Phytopathologia Mediterranea
The international journal of the Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

OPEN ACCESS

Citation: Issa T., Almadi L., Jarrar S., Tucci M., Buonaurio R., Famiani F. (2019) Factors affecting *Venturia oleaginea* infections on olive and effects of the disease on floral biology. *Phytopathologia Mediterranea* 58(2): 221-229. doi: 10.14601/Phytopathol_Mediter-10610

Accepted: January 19, 2019

Published: September 14, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Juan A. Navas-Cortes, Spanish National Research Council (CSIC), Cordoba, Spain.

Research Papers

Factors affecting *Venturia oleaginea* infections on olive and effects of the disease on floral biology

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Summary. The disease olive peacock eye was evaluated in organic olive orchards in Palestine (Asira, Burquin, Qabatia and Sir). In each orchard, six trees were randomly chosen (three pruned and three unpruned). Severity was estimated at 20 d intervals by determining the percentage of infected leaves in the upper, mid and lower parts of the canopy of each tree. Proportions were estimated of symptomatic leaves, and those with asymptomatic infections using the NaOH test. In spring, inflorescence length was determined just before flowering (white stage). The proportions of fruit set were also calculated on small, labeled branches. Among the different sites/orchards tested, there were significant differences in the infection level, which were related to climatic conditions. Pruning reduced the susceptibility of the trees to the disease. The infections were much greater in the lower parts of the canopy. Peacock eye greatly reduced inflorescence length and fruit set. *Venturia oleaginea* severity was negatively correlated with inflorescence development and fruit set, due to defoliation caused by the pathogen. Based on effects on fruit set, the disease could severely reduce olive yields. This is the first report showing quantitative relationship between olive defoliation caused by peacock eye and reproductive activity.

Key words. *Olea europaea* L., organic olive growing, pruning, peacock eye, *Spilocaea oleaginea*, Palestine.

INTRODUCTION

Peacock eye (olive leaf spot), caused by *Venturia oleaginea* (Castagne) Rossman & Crous (= *Spilocaea oleaginea*), provokes severe damage to olive in all olive-growing countries. In recent years, the incidence and severity of this disease have increased in Palestine, likely resulting from climate change (Salman *et al*., 2011). Peacock eye is considered the most important disease attacking olive trees in Palestine, where olive production is very important, contributing approx. 13% to the national income (Salman *et al*., 2011; Abuamsha *et al*., 2013; UNCTAD, 2015).

Venturia oleaginea mainly attacks olive leaves, sometimes fruit peduncles and rarely tender shoots and fruits (Agosteo and Schena, 2011). The first symptoms of the disease are small, roundish, black spots, which may be arranged in concentric circles with an olive-green to dark olivaceous centre and peripheral rings with reddish-brown to yellow or green halos (Agosteo and Schena, 2011). The infected leaves fall prematurely and defoliation negatively affects the vegetative and reproductive activities of the trees (Graniti, 1993). Tree susceptibility to the disease largely depends on environmental conditions (it is favored by high humidity of the air), plantation density, cultivar and leaf age (old leaves are less susceptible) (Graniti, 1993; Obanor *et al.*, 2008, 2011). Favourable conditions for disease development are temperatures between 15 and 20 °C and persistent rain. At these temperatures, duration of leaf wetness required for infections is the least. Progress of the disease is reduced by hot, dry conditions (Obanor *et al.*, 2008, 2011).

Although pruning is known to reduce humidity and shading within olive tree canopies, and consequently disease incidence and severity, no data are available on these effects. Therefore, the positive role of pruning for reducing peacock eye is hypothetical, but has never been experimentally demonstrated. Little information is available on the quantitative effects of factors that favour the disease, and on effects of the disease on the reproductive activity of the trees. Furthermore, no clear relationships are known regarding the effects of the disease on flowering, fruit set and final olive yields. It is known that the disease mainly attacks the lower parts of tree canopies, but no quantitative data are available to evaluate its distribution in the different parts of the canopies.

The aims of the present study were to: 1) acquire more knowledge on peacock eye in Palestine, and on environmental conditions which affect development and presence of the disease in different areas of the country; 2) establish and quantify the effects of pruning on the disease; 3) determine and quantify the distribution of the disease in the different parts of olive tree canopies; and 4) measure and quantify the effects of the disease on floral biology (inflorescence growth and fruit set). The acquired knowledge of factors affecting the development and severity of peacock eye will assist the definition of disease management strategies, particularly in relation to the different conditions occurring in olive producing countries of the Mediterranean area.

