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Full Length Research Paper

Evaluation of effect of *Ophiostoma novo-ulmi* on four major wood species of the elm family in Rasht (North West of Iran)

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Dutch elm disease is the most common and destructive disease of the elm family. The pathogen of *Ophiostoma novo-ulmi* will systematically lead to the blockage of xylems and cavities, and will finally lead to the development of wilt symptoms. The present study aimed to compare the diameter size of vessels and the number of xylary rays in four species: *Ulmus carpinifolia*, *Ulmus glabra, Zelkova carpinifolia* and *Celtis australis* as important factors in host resistance to elm disease. To do this, some samples were randomly collected from 10 cm above the place of seedling's inoculation. The test results showed that there was a significant difference at 1% probability level among the measured indicators in these four species, the maximum diameter of vessel and the maximum number of xylary rays belonged to *U. carpinifolia*. According to the results, *Ulmus campestris* was more sensitive towards Dutch elm disease as compared to the other three species, and *C. australis* had the greatest resistance to Dutch elm disease.

Key words: Dutch elm disease, xylary rays, vessel, resistance.

INTRODUCTION

Dutch elm disease was first observed in 1959 in forests of Golestan province, the Kansakuy and Kerenfecter Mountains, and then spread to other areas (Iraqi et al., 2008). In Iran, in addition to *Ulmus glabra*, this fungus has also affected *Zelkova carpinifolia* and *Ulmus carpinifolia* trees. In Qods Park of Rasht, Iran hundred-year-old *Z. carpinifolia* trees with a diameter of 1 m was affected by this disease (Iraqi, 2006).

As cited by Martin et al. (2005), over the past 100 years in Europe and North America, the prevalence of two Dutch elm disease epidemics by two fungal species of *Ophiostoma novo-ulmi* and *Ophiostoma ulmi* have affected elms. Moreover, considering the progress of the disease, they showed that pathogen spreads within the xylems of the tree and blocks them and leads to appearance of wilt symptoms which eventually causes death of the tree. The appearance of these symptoms is seemingly the result of the interaction between trees and fungal metabolites. Based on the studies conducted by Sola et al. (2005), elm bark-eating beetles (type *Scolytus* or *Hylurgopinus*) transmit the spore of fungal pathogen from diseased trees to healthy ones. Another way of disease transmission is through root grafting. In eugenic programs for elms' resistance to Dutch elm disease, thousands of elms have been annually inoculated with *Ophiostoma novo-ulmi*.

By growing and getting to the seedling stage and to a mature tree, an elm tree undergoes morphological and

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Table 1. Comparison of the number of xylary rays and vessel diameter average in the species studied

Species	Vessel diameter average (µm)	Number of xylary rays average (N)
U. carpinifolia	48/2240	7/88
U. glabra	38/1324	4/84
Z. carpinifolia	43/7344	5/76
C. australis	22/3140	3/12

*Each number is the mean of 25 replication.

physiological changes (Sola et al., 2005). During this period, phenology and anatomical characteristics of the wood of trees undergo a change. In one to three year-old branches of *U. minor* and *U. americana*, the average diameter of xylems as well as the position of vessels inside the annual growth rings are different at different ages (Sola et al., 2005). In one to two year-old branches, vessels are actually scattered vessels with smaller diameters, and 3-year-old vessels are ring-porous and thicker. It is reported that vessels with smaller diameters are one of the resistance factors against Dutch elm disease (Sola et al., 2005). Assuming that there is a significant difference between vessels' diameter in four important species of elm family against Dutch elm disease, the present research was conducted to compare vessels' diameter and the number of xylary rays in these three species as important factors in resistance to Dutch elm disease.

MATERIALS AND METHODS

Sampling

To perform this experiment, three-year-old seedlings with the same height (almost 2 m) of *U. glabra*, *U. campestris* and *Z. carpinifolia* prepared from the Parks and Green Space Organization of Rasht City were used. Seedling's inoculation was performed in early June, 2010. To do this, a small incision was made on the stem of the seedlings with a sterile scalpel, 0.5 ml of spore suspension x 10^6 1 ml isolated Onul, fungus *Ophiostoma novo-ulmi* was placed on a knife by a 5 ml hypodermic syringe.

This suspension was immediately drawn into the wood. The location of inoculation was then closed with parafilm for two days. Four to eight weeks after the seedlings' inoculation and the appearance of physical symptoms, such as wilting of the leaves and drying up of branch tips, some samples from 15 cm above the place of inoculation were randomly selected and transferred to the forest laboratory located at the Department of Natural Resources, University of Guilan, Iran.

