American-Eurasian J. Agric. & Environ. Sci., 15 (3): 380-387, 2015
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2015.15.3.12547

Spatial Pattern and Disease Severity of Charcoal Canker in Hyrcanian Forests, North of Iran

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Abstract: In recent years, charcoal cankeraffecting the oak species (*Quercus castaneifolia*) in northern forests' and (*Quercus persica*) in Zagros forests' of Iran.This research investigated the population density of diseased trees and interaction across the severity of disease symptomatology onQ. Castaneifolia in the Quroq Park Hyrcanian Mixed Forests of Iran. We used two-dimensional spatial analysis tools with data gathered in point-centered-quarter format in 2013 to quantify the population density of diseased trees to examine the spatial pattern of tree mortality and investigated co-occurrence severity of disease symptoms using paired quadrat covariance analysis. Eventually, the percent of dead crown (total number of dead branches), depth, area and canker number in 400 trees were recorded in seven transects. Results showed that nearly 60 % of the oak trees in the park are diseased, as for the severity of disease oak trees with high probability 36% of oak trees recorded in this study offing death. Dead trees and high disease severity were strongly clustered at broader scales (800 m), trees with only a limited bleeding and signs of fungal activity *Biscogniauxia mediterranea* occurred in clusters away from dead trees. Crown mortality and Beetle-infested trees co-occurred with mortality were strongly correlated. These findings suggest that clustered spatial patterns of charcoal cancer can be important for the control and management of this disease in oak forests.

Key words: *Quercus castaneifolia* · Spatial pattern · Charcoal canker · Two-dimensional spatial analysis

INTRODUCTION

In the Mediterranean area, the main risk seems to be related to a potential reduction of precipitation and the consequences of this is more susceptible to opportunistic organisms such as fungal pathogens in making trees [1]. Among these, *Biscogniauxia mediterranea* is well known as the causative agent of charcoal canker in oak and is a serious problem in oak forest [2-5]. Oak charcoal cankercaused by B. mediterranea is a common disease of oak worldwide. *Biscogniauxia mediterranea* is common cause of charcoal canker diseases and involved in the massive decline of Quercus spp. and other trees in forests of Europe, North America, Africa, New Zealand and Asia [2,4,6,7]. Despite the numerous studies that have been done for identifying the pathogen, but few studies have attempted to characterize the spatial pattern of this disease and which remains one of the most challenging aspects of studying this disease.

The analysis of plant diseases spatial patterns provides knowledge into the spatial characteristics of epidemics and allows biological and environmental hypotheses to beproposed to account for the associations among diseased plants. Spatial analysis of plant diseases has been used to describe spatial pattern propose improvements in management and control methods [8], identify some factors that influence the spread of plant diseases, [9] or improve sampling strategy [10,11].

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Inthisregard, [12], investigated spatial pattern dynamics of oak mortality affected by sudden oak death and the results oftheirresearch showed that early in the study period, dead trees were strongly clustered at smaller scales and after three years this clustering was less pronounced. Also, [13] used spatially explicit neighborhood models to predict the abundance of soil-borne pathogen and showed the pathogen abundance in the forest soil was not randomly distributed. [14] were implemented the bivariate point pattern analysis to examine thespatial patterns of beech thicket formation and beech bark disease spread.Their results indicatedthat disease severity of beech saplings was highest in close proximity to highly cankered canopy beech trees.

In 2011, the first reported of the charcoal cankeron *Q. castaneifolia* trees that represent a new host of *B. mediterranea*, in the Golestan forest in northern Iran. A few years after the first report, large scale outbreak in oak forests, especially in the forest parks clearly is visible. Nevertheless, we still do not know rate of oak mortality and density of tree infections in oak forests. We do not know how the charcoal canker disease extends its range and have no ability to predict which stands of trees are at high risk of infection.