MATERIALS AND METHODS

This study was carried out in the West Bank, which is a landlocked territory forming the bulk of the Palestinian territories and the State of Palestine. The West Bank climate is characterized by long, hot and dry summers with no or little rainfall from the end of April-beginning of May to October. However, rainfall and temperature vary according to altitude and location (Lodolini *et al.*, 2016, 2017). Four representative organic olive groves were chosen. They were located in Jenin and Nablus, which are the two provinces with the greatest concentration of cultivated olive. The groves were chosen to represent the diversity in topography, altitude and climatic conditions (temperature, relative humidity, rainfall), which characterize the study area. Two orchards were located in sub-areas (Sir and Qabatia) which are known to develop low levels of infection, and the other two were located in sub-areas with high levels of infection (Northern Asira and Burqin). The altitudes of these four areas are: Northern Asira 120 m above sea level, Sir 178 m, Burqin 233 m and Qabatia 268 m (PCBS, 2014). The olive cultivar in all four orchards was Nabali, which is the most important in Palestine. The trees were adult, spaced at 7 × 7 m and trained to the globe system. The orchards were managed with two to three tillages each year and organic fertilization. There were no treatments applied to control peacock eye.

Some climatic data (temperature, relative humidity of the air and rainfall) were recorded at meteorological stations near the olive orchards. In each orchard, six trees were randomly chosen. Three of the trees in each orchard were subjected to pruning before starting the investigation, while the other three were not pruned. This was to allow evaluation of the effects of pruning on peacock eye disease development. On each tree, nine branches were selected as follows: three in the upper part of the canopy (the upper third), three in the middle part (the middle third) and three in the lower part (the lower third). For each branch selected, all of the leaves were counted, and peacock eye symptomatic leaves were counted. These observations were repeated at approx. 20 d intervals. At the same time, the NaOH test was used to determine asymptomatic disease in leaves collected from branches close to the labelled branches. From each designated part of the canopy, three samples (one per tree) of 20 leaves were used. These leaves were immersed in a solution of 5% NaOH at 60–70°C for 2–4 min. The leaves were then examined and the numbers of infected leaves (recognizable by the appearance of dark circular spots) was recorded. The data collected from the labelled branches and the determination of the amounts of asymptomatic infection on leaves were used to calculate the proportions of fallen leaves (defoliation), symptomatic leaves and infected asymptomatic leaves for each of the three parts of the canopy. The observations on leaves were carried out from January to the first days of May 2016, and only leaves grown in 2014 and 2015 were considered. In particular, the defoliation caused by the disease on the labeled branches was determined by counting the number of nodes on branches grown in 2014 and 2015. Because each node produces two leaves, it was possible to calculate the number of leaves present on each branch in the absence of leaf fall. Leaves survive for approx. 2.5 years, so it was hypothesized that the leaves that were no longer on the labeled branches had fallen mainly because of peacock eye. Therefore, fallen leaves caused by peacock eye were taken as the difference between the number of leaves derived from the multiplication of the number of nodes × 2 and the leaves that were present at the last sampling (effective defoliation). Because all the *V. oleaginea*-infected leaves fall, the total potential percent defoliation was calculated as the effective percent defoliation plus the percent symptomatic leaves plus the percent infected asymptomatic leaves.

The new leaves that developed from March of 2016 were not considered because of the short period from bud sprouting to the last sampling at the beginning of May.

In spring, the number of inflorescences on the labeled branches was determined, and approx. 1 month after flowering the number of fruits was also determined. This was to allow calculation of the ratio between the number of fruits and inflorescences, to give the amount of fruit set.

Immediately before flowering (when the inflorescences were white), three samples of ten inflorescences per tree were collected from each of the three parts of the canopy, and inflorescence lengths were measured.

Data were statistically analyzed by ANOVA according to a factorial experimental design (site/time of observation/pruning/part of the canopy), and the averages were compared by the Student-Newman-Keuls Test. Some data were also presented as simple linear regressions of some variables.

RESULTS

Climatic parameters of the areas studied

Some climatic parameters of the experimental sites are summarized in Table 1. Temperature (T) and relative humidity (RH) of the air were available only for the Northern Asira and Sir sites, whereas rainfall was available for all four sites. Northern Asira had lower T and higher RH than Sir. Asira and Burquin had the highest rainfall amounts.

