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Hellenic Plant Protection Journal **15:** 80-86, 2022
DOI 10.2478/hppj-2022-0010

Biological parameters of grape phylloxera, *Daktulosphaira vitifoliae*, on local grapevine varieties in central Syria – Implications on their susceptibility

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Summary Biological parameters of phylloxera local strains as well as infestation were studied on Syrian grapevine varieties including Baladi, Salamone, Karawane, Hafarzale, Gharbe and the American resistance rootstock (ARR-B41) (*Vitis vinifera* x *Vitis berlandieri*). Root artificial infestation method revealed significant differences in phylloxera population between the majority of tested varieties comparing to the ARR-B41 rootstock. Hafarzale and Karawane have shown the lowest population of grape phylloxera, with a similar level of resistance to rootstock ARR-B41. Furthermore, Salamone and Gharbe are more conducive to grape phylloxera reproduction. Field investigation has shown significant differences between the local varieties and ARR-B41 in terms of the average number of galls "nodosities". Overall, Hafarzale and Karawane tended to form significantly fewer galls than the other varieties.

Additional keywords: B41 rootstock, galls, grape varieties, nodosities, phylloxera, tuberosities

Introduction

Syrian vineyards (*Vitis vinifera*) cover more than 70,000 hectares, producing about 540,000 tons of grapes annually (Makee *et al*., 2010). Since the end of the 19th century and the beginning of the 20th century, the grape phylloxera *Daktulosphaira vitifoliae*, Fitch (Hemiptera: Phylloxeridae), has spread worldwide through plant materials (Granett *et al*., 1985; Triska *et al*., 2018). In Syria, phylloxera was first discovered on the local grapevine variety Doumani in 1935 and caused catastrophe in the Douma region near Damascus. Subsequently, it invaded Syrian nurseries and quickly settled in Syrian vineyards (Balachawsky and Mesnil, 1935; Idris and Arabi, 2014).

Grape phylloxera is considered to be the most harmful pest to grapes. In Dara Province located in southern Syria, it was estimated that 82.5% of vineyards were infested with phylloxera (Al-Chaabi *et al*., 2012). Granett *et al*. (1996) reported that once a vineyard is infested with grape phylloxera, it is estimated to stop its production in about two to five years. Grape roots are damaged by phylloxera through the formation of flashy galls, "nodosities" on immature roots by the expansion of the root cortex, while on mature roots they are known as "tuberosities", and the root system weakens over time as a result (Kellow *et al*., 2004). These galls are used by phylloxera to extract stored nutrients, such as sugar and amino acids, and can support populations with high reproduction rates (Omer *et al*., 2002; Lawo *et al*., 2011; Idris and Arabi, 2014). Thus, the infestation of phylloxera destroys the root system of grapevine, while the new phylloxera type may adapt to resistant rootstocks (i. e ARR-B41) (Granett *et al*., 2001).

Several control methods including pesticides, radiation and soaking in hot water would be only useful in the isolation and treatment of quarantine grape phylloxera (Granett *et al*., 2001; Makee *et al*., 2008; Makee *et al*., 2010, Adam *et al*., 2012; Adam *et al*., 2013; Adam *et al*., 2021). Yet none of these methods are effective to control the insect. Grafting susceptible species onto American phylloxera-resistant rootstocks remains the most effective phylloxera control method

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(Fossen, 2005). Noteworthy, some resistant rootstocks have either lost their resistance or became less resistant to phylloxera after years of use (Granett *et al*., 1985). For example, the rootstock ARR-B41 which is still resistant in France, it is not resistant in California vineyards, creating and urgent need to replant new vines with a suitable resistant rootstock (Granett *et al*., 1983; De Benedictis and Granett, 1993; Makee *et al.,* 2004). In most cases, rootstocks usually restrict the growth due to incompatibility of roots between rootstocks and grafted vines, reducing the vigor of grafted varieties (Makee *et al*., 2010; Idris and Arabi, 2014). According to previous research, in Dara Province (southern Syria), 89% of grapevine varieties grafted onto American rootstocks resistant were incompatible (Al-Chaabi *et al*., 2012).