This work uses data gathered from ''point-centered-quarter'' [15] transects to First quantify the population density of trees with different disease symptoms across the study area and second to analyze the spatial patterning of the disease to determine the scale of clustering of symptoms across space, the degree to which severity of disease symptomstended to co-occur in the forest.

Thus, the overall objectives of this study are as follows: (1) to quantify the population densities of trees with different degrees of disease symptoms (disease categories) across the study area,(2) to determine the severity of co-occurrence of the crown and stem symptoms and (3) to determine the extent of clustering of the disease categories.

MATERIALS AND METHODS

Study Area: As shown in Figure 1, the study area is located at the Forest Park Qoruq (FPQ) in Golestanprovince of Iran (latitude = 54°43'00“, longitude = 36°52'00“). The park is about 600 ha in size, with elevations ranging from 120 m above sea level, to 600 m, have is north aspect (Fig. 1). Average slopes in the park are around 0-20% and extreme slopes can approach 50%. This predominantly north-facingslopeshas a semi-Mediterranean and temperate humid climate type characterized by rainy and temperate winters and dry season in July, August and September happens. Based on the composition, abundance and canopy cover, the forest trees are including oak in elevation ranges from 120 to 600 m above sea level, With70% coverage, hazel-hornbeam from 120-160, With25% coverage hazel at 120-140, With2.6% coverage hornbeam- hazel at 120-160 meters, With2.4% coverage are deployed. Additional overstory species include oak (*Q.* castaneifolia), hornbeam (*Carpinus betulus*), Persian ironwood (*Parritia persica*), Caucasian elm *(Zelkova carpinifolia)*, Persian maple (*Acer insigne*) and White Poplar (*Populus alba*). The understory is comprised of shrubs and small trees, including hawthorn, (*Crataegus monogyna*, *C. ambigua*), medlar (*Mespilus germanica*), Pomegranate *(Punica granatum)*, Blackberry (*Rubus persicus*), Butcher's Broom (*Ruscus hyrcanus*) and catbriers (*Smilax excelsa*).

Sampling of Oak Trees *(Q. Castaneifolia* Sp.) Were Collected: The advantage of using plot-less methods rather than standard plot-based techniques is that theytend to be more efficient. Point-centered quarter method is faster, requiring less equipment and may require fewerworkers. However, the main advantage is speed. The point-centered quarter (PCQ) method of transect data collection is one of the most popular vegetation sampling strategies [16,12]. Therefore, we collected data using the PCQ method in FPQ in the summer of 2013. with ArcGIS, Parallel linear transects were established approximately 200 m apart, vertically from top to bottom, which served as the anchor point for the seven transects. Sampling center points (nodes) were located every 100 m on each transect, with the first node starting 200 m from the anchor. Transects ranged from 4 to 22 nodes in length. The location of each center point was recorded with a global positioning system (GPS) device. At each node, cardinal compass directions defined four quadrants. The closest oak (>7.5 cm dbh) in each quadrant was labeled with a number, crown cover mortality percent, diameter at breast height (dbh) and disease status were determined. The distance from node center and azimuth of each tree were recorded. Only trees that were within a maximum distance of 50 m from a node center were recorded.

Symptomseverity Evaluation of Charcoal Canker: Visual disease symptoms for each of the trees [Oak trees *(Q. castaneifolia* sp.)] at each node were evaluated and recorded. Charcoal cankerdisease severity