Table 1. Climatic data of the experimental sites in Palestine examined in this study. Temperature and relative humidity values are means ± standard errors.

Area	Average air temperature (°C, Dec 2015 – May 2016)	Relative humidity of the air (%, Dec 2015 – May 2016)	Cumulated rainfall (mm, Sept 2015 – May 2016)
Northern Asira	15.1 ± 1.9	71.9 ± 4.7	560.7
Burqin			493.5
Qabatia			409.8
Sir	18.1 ± 2.1	61.7 ± 4.3	409.2

Amounts of leaf infection

Data on the effects of the factors (site/environment, time of observation, pruning, part of the canopy) on the amounts of leaf infection were statistically analyzed using a factorial design. There were statistically significant effects for all the factors, but significant interactions between factors were very few, so these are not presented.

Effects of the different sites

All the parameters used to evaluate the amounts of infection were significantly affected by the site/environment (Figure 1). The percentages of symptomatic leaves and infected asymptomatic leaves were greatest in Northern Asira, followed by much lower values in Burqin and then lower still for Qabatia and Sir. A similar trend was observed for effective and total potential defoliation. The amounts of effective and total potential defoliation were very high in Northern Asira and high in Burqin.

Effects of time

The percentage of symptomatic leaves decreased from the beginning of the experiment (January) to February, and then increased and reached a maximum in March before decreasing thereafter (Figure 2). A similar pattern was observed for the percentage of infected asymptomatic leaves.

Effects of pruning

Pruned trees had lower proportions of symptomatic and infected asymptomatic leaves than unpruned trees (Figure 3). A similar result was observed for both the

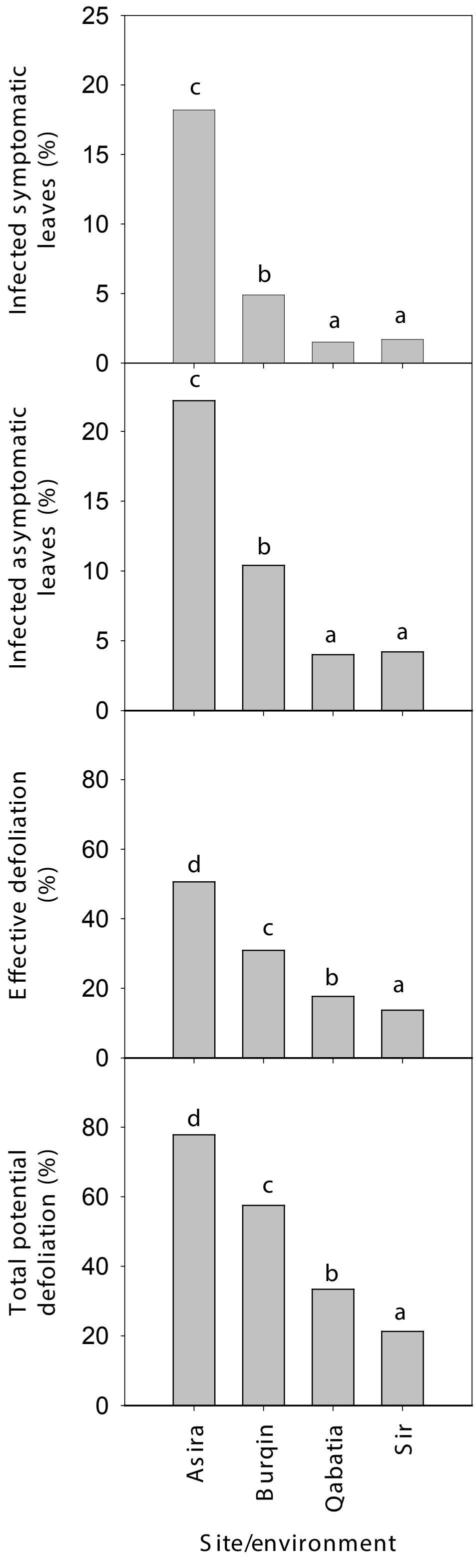

Figure 1. Means of olive tree defoliation and *Venturia oleaginea* infections as influenced by different sites/environments in Palestine. Means accompanied by the same letter are not significantly different ($P \leq 0.05$).

