants without defoliation and pinching showed more root development than plants with defoliation and pinching. Under normal conditions, 75% of the assimilates present in the plant are utilised for main stem growth and root development. However, pruning in evergreen plants leads to a loss of assimilation, which affects the distribution of assimilates and causes the plant to redistribute its assimilates. For this reason, plants that redistribute assimilates have been reported to reduce root development by sending less assimilate to the root zone (Wilson 2000; Moot *et al.* 2021). In fact, plants can experience C limitation by reducing C assimilation due to defoliation (Bardgett and Wardle 2003). To overcome this temporary C limitation, plants allocate more C aboveground for photosynthetic tissue regrowth at the expense of belowground C allocation. In the long term, however, these plants must continue to allocate C belowground to take up nutrients and water (Schmitt *et al.* 2013). Therefore, belowground C allocation for root biomass production may depend on the intensity of defoliation (Zhou *et al.* 2021) and nutrient availability in the soil (Liu *et al.* 2021).

In this study, the application of complete defoliation (100%) affected the development of the roots and the upper parts of the plant the most due to the complete cessation of photosynthesis. In fact, the carbon decline caused by leaf fall impairs the ability of roots to meet their carbon needs. Plants often respond to leaf loss by slowing or stopping root growth or by redistributing carbon to establish a functional balance between roots and shoots (Mukherjee *et al.* 2015; Wiley *et al.* 2017). In *Sesbania* and *Populus*, for example, physical leaf damage alters the pattern of resource allocation to various vegetative and reproductive organs (Marshall *et al.* 2005; Stevens *et al.* 2014). In addition, manual removal of leaves 6 weeks before natural leaf fall in apples resulted in severe root mortality within 2 weeks (Head 1969). A similar reaction was observed in blackcurrants (Atkinson 1972). However, photosynthesis in the remaining leaves can increase by 25 to 75% after defoliation, especially after partial defoliation (Pinkard *et al.* 1998; Wilson 2000; Korpita and Orians 2014; Qiu *et al.* 2016; Eyles *et al.* 2016). Indeed, it has been reported that the

loss of leaf area in ‘Fuyu’ date palms and cherry trees can be compensated to some extent by increasing the activity of the remaining leaves (Layne and Flore 1995; Choi 2004). This study showed that 25% defoliation did not cause a significant decrease in the characteristics of the upper part of the plant except for plant width and number of leaves, but it stopped shoot growth and increased characteristics such as shoot length, leaf width, and height. This is thought to be an indication that the remaining leaves on the plant are trying to compensate for the loss by increasing the rate of photosynthesis. In contrast, it was observed that the loss of root characteristics could not be compensated. It is thought that the reason for this is that the plant reduces the assimilates supplied to the root zone to compensate for the loss of the upper part. In their study on ‘Fuyu’ persimmon (*Diospyros kaki*), Choi and Kang (2005) found that the increase in defoliation at 0%, 25%, 50%, 75% and 100% defoliation treatments gradually reduced all measured parameters in all plants. Complete defoliation (100%) significantly reduced the dry weight of the roots. The results obtained by the researcher support this study.

Considering the results of previous studies, it was found that boxwoods responded to pinching and defoliation in a manner broadly similar to previous studies. Also, the compensation mechanism for losses caused by pinching and defoliation reported in previous studies has similar characteristics in boxwood. In addition, it has been shown that boxwoods that suffer leaf loss due to diseases and pests such as the boxwood borer and boxwood blight can respond by rapidly producing new leaves and shoots to compensate for these losses. Thus, the box trees are given another chance to survive.

Performance of Modeling the Architecture of the Root System

Depending on the effect of defoliation and pinching applications, the number of crossings was defined as an important classification node in the structure of the trees. The number of crossings decreased the most with 100% defoliation compared to the control with 78%. ML made the correct prediction on this issue. Thus, if an automatic classification system based on classifiers is developed, the number of crossings can be used as the main criterion in evaluating the impact of applications such as defoliation and pinching. ML classification accuracy achieved the third lowest accuracy level, with logistic regression at 84%, PART at 76% and J48 at 72%. The LR algorithm enables the prediction of the future value of the dependent variable based on the input data of the dependent and independent variables (Karaca and Karacan 2016). In addition, in the study conducted by Suganya (2020), which is similar to the results of this study, logistic regression was found to be the best algorithm with 100% accuracy for predicting product yield. It has also been reported to be one of the best methods used in agricultural product estimation (Kumari *et al.* 2016). In addition, Kavdir and Guyer (2008) used artificial neural networks, MLP, and DT classifiers for apples and reported the highest classification accuracy (83.3%) for MLP followed by (75.6%) for DT. It was reported that the accuracy of six olive oil classifications based on biochemical properties ranged from 81.6% to 85.7% (Gumus *et al.* 2018). The results obtained by the researchers seem to be different. The results obtained in this study also varied depending on the classifier.

CONCLUSIONS

1. Next to the roots, the leaves are the most important organ of plants when it comes to plant development. Defoliation or pinching can occur both in nature and in cultivation.

In addition, many techniques such as pruning, pinching, defoliation, and chemical applications are used to control plant growth in shrub and tree-like plants used as ornamentals. While this study showed that boxwood could compensate for leaf loss in an 80-day period, it could not compensate for nutrient and root loss to the same extent.

2. After defoliation, boxwood rearranged its assimilation distribution to produce new leaves.
3. Increased critically important nutritional elements in the root and leaf by providing nutrient mobilization in the root and leaf.
4. Although different results were obtained in some applications, it was generally observed that as the severity of defoliation increased, there was a decrease in above-ground parts, root architecture characteristics, nutrient content, and chlorophyll quantity.
5. The greatest decrease in the characteristics studied was generally observed with 100% defoliation. It was also found that the plant was able to compensate for its losses to a greater extent than in other applications with 25% defoliation. It has thus been shown that box trees can respond to leaf loss, which can be caused mainly by physical and biological influences, even if they suffer complete leaf loss.
6. Logistic regression, one of the machine learning (ML) methods, showed sufficient potential in predicting the root architecture of box trees. Furthermore, it was possible to evaluate the input variables defoliation and pinching with these algorithms. 100% defoliation as the input variable and the number of crossings as the output variable were found to be the most important parameters by the machine learning algorithms. Root development in plants is essential for plant development quality. It is expected that the study will contribute to facilitating the estimation process by determining the best model. In addition, being able to predict the applications' results can save time and money. As a result, ML models were found to be effective in predicting the effects of defoliation and pinching variables on root architecture.

ACKNOWLEDGMENTS

The author thanks Dr. Elif Öztürk, who analyzed plant nutritional elements in the study, for her contributions.

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Article submitted: July 6, 2024; Peer review completed: August 7, 2024; Revised version received and accepted: August 14, 2024; Published: August 26, 2024.

DOI: 10.15376/biores.19.4.7450-7477