This study examined the population dynamics of grape phylloxera on five local grapevine varieties planted in central region of Syria and the resistant rootstock ARR-B41. Biological parameters of phylloxera, i.e., fecundity, egg laying period, development time of nymphs, number of galls "tuberosities", and survival rate of adults were considered. In addition, the number of galls "nodosities" located on the roots during the growing season were evaluated in the field.

Materials and Methods

Establishment of phylloxera colonies

Grape phylloxera was originally collected from the infested roots of local grapevine varieties in the middle region of Syria. Colonies of phylloxera were established in our laboratory according to protocols of previous work (Idris and Arabi, 2014).

Biological parameters of phylloxera and infestation in the laboratory

Healthy fresh roots with a diameter of 4-7 mm and a length of 5-7 cm from local grape varieties (Baladi, Salamone, Karawane, Hfalzale, Gharbe) and the American phylloxera-resistant rootstock ARR-B41 were collected from the field. Twelve roots of each variety were cleaned with tap water and wrapped separately with wet cotton wool. Ten phylloxera eggs from the laboratory colony were placed on each root (total of 120 eggs in each local grape variety and ARR-41B). Three to four infested root pieces were transferred onto a wet paper in plastic Petri dishes (diameter=12 cm, depth= 1 cm) as described by Makee *et al*. (2010) thus there were four replicates per variety. Ventilation was possible through a cloth–screened hole (1-1.5 cm) at the Petri dish lid. The dish edges were fixed with parafilm, kept in plastic boxes with firmly fitting lids, and brooded in incubator at 25°C and 75% relative humidity.

The infested roots were examined under a microscope for newly hatched nymphs, and the survival rate of adults were calculated using the following formula: % = (number of eggs hatched - number of nymphs that died/ number of eggs hatched) x 100. A total of 25 females were selected from the four replicates of each variety group (5 root pieces of each group X 5 females on each root) and the roots were then transferred to other plastic Petri dishes, held in plastic boxes with tightly fitting lids under the same conditions as described above. The following reproductive parameters were measured for each female: fecundity (number of eggs), oviposition (period during which eggs are laid), and developmental time (from egg to egg). Observations were made of the number of tuberosities that formed on the roots.

Infestation in field conditions

The experiment was carried out at about 12 kilometers north of Homs (Al Mukhtareyah: 34°80'42. 37" N 36°73'96. 97" E) (approximately 539 mm of rainfall during the growing season from November 10 to May 30. Stems of local varieties Baladi, Salamone, Karawane, Hafarzale, Gharbe and ARR- B41 were selected (n=228). After immersion in a 2000 ppm solution of Indole Butyric Acid (IAA) for 2 minutes, 48 stems of each variety were planted in 10-L plastics pots with sterile moist soil. Four months later, plants infested with phylloxera eggs (50 eggs per plant) were planted in the field, one square

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meter apart, line by line. In the following year, 12 plants of each variety were uprooted, root samples were collected randomly from each plant (about 100 cm), cleaned roughly on the spot and stored at 7°C for transportation to the laboratory. The number of nodosities was determined by a microscopic examination of each root.

Statistical analysis

The statistical analysis was done using the STATISTIC program version 6 (Statsoft, Inc. 2003). Data were subjected to analysis of variance at 5% significance level (P= 0.05). Data were transformed into square roots where appropriate. Significance of differences between means, were performed according to Tukey HSD test.