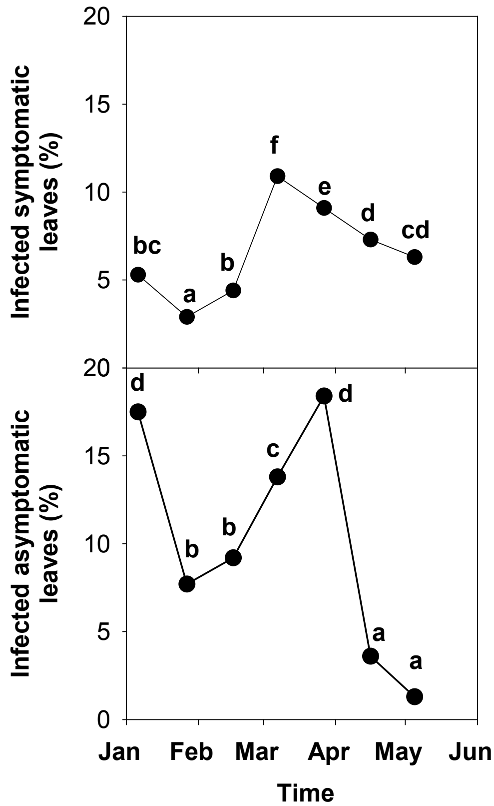

Figure 2. Mean proportions of olive leaf infections by *Venturia oleaginea* as influenced by the time of observation. Means accompanied by the same letter are not significantly different ($P \leq 0.05$).

effective and total potential defoliation proportions.

Effect of leaf position in the canopy

The proportions of symptomatic leaves and infected asymptomatic leaves were both affected by the position of the leaves in the tree canopies (Figure 4). The greatest infection percentages were always in the lower parts of the canopies and the least infection was in the upper parts. The greatest percentages of effective and total potential defoliation were in the lower parts of the canopies.

Floral biology and fruiting

All the factors considered in this study affected the mean length of the olive inflorescences (Figure 5). The longest inflorescences were found in Qabatia, whereas the shortest were in Northern Asira. Intermediate inflorescence length occurred in Burqin and Sir. The inflorescences were longer on pruned trees and in the upper parts of the canopies. Fruit set was significantly affected

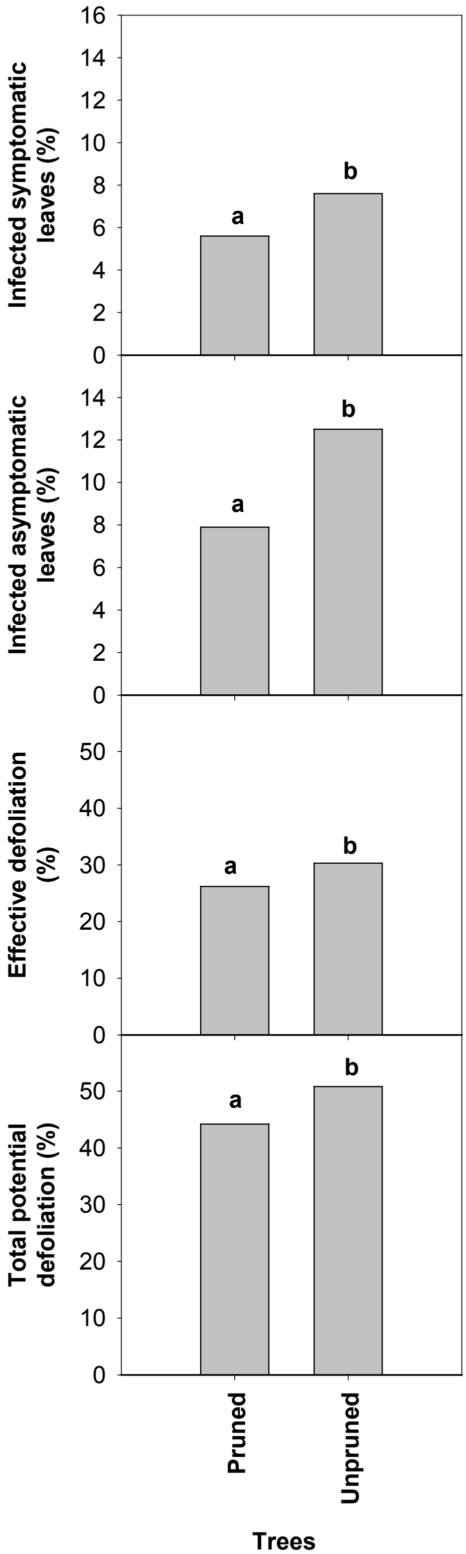

Figure 3. Means of olive tree defoliation and *Venturia oleaginea* infections as influenced by pruning. Means accompanied by the same letter are not significantly different ($P \leq 0.05$).