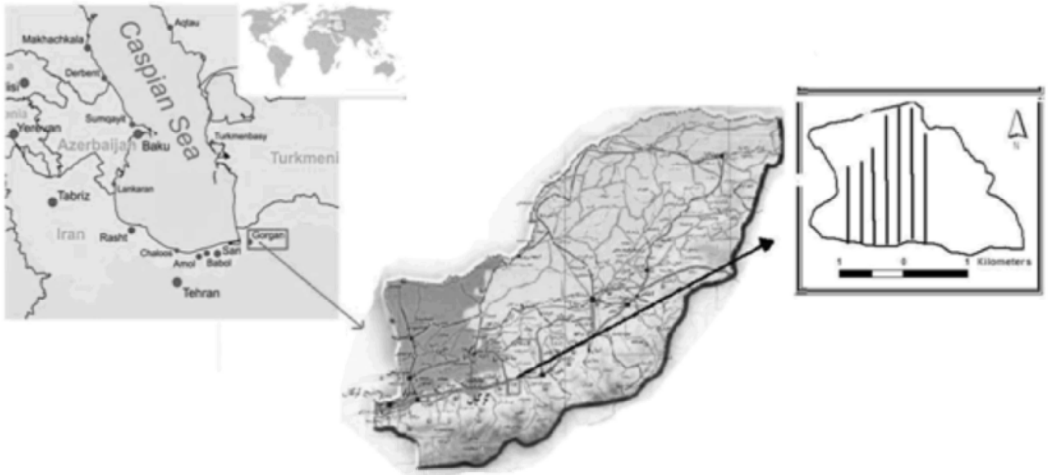

Fig. 1: Qroq Park study area in Gorgan province, Iran and showing transect locations

Table 1: Damage severity classification based on the morphological disease symptoms and characteristics of tested Oak trees (Q. castaneifolia sp.).

Classifieds*	Crown mortality percent in damage classes	Disease symptoms on tree trunk
1	1-5	asymptomatic
2	6-20%crown mortality	Only a limited bleeding and signs of fungal activity B. mediterranea were found.
3	21-35%crown mortality	Bleeding and Cankerdevelopment; Twigbeetleshave causedlightdamage.
4	35-55% of the crown mortality is sparse andweak.	Bleeding, Canker development, part of the trunk bark isolated, deep wounds, and range of wood-eating beetle activity increased and the tree cannot restore your health and vitality.
5	56-100% crown mortality	The trees are dead or dried and drying is and on more than 60 percent of the evidence trunk Twig beetle activity was observed.

Classification followed the method described by [17]

on individual trees was ranked from 1 (no disease) to 5 (dead) based on stem health and crown loss. Table (1) includes of 1: asymptomatic; 2: Only a limited bleeding and signs of fungal activity *B. mediterranea* were found. 3: bleeding plus beetles; 4: bleeding plus beetles plus deep canker and wood-eating beetle activity has increased range; 5: dead [17,12]. We analyzed the data from the PCQ method in two ways: estimated the population density of trees across the study area and then examined the spatial pattern of crown mortality and associated symptoms.

Population Density Estimation: The population density of oak trees at each of five different symptom severity was estimated from the PCQ data in three steps. First, the mean of all node center-to-plant distances (the mean distance from the center node to all four sample trees) was calculated for all sample points, regardless of symptom, using the following method [16]:

$$\sum_{i=1}^{n}\sum_{j=1}^{4} R_{ij}$$

Where R_{ij} is the point-to-tree distance at point *i* in quarter *j, n* isthe number of sample points along the transect, *4n*is the number of samples one for each quarter at each point, *i* isa particular transect point, where i = 1,...n, *j*isa quarter at a transect point, where j = 1,...4.

$$\bar{r} = \frac{\sum_{i=1}^{n}\sum_{j=1}^{4} R_{ij}}{4n}$$

Where

$\tilde{r}$ is mean distance.

$$\hat{\lambda} = \frac{1}{\bar{r}^2}$$

Where $\tilde{\lambda}$ is absolute density (trees/m^2).

