Three Pistachio Species Evaluated For Resistance to the Common Pistachio Psylla, *Agonoscena pistaciae*

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Abstract - Two wild pistachio species and the cultivated pistachio were evaluated for resistance to the common pistachio psylla, a major pest of pistachio trees, in a laboratory trial. The results of this study showed the causes of resistance in tested materials. It was found that the cultivated pistachio, *Pistacia vera* with high fruit quality, were more favorable to the common pistachio psylla than were wild pistachio species with poor nut quality.
Key words: *Pistacia*, pistachio pest, psylla, resistance

I. Introduction

Pistacia Linnaeus is mainly a subtropical genus comprising some 10 species of dioecious trees and shrubs. Geographically, the largest concentration of *Pistacia* species is found in West Asia and in the Mediterranean region [23]. Wild species are important in the development of pistachio varieties because they provide rootstocks resistant to biotic and abiotic stresses, and tolerant of drought and poor soil conditions. It is these characteristics that make this crop and its wild relatives suitable for planting in marginal lands [17]. Three *Pistacia* species occur naturally in Iran, including; *Pistacia atlantica* Desf., *Pistacia khinjuk* Stocks and *Pistacia vera* Linnaeus [19]. *Pistacia atlantica* is one of the most widely distributed wild species. In addition it is divided into four ecogeographic races: subs*P. cabulica* (Stocks); subs*P. mutica* (Fischer & C.A. Meyer); subs*P.* kurdica (Zohary); and subs*P. atlantica*. Seeds of *P. atlantica* can be used for seedling production and extraction of oil. Resin is also considered to be a valuable product of these trees. *Pistacia khinjuk* is rarely a dominant species in the environments where it occurs. It thrives in dry steppe-forests or steppe formations, mostly in stony places and in rocky mountain terrains [23]. In Iran, due to extensive destruction of trees, this species is now only found in very inaccessible places such as rocky cliffs that are otherwise unsuitable sites for trees. Sheibani [19] reported three varieties of this species including; var. *heterophylla* Bornm, var. *populifolia* Boiss, and var. *oblonga* Bornm. However, in Turkey two categories (large-fruited and small fruited types) of "*khinjuk*" were identified. Large *khinjuk*s have high oil content and they are thus popularly called "fatty *khinjuk*s" [12]. In Iran, similar to its neighboring countries, both of the above mentioned wild species (i.e. *P. atlantica* and *P. khinjuk*) are suffering from the effects of erosion caused by human activities, particularly overgrazing and harvesting for use as firewood or charcoal. In contrast, *P. vera* is a very important plant species in Iran, and pistachio nuts are currently the primary agricultural export product. Currently, over 300,000 ha of pistachio orchards comprising about 45 indigenous cultivars are under commercial management. Pistachio growers experience many serious problems; pests and diseases are considered one of the main causes of tree damage and reduced yields.

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer, is one of the most serious insect pests of cultivated pistachio, *P. vera* in Iran [13 & 14]. This insect is distributed throughout the country in both pistachio producing regions and wild pistachio growing areas. In addition to A. *Pistacia*e, two other psyllid species, *Agonoscena bimaculata* Mathur and *Megagonoscena viridis* (Baeva), occur in Iran. *Agonoscena bimaculata* was only found on *P. khinjuk* but M. *viridis* attacks all three pistachio species (*P. atlantica*, *P. khinjuk* and *P. vera*) that grow in Iran [5 & 16]. However, these later two psyllid species are considered economically unimportant now [16]. Although several species of predacious arthropods attack common pistachio psyllids, their impacts are insufficient for pest control. Psyllid resistance to insecticides has recently developed rapidly [13 & 15]. Host plant resistance would therefore be a valuable strategy that would significantly reduce production costs. Nevertheless, resistance to the common pistachio psylla has not been studied in Iran and no information is available from other pistachio producing countries.

Laboratory experiments have been widely used to evaluate the susceptibility or resistance of various plants against their key pests. Butt et al. [6] suggested that resistance to psylla can be readily identified in pear genotypes in a 24 h bioassay. They reported that a detached leaf test is useful for following the fate of individual nymphs and for limiting their movement for ease of observation. In addition, the test is useful as a bioassay of field grown material. A leaf-section bioassay was also reported by Wünn et al. [21] and this method was successfully used for evaluating rice stem borer resistance in transgenic rice [22].