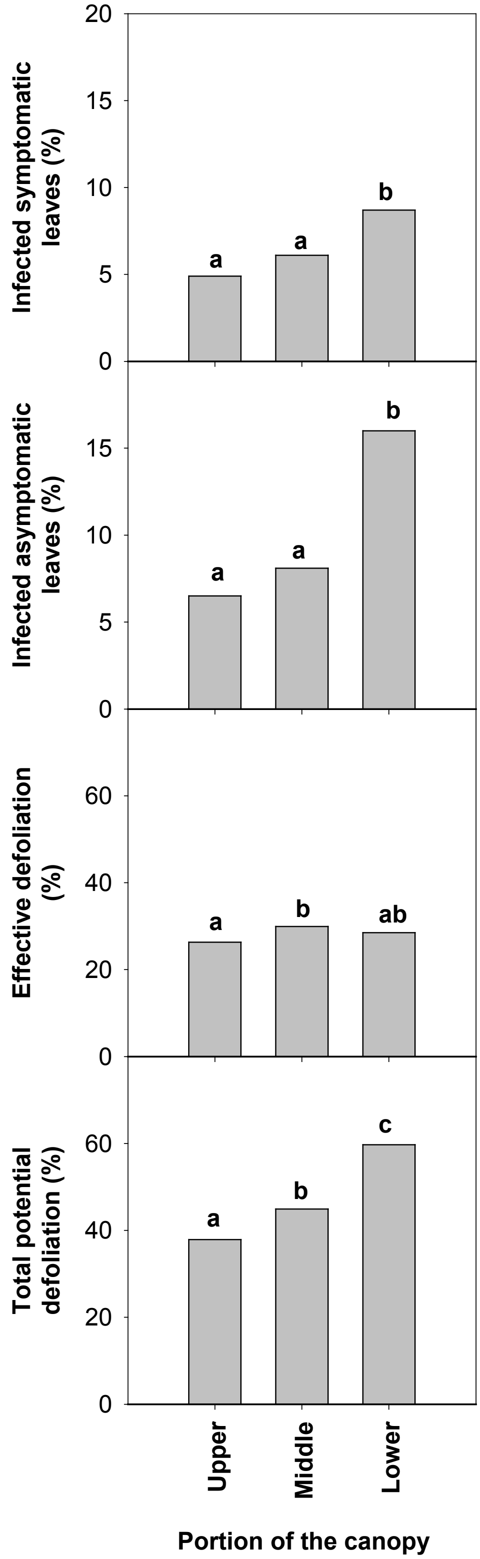

Figure 4. Means of olive tree defoliation and *Venturia oleaginea* infections as influenced by three different tree canopy positions (upper, middle and lower). Means accompanied by the same letter are not significantly different ($P \leq 0.05$).

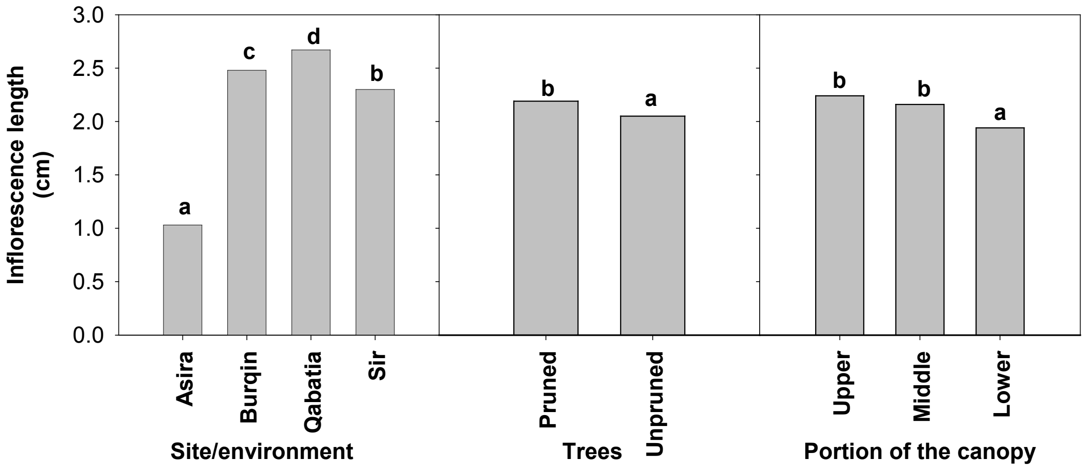

Figure 5. Mean olive inflorescence lengths as influenced by the site/environment, pruning and portion of tree canopy. Means of each factor accompanied by the same letter are not significantly different ($P \leq 0.05$).