Given that the species mentioned are considered as hard woods, the pieces were boiled in water for 1 to 2 h in order to soften them for cutting. Next, they were cut into 2 x 2 x 2 cm pieces. Finally, microscopic cuts with a thickness of 15 micrometers in certain directions were obtained using a microtome (Reichert type) (Parsapajoh and Schweingruber, 2001).

In this experiment, some healthy seedlings inoculated with sterile distilled water were also used as controls. After sectioning, staining the samples based on Parsapajoh and Schweingruber (2001) method was done according to the following order: Placing the sections into a 5% bleach solution for 15 to 30 min, washing the cuts with distilled water in a way that the smell of the solution is totally eliminated, placing the cuts in 1% safranin for 3 to 5 min, washing with sterile distilled water, washing the stained cuts in 50% alcohol, then 75% alcohol, washing the cuts in 96% alcohol for two to three times to remove the dye, placing the sections into xylene solution for the transparency and drying of the sections, and finally, placing the sections on a lam containing a drop of Canada balsam glue to make a permanent slide.

Measuring the desired anatomical indices

After preparation of microscopic slides, vessel's diameter and the number of xylary rays in *U. campestris*, *U. glabra*, *Z. carpinifolia*, and *Celtis australis* were measured under OLYMPUS (BX51) microscope. To do this, vessel's diameter and the number of xylary rays of each species were counted. The diameter of vessels was calculated based on micron. The same procedure was repeated 25 times. SPSS statistical software was used to analyze the obtained results. First, data following a normal distribution was examined using Colmogrove-Smirnove test, and homogeneity of variances was examined using Loon test. According to the normality and homogeneity of the data, one-way analysis of variance (ANOVA) was used to investigate the difference or non-difference of the indices of vessels' diameter and the number of xylary rays. After it was found that there was a significant difference between the relevant indices, Duncan test was used for multiple comparisons of means.

RESULTS AND DISCUSSION

The diameter of xylems and the number of xylary rays for four species of *U. campestris, U. glabra, Z. carpinifolia,* and *C. australis* were measured (Table 1). The analysis of the data obtained indicates that there was a significant difference at 1% probability level between different species of elms in terms of the diameter of xylems so that *U. campestris* had the greatest diameter and after that, *Z. carpinifolia, U. glabra*, and finally, *C. australis* have the smallest diameter (Table 2, Figure 1, Figure 3). The results also indicated that there was a significant difference at 1% probability level between different species of elms in terms of the number of xylary rays so that the maximum and minimum number of xylary rays belonged to *U. campestris* and *C. australis*, respectively (Table 3, Figure 2).

The results of this research are consistent with the works of other researchers (Safdary and Golchinfar, 2011; Iraqi and Rahnama, 2008). Because of the smaller vessels in resistant elm, the fungus could pass through the additional pores. Also, resistant elm has smaller

Table 2. Analysis of variance table of vessel diameter in species studied

Parameter	Sum of squares	df	Mean square	F
Between groups	9583.000	3	3194.000	9621689.015**
Within groups	0.032	96	0.00	
Total	9583.000	99		

**Significant at 1% level (P=0.01)

Table 3. Analysis of variance table of the number of xylary rays in species studied

Parameter	Sum of squares	df	Mean square	F
Between groups	294.000	3	98.000	714.000**
Within groups	13.000	96	0.00	
Total	308.000	99		

**Significant at 1% level (P=0.01)

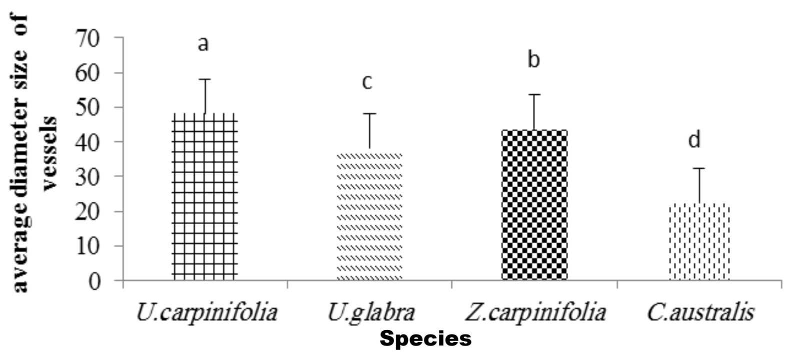

Figure 1. comparison of vessel diameter average in the species studied

groups of vascular bundles that are separated by paranchyma tissue (Solla and Gil, 2002). According to the susceptibility of *U. campestris* species and high resistance of *C. australis* species to Dutch elm disease, the results indicated that anatomical indices of vessels' diameter and the number of xylary rays could be one of the main factors in the incidence of degrees of resistance or host susceptibility to the pathogen in various species of elm. According to the studies, *U. carpinifolia* species, as compared to Chinese elm species, has a greater diameter and is highly susceptible to Dutch elm disease (Iraqi and Rahnama, 2008). In a study, Solla and Gil (2002) also showed that susceptible species of *U. campestris* (*U. minor*), as compared to the hybrid species resulting from crosses of *U. carpinifolia* and Siberian elm (*U. minor* x *U.pumila*), has a greater diameter (Solla and Gil, 2002). By doing pathogenicity tests, they also proved that three-year-old seedlings of the mentioned elms, as compared to two-year-old seedlings, cause more withering due to bigger vessels.