It seemed possible that among the various species and cultivars of *Pistacia* there existed some variation in resistance to psylla. Therefore, laboratory and field screening would be useful for evaluating their attractiveness to oviposition and susceptibility to psyllid population development. The present experiment is the first study using a detached leaf bioassay procedure to screen pistachio species for psylla resistance. This study was undertaken to evaluate three pistachio species from Iran for resistance to common pistachio psylla. Our intention was to develop a fast, reliable bioassay for screening *Pistacia germplasm* for pistachio psylla resistance.

II. Materials and Methods

The bioassay procedure was modified from techniques described for *Pyrus germplasm* in several studies [6, 2 & 1]. All laboratory trials were conducted under controlled conditions (27.5±0.5°C, 55±5% RH and 16:8 L:D). Leaf-disc cages were used in the development and reproduction experiments. A cage was made from a plastic Petri-dish (52 mm in diameter) with a 20 mm diameter hole in the middle of the lid, and covered by a piece of fine net (2 mm mesh) to provide ventilation. Agar media (8g/l) was used as a source of moisture for the plant leaf. Young and fully developed leaves were cut to the same size as the dish and placed, lower side down, on the 3 mm thick agar media covered by filter paper. Eight leaf-disc cages and two small glass jars containing a saturated magnesium nitrate salt solution (for maintaining relative humidity 55±5%) were placed in a plastic box inside an incubator. Lighting in the incubators was provided by white fluorescent lamps with light intensity of about 13 watts/m2 in the cabinet area. Leaves of each pistachio species were collected from bearing pistachio trees, including *P. atlantica* subspecies *cabulica*, *P. khinjuk* variety *heterophylla* and *P. vera* cultivar Akbari, in the experimental collection at the Pistachio Research Institute of Iran, located in suburb area of Rafsanjan.

A laboratory colony of pistachio psylla was maintained on potted and grafted pistachio (cultivar Akbari) seedlings. Three adult psyllid pairs (male and female) were collected from pistachio orchards and released on caged seedlings and given the opportunity to reproduce for two generations under laboratory conditions (27±2°C and 16:8 L:D).

Nine pairs of adult psyllids (5-6 days old) were removed from the colony and placed in three leaf-disc cages (separately for each plant species) under controlled conditions and allowed to lay eggs for 24 hours. Leaf-disc cages were changed after the first 12 hours. Leaf-discs containing psyllid eggs were examined three times a day (every 8 hours) to determine the egg incubation period and newly hatched nymphs were removed and individually placed in fresh leaf-disc cages using a fine hair brush. Nymphs were gently prodded until they retracted their mouthparts and began to walk. Experiments to measure the developmental time of psyllid nymphs were started using 150 newly emerged nymphs. The psyllid nymphs were also transferred to fresh leaf-disc cages at two day intervals using a fine camel hair paint brush. The development of the nymphal stages was checked once a day and the duration of each stage was recorded until adult emergence. For each adult, the sex was recorded and the body size (body length from forehead to the end of wings) was measured using a dissecting microscope equipped with an ocular micrometer. The number of dead and missing nymphs was recorded, though missing nymphs, such as those stuck in agar medium throughout the experiment or those that escaped during the 2nd to 5th instars, were not included in overall data. The entire experiments for egg incubation period and nymphal development were replicated twice.

The daily fecundity of pistachio psylla was determined by pairing newly emerged adult male and female psyllids in pistachio leaf-disc cages (54 mm diameter). At the same time, each day each pair of adult psyllids was carefully transferred to a fresh leaf-disc cage using a small aspirator, and all eggs laid on the old leaf-discs were counted. The male was replaced if it was disabled or dead. The adults examined in this study were reared in the development trials and they were standardized by using similar adult size (body length). In this experiment, the fecundity of female psyllid was determined for first 12 days of its life span.

Data obtained from these development and reproduction experiments (developmental time, mortality, sex ratio and fecundity) were compiled in a life table for the first 12 days of adult life span under controlled conditions. Life table parameters were calculated using a QBASIC program [11]. From this, the parameter "intrinsic rate of natural increase (r_m)" [4] was determined.