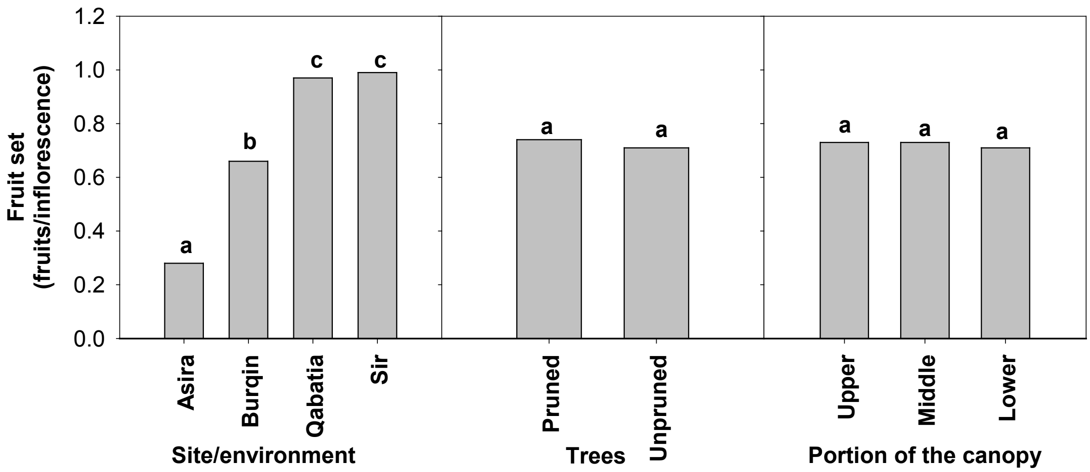

Figure 6. Mean fruit set values for olive trees as influenced by the site/environment, pruning and portion of tree canopy. Means of each factor accompanied by the same letter are not significantly different ($P \leq 0.05$).

by the site/environment, but was not affected by pruning or position in the tree canopies (Figure 6).

DISCUSSION

This investigation has shown a significant influence of all the considered factors on the severity of peacock eye occurring in olive orchards.

The effects of site/environment were most likely due to the different climatic conditions at the different experimental sites. In particular, Northern Asira, where the greatest amounts of infected leaves and defoliation were found, had the greatest rainfall and high values of RH, along with relatively cool temperatures. These conditions are particularly favourable for peacock eye development, as conidium production by *V. oleaginea* is optimal at 15°C, especially when humidity is high (Azeri, 1993; Guechi and Girre, 1994; Obanor *et al.*, 2008). In contrast, the lowest amounts of infected leaves and defoliation were observed at Sir, where the lowest rainfall and RHs, and the highest temperatures, were recorded. Using the rainfall data, which was available for all four sites, the important and positive influence of high humidity resulting from high rainfall on disease development was demonstrated. There was a significant positive linear correlation between the cumulated rainfall at the four sites and the levels of infection and defoliation that were recorded (Figure 7). For rainfall, the period