Finally, the population density of oak trees at a particular symptomseverity level is calculated as the product of the overall population density $\tilde{\lambda}$ and the percentage of trees at that symptomseverity level. Thus, the population density of symptom severity level *i* was calculated by:

$$\widehat{\lambda_i} = \frac{\text{Quarters with symptom severity level } i}{4n} \times \hat{\lambda}$$

Where $\tilde{\lambda}_i$ is absolute density λ_i of symptom severity level *i* relative density of each symptom severity level is the percentage of the total number observations of that symptom severity level,

$$q_i = \frac{n_i}{n} \times 100\%$$

Where q_i is relative density of each symptom severity level *I*, n_i is sample size of trees at symptom severity level *i* (*i* = 1, 2, 3, 4, 5) and *n* is the overall sample size.

Spatial Pattern Analysis of Mortality: Methods of descriptive spatial pattern analysis (SPA) allow the online community to use a continuous transect samples which are quadrate. In the quadrate continuous transect for each species or ranking of diseases in a population or species characteristics (canopy cover, biomass) required to be recorded. Quadrat variance methodswhich include paired quadrate variances (PQV), ‘‘two-term local quadrat variance’’ (TTLQV) as well as 3TLQV and others, are used predominantly for one-dimensional regularly-spaced transect data. Since the blocks are contiguous, block size is identical to the distance among block centers. The idea is that a peak in variance on this graph will indicate maximum contrast between patch and gap, if there is a stationary process with constant cluster size, at a distance equivalent to approximate cluster size. [18,19,12]. Our original transect data were collected using a PCQ sampling method, where each transect consisted of a number of nodes with four observations recorded at each node’s four quarters. In order to apply the quadrate variance methods to the transect data for detection of the scale of disease clustering, we adjusted the PCQ transect data in two steps: first, we treated the nodes along each transect as the centers of the quadrates and the width of quadrates is determined as the maximum point-to-plant distance. Second, the quarter-wise observations were converted to quadrate-wise observations by replacing the four-quarter records with the mean or maximum value for each node. We applied two quadrat variance analysis methods including two-term local quadrat variances (TTLQV) and three-term local quadrat variance (3TLQV), to the adjusted transect data. Unlike earlier methods, TTLQV is not restricted to detecting pattern on a scale of 2n blocks (Hill 1973). The two methods were defined in the following formulas [20,21]:

$$3TLQV(r) = \frac{\sum_{i=1}^{n+1-3r}\left(\sum_{j=i}^{i+r-1} x_j - 2\sum_{j=i+r}^{i+2r-1} x_j + \sum_{j=i+2r}^{i+3r-1} x_j\right)^2}{8r(n+1-3r)}$$

$$TTLQV(r) = \frac{\sum_{i=1}^{n+1-2r}\left(\sum_{j=1}^{i+r-1} x_j - \sum_{j=i+r}^{i+2r-1} x_j\right)^2}{2r(n+1-2r)}$$

Where xj is the observation on the j-th quadrat; n is total number of quadrats; r is the scale parameter, which is the integer-times of the quadrat size. The peaks of the variance in 3TLQV, TTLQV and PQV can be interpreted as the scale of the pattern. In our case, the peak in each graph represents the size of the clustering and the height of the peak indicates strength of clustering.Paired quadrat variances is mathematically identical to a one-dimensional variogram analysis, detecting the scale of the pattern under investigation [22]. Just as the variogram can be extended to a cross-variogram for covariance analysis, the PQV can also be extended to a paired quadrat covariance (PQC) in order to study the co-variance among oak trees at different symptom severity. To do this, the PCQ transect data were adjusted as follows: for each symptomseverity level, the quadrat value at each node was recorded as 1 or 0 (presence or absence of a symptom). This generated five binary transects of quadrats, each of which corresponded to one symptom. The PQC is similarly defined in the formula:

$$V_{PQC}(i,j,r) = \frac{\sum_{k=1}^{n-r}(x_{i,k} - x_{i,k+r})(x_{j,k} - x_{j,k+r})}{2(n-r)}$$

Where x_i, k and x_j, k is the presence/absence values on the k-th quadrat for symptom severitylevel i and j, respectively; n is total number of quadrats; r is the scale parameter, which is the integer-times of the quadrat size. We used the formulae above to analyze data along the longest transect in our study area.