Results and discussion

Grape phylloxera infestation intensity is affected by environmental factors such as temperature, humidity, soil, grape variety and genotype (Makee *et al*., 2010; Powell, 2012; Hoffmann *et al*., 2016). The assessment of biological parameters is a good method of determining the grapevine root susceptibility to phylloxera (Idris and Arabi, 2014). The results showed significant differences between local grapevine varieties in Syria and the resistant ARR-B41 rootstock for biological parameters including, fecundity, oviposition (egg laying period), developmental time of nymphs, and number of tuberosities ($F= 441.35$, $df= 5$, $P<.005$; $F= 223.2$, $df= 5$, $P< 0.005$; $F=25.8$, $df= 5$; $P<0.005$; $F=55.6$; $df= 5$, $P<0.005$, respectively) (Fig. 1). These biological parameters were significantly lower in Akrawane and Hafarzale compared to the other local varieties. In contrast, on these varieties and ARR-B41, it took the same amount of time for phylloxera nymphs to reach adulthood. According, to the study by Makee *et al*. (2010) local varieties phylloxera reproduction abilities on their roots. The survival percentage of phylloxera adults on roots was not significantly different between the Hafarzale variety and ARR-B41, but it was low in comparison with the other local varieties ($F= 182.3$, $df= 5$, $P<0.005$) (Fig. 2).

The number of nodosities differed significantly between the local varieties and ARR-B41 in spring, summer and autumn ($F=179.08$, $df=5$, $P<0.0001$; $F=176.216$, $df=5$, $P<0.0001$; $F=102.96$, $df= 5$, $P<0.0001$, respectively), and Hafarzale was the variety with the lowest nodosities (Hafarzale, ARR-B41, Baladi, Gharbe, Karawane, Salamone). Root galls significantly increased in spring and summer but decreased in autumn (Fig. 3). In winter, none of the studied varieties formed nodosities on the roots (Fig. 3). There is a strong correlation between the adaptability of grapevine varieties to phylloxera reproduction and the number of nodosities (Lawo *et al*., 2011).

Grapevine genotype, virulence of phylloxera strains and growing season conditions, i.e., dominant temperature in winter, are key factors influencing the grapevine resistance to phylloxera. According to Hoffmannet *et al*. (2016), phylloxera forms galls on suitable vine roots due to the effect of two main parameters, the plant genotype and the growing season. No formation of root galls in the examined local grapevine varieties during winter indicates that local season conditions influence the insect distribution and lifespan. In central Syria, the temperature can drop to -10°C for many days during winter, and the mean temperature is 1-3°C for several months. These low winter temperatures may play a crucial role in preventing phylloxera from reproduction due to mortality from freezing (Korosi *et al*., 2012). In temperate climates, phylloxera can survive winter in high density on grapevine roots even on American resistant rootstocks (Hoffmannet *et al*., 2011; Hoffmannet *et al*., 2016).

Omer *et al*. (1997) suggest a reduced direct impact of environmental conditions, such as the impact of temperature on phylloxera's population dynamics. As noted by Eissenstat *et al*. (2005), the majority of root growth in grapevine occurs between spring and summer and the roots usually do not grow near the harvest time or the dormant