Moreover, in a study, it was found that there is a strong direct relationship between the simul-taneity of the forma-tion of vessels with greater diameters, appearance of beetle pathogens which mainly happens in late spring and early summer, and susceptibility of trees (Solla et al., 2005a). The research also shows that there is less structure of xylary rays (regions with rays) in resistant elms. Therefore, the nutrients needed for fungal growth are less available for the pathogen (Martin et al., 2009). According to the Quellette et al. (2004) findings, formation tyloses in elm prevent the movement of the fungus to other elements. In addition to the effect of xylem vessel diameters on disease progress, other hydraulic xylem aspects might be considered when discussing DED pathogenesis. Size of vessels is determined not only by diameter of vessel elements but also by their length. Moreover, the proportion of vessels with large diameters can favor xylem invasion by the fungus along the sap flow and deter the tree's ability to compartmentalize the disease (Solla et al., 2005b).

Therefore, the role of xylems in providing different levels of hosting for Dutch elm disease cannot be ignored because many studies have shown that even some populations within a species of elm family that are, to some degree, resistant to different isolates of Dutch elm disease pathogen have also smaller vessels as compared pared to susceptible populations of the same species (Solla and Gil, 2002). Therefore, the results show that

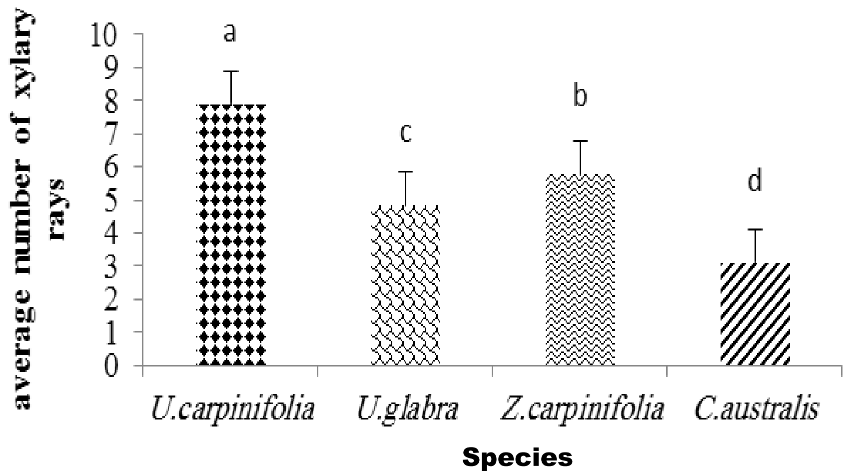

Figure 2. comparison of the number xylary rays average in the species studied.

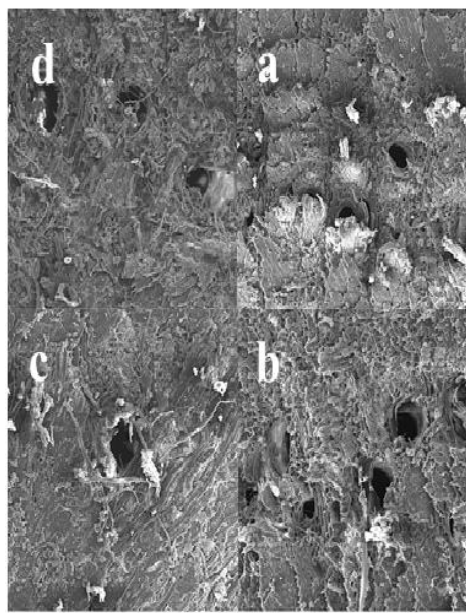

Figure 3. comparison of vessel diameter in a) C. australis, b) U. glabra ,c) Z. carpinifolia d) U.carpinifolia- the cross section (200micrometer).

in forested and urban areas, resistant ecotypes of susceptible species, such as *U. carpinifolia*, *Z. carpinifolia*, *U. glabra*, and *Ulmus* densa (a resistant variety of *U. carpinifolia*) and *C. australis*, which are both completely resistant to Dutch elm disease, can be identified and selected by measuring these indices without doing pathogenicity tests in the early stages of selecting ecotypes.

The results of this research can lead us towards the best method of managing this disease, that is, identification and propagation of species and varieties resistant to the disease.

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