RESULTS

Population Density Estimation: In total, data from 400 trees with an average diameter of52/55 cm with a diameter range of 15-140 cm in seven transects with 102 nodes, were registered. The mean node center-to-plant distance (for all oaks) is 17.86± (11.86) m and the population density of all oak trees in the study area is 32.2 trees/ha. The analysis results showed that severity of disease symptoms and mean (trees/ha) was similar in the all transects and the differences were notsignificant.

The tree density characteristics mean that disease state of oaks is reported for all transects in Table 2. The population density of trees at different symptom severityis reported in Table 2 and 3, the results showed that number, percentage and density of trees with all symptoms, absolute frequency and density, (trees/ha),

Table 2: Tree density, mean (trees/ha) and the mean grade of severity of disease symptoms

Transect	No. of nodes	Mean(trees/ha)	Standard deviation	Minimum-maximum	Mean disease state ± (standard deviation)
1	14	17.55	12.63	1.4-53	2.66±1.09a*
2	14	18.61	10.32	1-38.8	1.87±1.21a
3	17	17.38	9.96	3.7-49.48	2.40±1.4a
4	22	19.65	11.53	1.7-47	2.42±1.7a
5	16	17.28	12.75	1-56	2.2±1.48a
6	15	19.11	11.43	2.26-53.5	2.23±1.35a
7	4	21.40	14.47	3-52	2.53±1.65a

Table 3: Density and abundance of trees in different disease severity

Damage classification	1	2	3	4	5
Frequency	166	102	50	38	56
Absolutely frequency	76.3	57.7	35	26	35
Relative density	40.2	24.65	12.2	9.35	13.6
Absolutely density (tree/h)	12.9	7.9	3.9	3	4.4

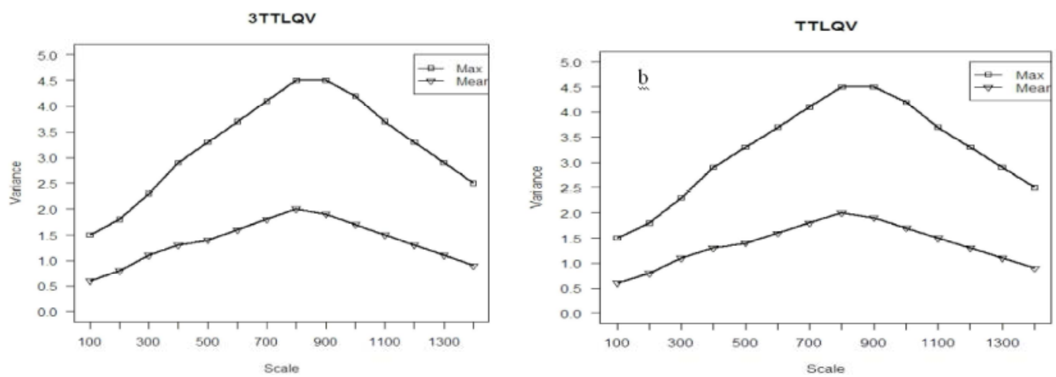

Fig. 2: Result of transect analysis for clustering of overstory mortality: a TTQLV analysis of the longest transect using the mean symptom severity from each node and the maximum symptom severity from each node; b 3TQLV analysis of the longest transect using the mean symptom severity from

trees without symptoms (1), are much higher than other treesand trees with 31-45% of the crown mortality and Canker development who cannot restore your health and vitality arethe lowest. With increasing disease severity, frequency and density of diseased trees are reduced, but a special assembly oak dead (5) than other trees were observed (table 3).the results also showed that 60 percent of the trees are disease, 13.6 percent were dead. So that 21.5% of these trees cannot recover his health hand then dried in the near future.