III. Results and Discussion

In all laboratory studies reported here, pistachio psylla readily developed and reproduced on all three *Pistacia* species. Although the development time, survival of immature stages, as well as fecundity of adult psyllids were significantly different among the three host plants, none of the three pistachio species appeared to be a resistant host. Nymphs that fed generally remained at one site and fed continuously for the duration of the experiment, while excreting copious amounts of honeydew. However, some nymphs moved about, either leaving the leaf (missing) or dying after feeding. The majority of the nymphs (about 65%) on cultivated pistachio fed on leaves and developed to adults. In comparison, more than 80% of nymphs on wild pistachio leaves either died during the immature stages or moved off the leaves (missing) during the first 24h of 1st instar (Fig. 1). The fate of the missing nymphs could not be determined, although considerable differences were found between the numbers of missing nymphs on the three treated plants. However, the high percentage of missing nymphs on wild pistachio species might indicate that this rejection was caused by feeding inhibitors or the absence of sufficient feeding stimulants.

Table 1 Developmental period, adult body length (mm) and reproduction of *Agonoscena pistaciae* on three host plant species in controlled conditions (27.5±0.5°C, 55±5% RH and 16:8 L:D).

Pistachio species examined	Developmental period (day±SE)			Adult size (mm±SE)		Fecundity (egg±SE)
	Male	Female	(Male+female)	Male	Female	
P. atlantica	17.5±0.61b (9)	18.3±0.66b (10)	17.9	1.42±0.027b	1.59±0.037b	68.9±6.33b (10)
P. khinjuk	20.1±0.8a (11)	20.9±0.65a (13)	20.5	1.38±0.013c	1.44±0.030c	66.6±2.8b (10)
P. vera	13.9±0.41c (31)	14.9±0.49c (29)	14.4	1.51±0.010a	1.66±0.012a	181±6.96a (10)
P value	0.001	0.001		0.001	0.001	0.001

Means in each column followed by the same letter are not significantly different in one way ANOVA, using LSD-tests at P=0.05.
The figures in parentheses indicate the number of replicates.
Fecundity was examined for the first 12 days of adult life span。

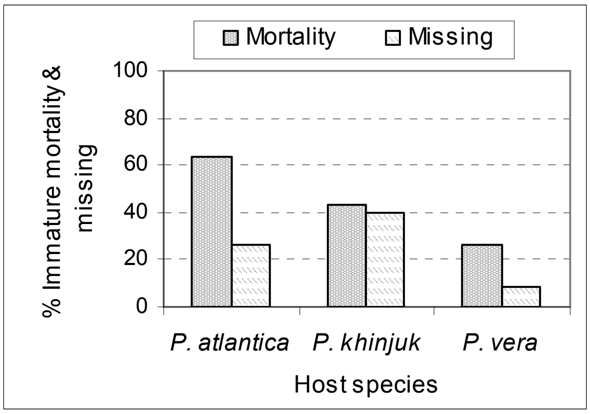

Fig. 1. Total immature psyllid mortality (egg to adult) and missing (not feeding) first instar nymphs (%) on three pistachio species in controlled conditions. 150 first instar psyllid nymphs were used at establishment.

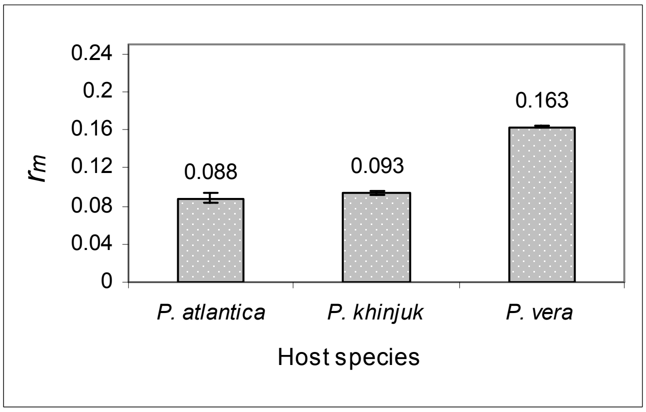

Fig. 3. Influence of host plant on the intrinsic rate of natural increase (r_m) of the common pistachio psylla in controlled conditions. Psyllid fecundity examined during the first 12 days of adult life span. Bars show standard errors (n=10).