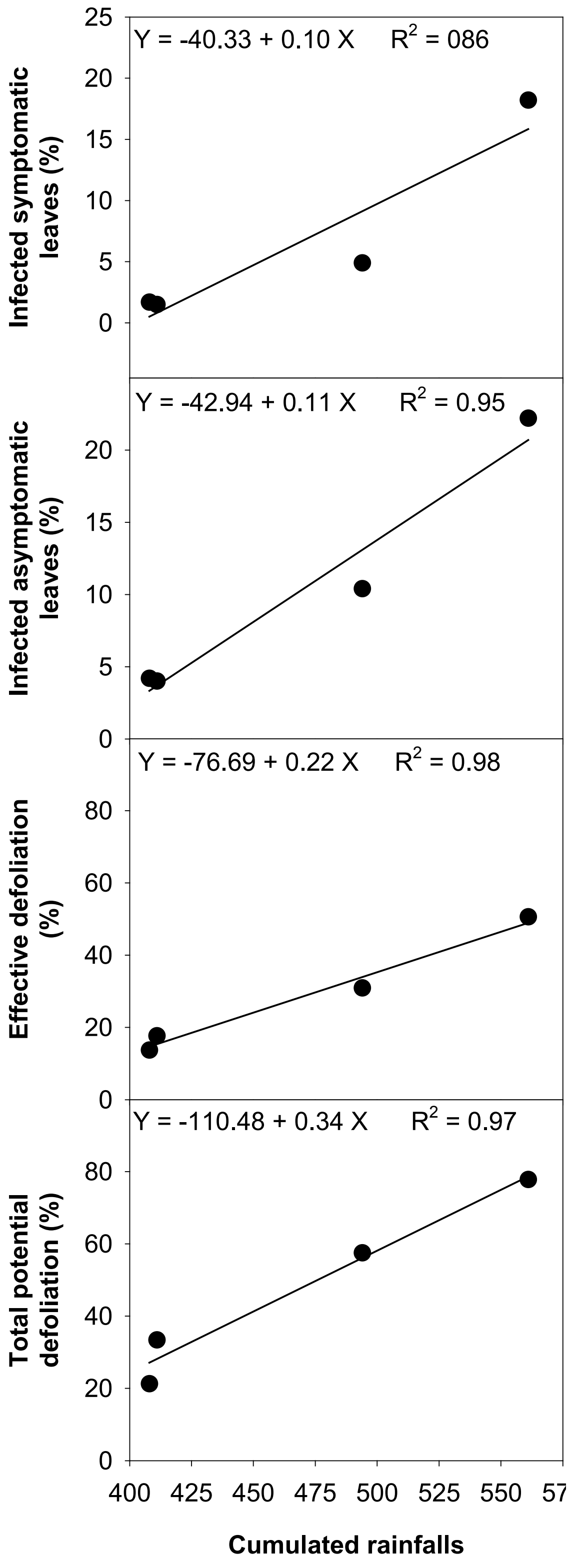

Figure 7. Relationships between cumulated rainfall in the four areas of Palestine (Asira, Burqin, Qabatia and Sir) and infections/defoliations caused by peacock eye of olive.

from September to May was considered as it is when most of the rain occurs and so it is representative of the climatic conditions of the area. Therefore, environmental factors, in particular RH and T, appear to be the major elements in determining differences in the infection and spread of peacock eye disease among the considered sites. Indeed, high humidity and relatively cooler conditions are the most suitable for the disease (Salerno, 1966; Saad and Masri, 1978; Graniti, 1993; Obanor *et al.*, 2008; 2011). These results are similar to the those of previous observations in Palestine, which showed high levels of *V. oleaginea* infection in Nablus where climatic conditions were similar to Northern Asira, and low levels of infection in Tubas where climatic conditions are similar to Sir (Salman *et al.*, 2011).

The trends of infected leaves, both symptomatic and asymptomatic, showed maximum values from the end of winter to beginning of spring. This is when very favourable temperature and humidity conditions support rapid development of peacock eye (Guechi and Girre, 1994). The subsequent decreases in the percentages of diseased leaves were probably due to losses of the infected leaves from the trees.

Lowest levels of infection were observed in pruned trees compared with unpruned ones. This can be explained by considering that thinning of the vegetation creates better conditions for lightening and aeration of the internal parts of the canopy, with a consequent decrease in RH, which is a very important factor in favoring leaf attack by *V. oleaginea* (Obanor *et al.*, 2008; 2011; Al-Jabi, 2013). However, even if the effect of pruning was supposed, the present study has given the first experimental evidence that pruning can reduce the severity of the peacock eye in olive.

Our results clearly showed gradients of peacock eye severity from the lower to upper parts of olive tree canopies. Amounts of infected leaves and defoliation decreased from the lowest to the upper parts of the canopies. This is likely to be because the lower canopy parts retain the greatest air humidities, while upper canopy parts have more light and are well-ventilated.