Spatial Pattern Analysis of Mortality: The results from twomethods including TTLQV and 3TLQV of transect analysis are similar. The 3TLQV and TTLQV plots show strong clustering of crown mortality in 800 m, as shown in Figure 2. 3TLQV and TTLQV plots of both mean symptom severity number of 5 (trees from all four quadrants at a node had dead crowns) and maximum of 5 (at least one of the trees in the four quadrants at a node had a dead crown) showed strong clustering at 800 m, or in other words (Fig 2), distinguished clusters with large open spaces between overstory mortality clusters are available.

Shown in Figure 3, compared co-occurrence in space for trees with the symptoms: 1(asymptomatic) and 5 (trees are dead or dried), 2 (limited bleeding) and 5 (trees are dead or dried), 3 (Bleeding, Canker development) and 5 (trees are dead or dried), 4 (Bleeding, Canker development, part of the trunk bark isolated) and 5 (trees are dead or dried). Figure 3 shows a co-occurrence 1(asymptomatic) and 4 (Bleeding, Canker development, part of the trunk bark isolated), 2 (limited bleeding) and 4 (Bleeding, Canker development, part of the trunk bark isolated), 3 (Bleeding, Canker development) and 4 (Bleeding, Canker development, part of the trunk bark isolated), by using PQC analysis. The strength of the association with mortality is in relation to the ‘‘0’’ variance x-axis, where a strong association is positive and a weak association is negative. Strong association of symptom severity 4 (Bleeding, Canker development, part

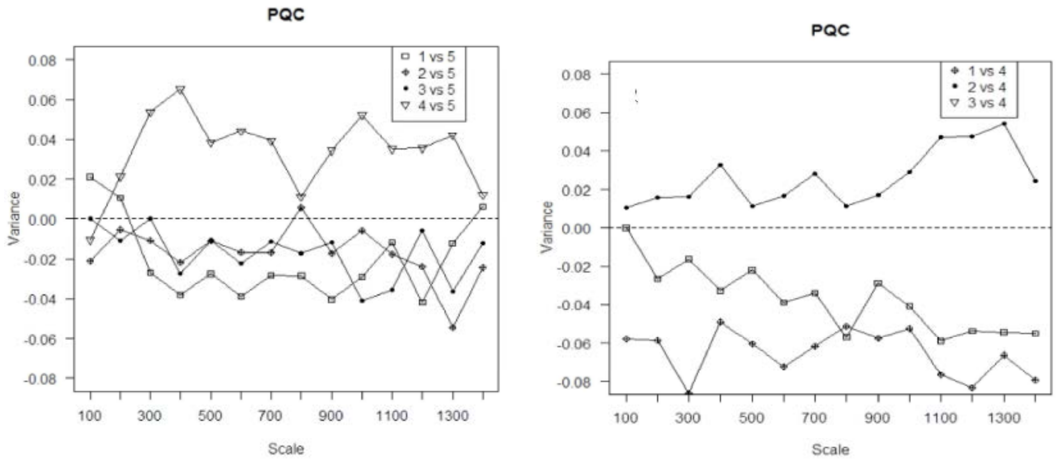

Fig. 3: Results of PQC: a: data comparing nodes with bleeding and mortality, symptom severity 5, and symptoms 4 and 5; b: data comparing nodes with symptom severity 1 and 4, symptom severity 2 and 4, and symptom severity 3 and 4.

of the trunk bark isolated), with mortality as tree was observed, (Fig.3, b), However, the association wasn't observed between dead trees and other trees.

Association of symptom severity 3 (Bleeding, Canker development) and with trees at 4 (Bleeding, Canker development, partof the trunk bark isolated) were observed, (Fig.3, a). However, the association wasn't observed between trees at 4 (Bleeding, Canker development, part of the trunk bark isolated) and other trees with Only a limited bleeding and signs of fungal activity *B. mediterranea* were found and asymptomatic trees. trees with bleeding, canker development, part of the trunk bark isolated was strongly correlated with dead trees across all scales and again trees with Bleeding, Canker development, twig beetles have caused light damage, with trees at 4 (bleeding, canker development, part of the trunk bark isolated) have a strongly correlated. Bleeding on trees occurred in clusters away from dead trees and the presence of *H. thouarsianum* was strongly related to overstory mortality through time [23].