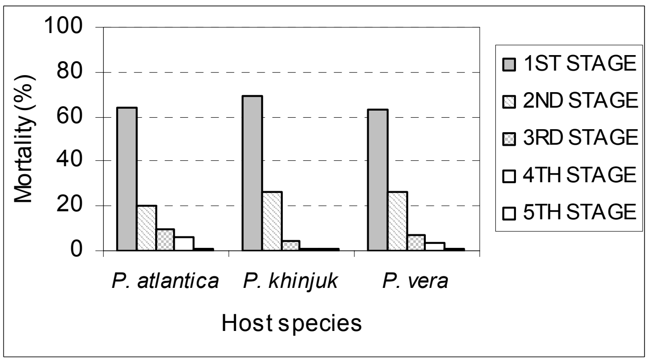

Fig. 2. Mortality of psyllid nymphal stages when feeding on three pistachio species in controlled conditions.

In this regard, Butt *et al*. [6] found most of the pear psyllid, *Cacopsylla pyricola* (Foerster) nymphs on resistant pear genotypes left the host leaves during the first 24 h of their experiment. In addition, the chemical composition of plants has previously been demonstrated to affect their susceptibility to insect pests [8, 6 & 7].

The developmental period of immature psyllids on *P. atlantica* and *P. khinjuk* was significantly longer than on *P. vera* (Table 1). The greatest nymphal mortality of psyllids occurred among first instars on all 3 pistachio *germplasm*s, and it was followed by mortality among 2nd instars (Fig. 2). Although some of this initial mortality may be attributed to the shock of being moved, there is evidence that total mortality of psyllid nymphs was much lower on *P. vera* than on the other two pistachio species (Fig. 1). Adult psyllids that developed on *P. vera* were also significantly larger than those on the other two pistachio species (Table, 1).

Pistachio psylla ovipositional response showed that all pistachio plants were attractive but differed significantly among species. Two levels of egg-laying during the 12 days of oviposition could be distinguished (Table 1). Adult psyllids laid the smallest egg masses (67 & 69 eggs) on *P. khinjuk* and *P. atlantica* respectively. In comparison 181 eggs were laid on *P. vera*. The intrinsic rate of natural increase (r_m) was used as the measure of psyllid performance on each pistachio species. Among tested plant species, r_m values of psyllids on *P. vera* (0.163) was significantly larger than for psyllid on *P. atlantica* (0.088) and *P. khinjuk* (0.093) (Fig. 3). In this regard, Ruggle and Gutierrez [18] conducted life table experiments for the spotted alfalfa aphid, *Therioaphis trifolii* f. *maculate* (Buckton) on resistant and susceptible cultivars at different temperatures, and the *maculate* statistic was used to assess the effects of host plant resistance of different cultivars because results were comparable.

The present study showed that antibiosis resistance and lower preference for oviposition occurred together in wild pistachio species. But, Berrada et al. [3] demonstrated that antibiosis resistance and nonpreference for oviposition did not operate similarly in pear species.

Overall, The extended development time, higher rate of mortality for immature instars and smaller size of adults as well as lower fecundity potential of psyllids on wild pistachio species all indicate the lower suitability of these plants as hosts for the common pistachio psylla. These results are in agreement with my observation in natural conditions, where the density of psyllid eggs and young instar nymphs on *P. atlantica* and *P. khinjuk* is relatively high in early spring, but the population usually quickly decreased over two weeks time. Usually the density of psyllids remained low thereafter on these plants. Although, biocontrol agents cause large effects on psyllid population in early spring, plant nutritional qualities might be also responsible for psyllid mortality when feeding on wild pistachios. From the present study it might be concluded that both reduced preference for oviposition and antibiosis contributed to moderate resistance in two wild pistachios tested. Resistance to pear psylla is found mainly in wild *Pyrus* species, originating from Asia and Europe [20, 9, 10 & 2]. More research should be conducted to demonstrate the correlations between pistachio psylla population growth in the field and antibiosis and oviposition preference measured in laboratory studies. This study indicated that the cultivated pistachio, *P. vera*, with high fruit quality, was more favorable to the common pistachio psylla than two wild pistachio species with poor nut quality. Mehrnejad (unpublished) found that all high-quality cultivars of *P. vera* are susceptible, but there are some sources of resistance in cultivars with low nut quality. Therefore, broad examination of cultivated pistachio cultivars is needed to screen these plants for psylla resistance. Sources of resistance within this gene pool might be used more effectively in a breeding program than sources from wild pistachio species because of the similarity in fruit characteristics within *P. vera*.

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