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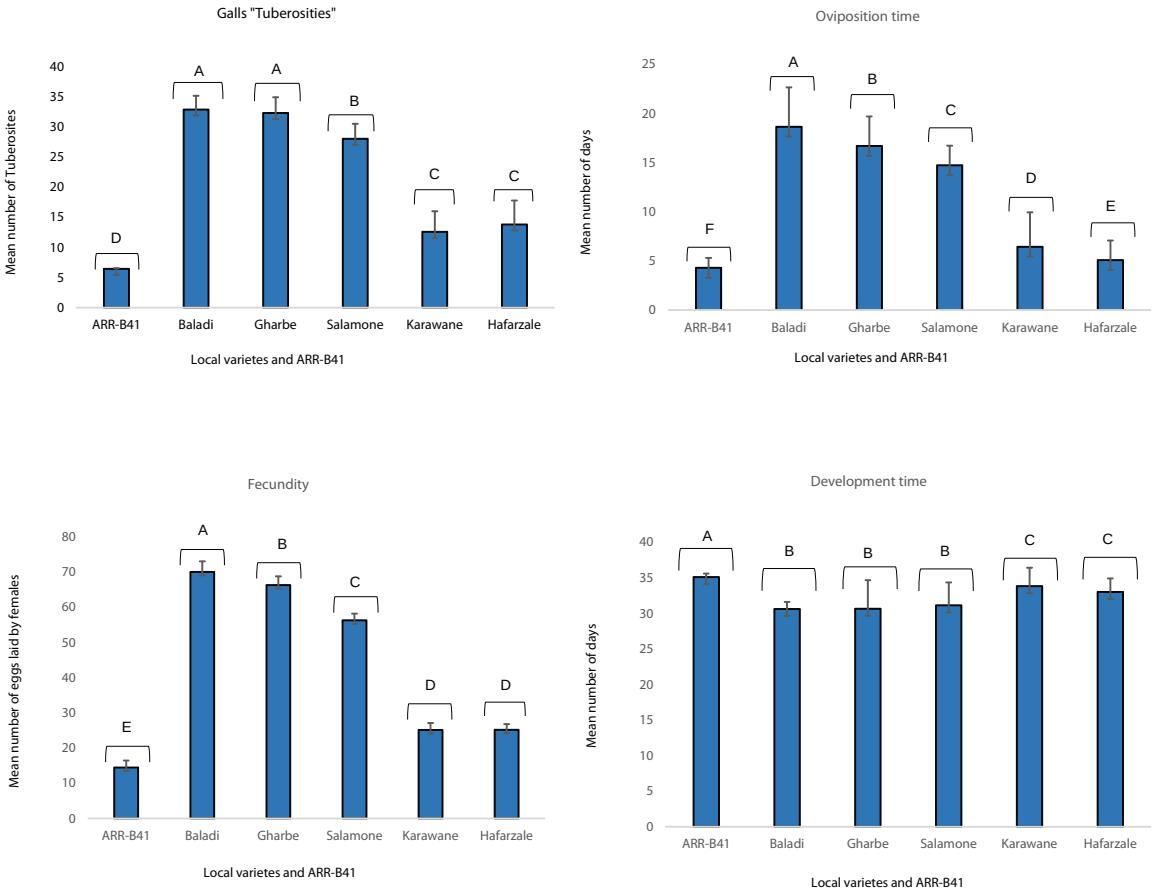


Figure 1. Biological parameters of phylloxera on local grapevine varieties in Syria and the phylloxera-resistant ARR-B41 rootstock. Different capital letters on columns indicate significantly different means ± standard deviation at $P < 0.05$ (Tukey HSD test).

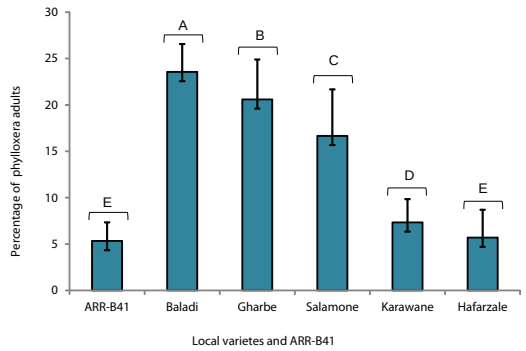


Figure 2. Survival percentages of phylloxera adults on local grapevine varieties in Syria and the phylloxera-resistant ARR-B41 rootstock. Different capital letters on columns indicate significantly different means ± standard deviation at $P < 0.05$ (Tukey HSD test).