DISCUSSION

This work provides an estimate of the density of symptomatic trees at different severity of disease across a forested landscape affected by charcoal canker. The results showed that 60% of the trees surveyed area is infected by the charcoal canker so that 36 percent of those with dry or very likely in the next few years will dry. According to these results, Forest Park Qoruq can be one of the foci of charcoal canker, in hardwood forests of northern Iran introduced because less than two years have passed since the identification and reporting of pathogen charcoal canker forest north of the country, so it has the spread and severity of disease. More than half of the oak trees in the study area were registered, brought to the mortality. More than half of the oak trees in the study area were infected to the disease or 26% of them are dry or drying up. Also our results indicate that disease severity of charcoal canker in the oak forest is not randomly distributed. Therefore, based on the results obtained, we can acknowledge that the in the earlier epidemic, dead trees were strongly clustered at large scales (800-1000) m, [12,14]. The results showed that the mean severity of disease symptoms in the study area is 2.37 and no significant difference in the transects. Uniform distribution of the severity of the disease, it may indicate keeping pace of destruction local biotic such as human [24] and pest (*Lymantria dispar)* and abiotic (drought) with charcoal canker [25,26]. There are many tensions in the area is likely to cause epidemics of the disease so that they will accelerate its expansion every day.

The results of relationship between severity of disease symptoms classifications of dead oak trees using paired quadrat covariance (PQC) showed that trees with high damage diseases have a strong association with dead trees so that healthy trees or low severity of disease severity were far from dead trees and the trees were not significantly associated [12, 27-29].Close relationship with severity of trees damaged and dead treesare perhaps indicative of the prevalence of disease in a specific location which may be due to differences in microclimate conditions and disease resistance of trees against each location [29-31]. Similar results have been reported in other plant ecosystems exhibiting passive pathogens dispersal, whereby the dispersion slope favor new infections closer to the source of inoculum [32,33]. However, interpretation of the role of fungi and insects, parasites and other biotic and abiotic factors in oak death are difficult because of many factors and interventions involved in oak mortality. Many of these organisms can cause physiological abnormalities or generally in duce mild symptoms that are not easily visible but enough to in duce handicapping trees and increase their susceptibility to infection with other parasites or poor environmental conditions are able. According to the results of this study revealed that the mean level of 800m clusters of trees with crowns dried to produce can be a good guide for identifying and mapping the oak mortality. The strong correlation between the severity of damage to trees and dead trees, dead trees may indicate that the focus of infection or disease, which provides optimal conditions for fungal growth and sporulation of *B. mediterranea* large inoculums to infest trees provide that place and trees near the center will be more pollution contamination.

The evidence presented in this study, new insights into the highly complex spatial distribution and co-occurrence severity of charcoal canker disease in oak forest.Because we have shown that the trees with high damage severity have closely related to each other. These findings might be useful in the control charcoal disease and restoration of forests affected by pathogen, which oak decline was cluster occurs. Specifically, these findings confirmed the results of research which stated that infected trees that have more than 15% lost their crown should becutandburnedto the groundnearestthe trunkand if possiblechump alsoberemoved andburnedfrom the increased pathogen inoculation and preventthe spreadof disease. Collectively,there these analyses suggest that strong correlation between dead trees and spread to nearby trees and spatial pattern of the disease showed that foci of infection are likely to occur through contamination in the forest. Therefore, it is essentialto identifyfoci ofinfectionand should bemanagedtopreventthe outbreak. It is also necessary in areas where outbreaks are likely to be repeated inspections before the outbreak of infection foci in the forest to prevent.

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