All the factors investigated in this study influenced the length of olive inflorescences. To determine if these differences were associated with pathogen infections, the relationship between the percentage of effective defoliation and inflorescence length was evaluated by using the average values of the combinations of the following factors: site/pruning/part of the canopy. A significant linear correlation ($R^2 = 0.66$) was found between these two parameters (Figure 8). This indicates that olive inflorescence length was related to the number of leaves and that the shorter length of the inflorescences in Northern

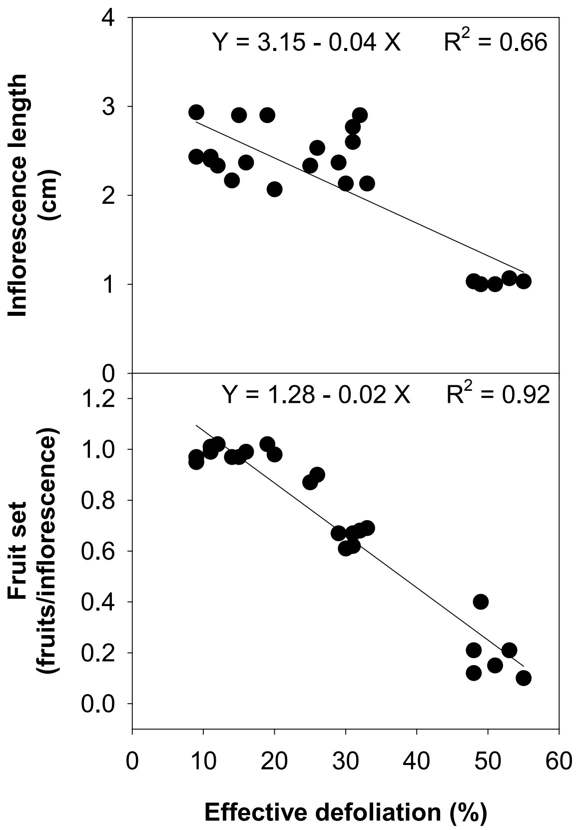

Figure 8. Relationships between effective olive tree defoliation caused by peacock eye with inflorescence lengths and fruit set. Each value represents the average of all the combinations of three factors (site, pruning, portion of canopy).

Asira, in unpruned trees and in the lower parts of the tree canopies were associated with the higher levels of defoliation caused by peacock eye.

The differences in fruit set can also be analyzed by evaluating the relationships between the proportions of effective defoliation and fruit set using the average values of the combinations of the following factors: site/pruning/part of the canopy. Again, a significant, negative, linear correlation (R^2 = 0.92) was found between these two parameters. This means that the differences in fruit set were also significantly associated with defoliation caused by the peacock eye disease. Examination of this relationship also indicates the effect of defoliation on the potential yield of the trees and thus the economic damage caused by the disease. Indeed, it is possible to see that depending on the level of defoliation, the number of fruits can be reduced up to one fifth (20%), when defoliation reaches values around 50%, compared to those obtainable in conditions of very low defoliation. This means that the impact of the disease on the potential production of the trees could be very high. Further investigations are required to determine the effects of peacock eye on olive fruit growth and possible fruit drop.

CONCLUSIONS

The results of this study indicate that variability of the environmental conditions in the different areas of Palestine is enough to determine differences in the susceptibility of olive to peacock eye attacks. Therefore, even though Palestinian Territories have a limited extension, there are significant environmental differences among the different areas, which determine significant changes in the susceptibility to peacock eye, which must be considered in applying site-dependent strategies for disease control.

Pruning can reduce the susceptibility of olive trees to peacock eye. Infection was generally greater in unpruned than pruned trees. To the best of our knowledge, this is the first experimental evidence showing that pruning has a positive role in reducing this disease. In Palestine the importance of olive pruning is often underestimated. Hence efforts have to be made to train farmers to apply this practice in an optimal/regular way, also taking into consideration its importance in controlling important biotic adversities, such as peacock eye disease.

There is a large gradient within olive tree canopies for susceptibility of leaves to *V. oleaginea*. Disease severity is much greater in lower than upper canopy parts. This information is important for optimization of disease control methods. Treatments must ensure uniform and complete coverage especially of the middle and lower parts of the tree canopies.

Peacock eye can reduce the lengths of olive inflorescences and fruit set. This is the first report showing direct and linear influence of defoliation caused by peacock eye on inflorescence length and fruit set, and these negative effects are associated with defoliation caused by the disease. Based on the effects of the disease on fruit set, it can be concluded that peacock eye can cause severe reductions in olive fruit yields.

The results of the present study increase knowledge of factors that affect the susceptibility of olive to infection by *V. oleaginea,* and of effects of defoliation caused by the pathogen on olive tree production. This information, besides being important for a better knowledge of the biology of peacock eye in Palestine and other Mediterranean countries, can be useful for the development of effective strategies for its control.

ACKNOWLEDGMENT

This paper was developed from the Master of Science thesis of Tahreer Issa.

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