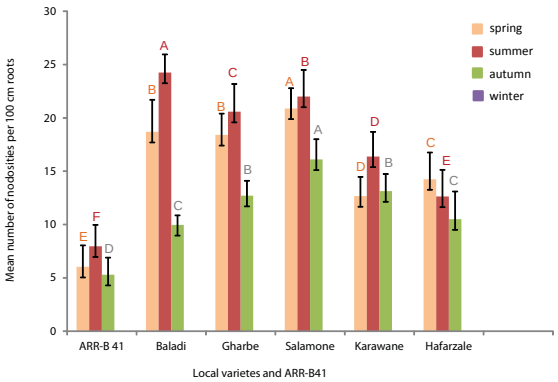


Figure 3. Number of galls "nodosities" on roots of local grapevine varieties in Syria and the phylloxera resistant ARR-B41 rootstock during the growing season. Different capital letters on columns of the same color indicate significantly different means ± standard deviation at $P < 0.05$ (Tukey HSD test).

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season when the soil is cold. Grapevine is the only host plant for grape phylloxera, so its nutritional value and chemical composition changes during the season and consequently the composition of food sources may affect the life cycle of phylloxera. In addition, the plant-mediated life cycle stages may also be altered by the microbial root and soil community structure or symbiosis association (Huber *et al*., 2007; Vorwerk *et al*. 2007). Therefore, we believe that the formation of galls is affected by the growth time of roots (spring to summer) resulting in intensive propagation of phylloxera on new roots, and the development of phylloxera stages is related to the state of the root system (Balbiani, 1874; Cornu, 1878). The varieties Hafarzale and Karawane, which had fewer galls compared to the other local varieties, may be considered for use as rootstock vines resistant to phylloxera infestation and reproduction.

Conclusion

The assessment of sensitivity to grape phylloxera infestation of five native grape varieties in Syria in comparison to the resistant rootstock ARR-B41, based on biological parameters of the insect and gall formation capacity under the same conditions, suggests that Hafarzale and Karawane can be included in the list of resistant grape varieties against phylloxera.

The authors acknowledge Professor I. Othman, General Director of Syrian Atomic Energy Commission, and Dr N. Mirali, Head of the Molecular Biology & Biotechnology Department, for encouragement and supporting of present work. Thanks are extended to Dr H. Makee to initiation of the work for feedback and to Dr Mohamad Hawat for revising the manuscript.

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Received: 28 September 2021; Accepted: 23 June 2022

Βιολογικές παράμετροι της φυλλοξήρας της αμπέλου, *Daktulosphaira vitifoliae*, σε τοπικές ποικιλίες αμπέλου στην κεντρική Συρία – Επιπτώσεις στην ευαισθησία των ποικιλιών

I. Idris, A. Asaad, K. Houssian και I. Kalefa

Περίληψη Μελετήθηκαν οι βιολογικές παράμετροι τοπικών στελεχών της φυλλοξήρας της αμπέλου καθώς και η προσβολή στις ποικιλίες αμπέλου της Συρίας, Baladi, Salamone, Karawane, Hafarzale, Gharbe και στο Αμερικανικό ανθεκτικό υποκείμενο ARR-B41 (*Vitis vinifera* x *Vitis berlandieri*). Η τεχνητή προσβολή στις ρίζες έδειξε σημαντικές διαφορές στον πληθυσμό της φυλλοξήρας μεταξύ της πλειονότητας των ελεγχόμενων ποικιλιών σε σύγκριση με το υποκείμενο ARR-B41. Οι ποικιλίες Hafarzale και Karawane είχαν τον χαμηλότερο πληθυσμό φυλλοξήρας, με επίπεδο αντοχής παρόμοιο με αυτό του υποκειμένου ARR-B41. Επιπλέον, οι ποικιλίες Salamone και Gharbe φαίνεται να ευνοούν περισσότερο

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την αναπαραγωγή της φυλλοξήρας. Το πείραμα στον αγρό έδειξε σημαντικές διαφορές μεταξύ των τοπικών ποικιλιών και του ARR-B41 όσον αφορά τον μέσο αριθμό όγκων. Συνολικά, οι ποικιλίες Hafarzale και Karawane έτειναν να σχηματίζουν σημαντικά λιγότερους όγκους από τις άλλες ποικιλίες.

Hellenic Plant Protection Journal **15:** 80-86, 2022

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