ANTIXENOSIS COMPONENT OF RESISTANCE IN POPLAR SPECIES AND CLONES (*POPULUS* SPP.) TO THE WILLOW AND POPLAR LACE-BUG, *MONOSTEIRA UNICOSTATA* (MULSANT & REY) (HEMIPTERA: TINGIDAE)

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ABSTRACT: During the year 2007, antixenosis resistance mechanism of 4 poplar species, including *Populus alba*, *P. deltoides*, *P. euramericana* and *P. nigra*, and 15 related clones was evaluated against the willow and poplar lace-bug, *Monosteira unicostata*, one of the most important pests of these trees. Poplar cuts, bearing 3-5 uniform and similar leaves, of each species were taken. The adult bugs previously developed under natural conditions on one poplar species, *P. alba*, were collected and released in a designed olfactometer while placing in a germinator (at temperature 24±0.1 °C, 50 % relative humidity, and a photoperiod of 16L:8D). The experiment was carried out with 10 replications. The numbers of male and female bugs attracted to each poplar clones were counted and recorded after 24 hours. Statistical analysis revealed a significant difference ($P<0.01$) in numbers of attracted lace-bugs to the poplar species and clones. The most lace-bug numbers were attracted to *P. nigra* and *P. alba* respectively, and the lowest attraction of lace-bugs was observed in *P. euramericana* and *P. deltoides*. Among the poplar clones, the comparison of means showed that *P. n.* 42.78, *P. n.* 56.53 and *P. n. betulifolia* attracted the most adult bugs and were considered as the most susceptible clones, while *P. d. missouriensis*, *P. e. vernirubensis* and *P. e. triplo* were the most resistant clones.

KEY WORDS: Poplar, clones, *Monosteira unicostata*, resistance, antixenosis, olfactometer.

Poplar species (*Populus* spp.) are important fast-growing trees in forest areas and landscapes in all regions of Iran. More than 200 species of arthropods, including insects and mites, are active organisms on poplar trees in the country (Sadeghi, 2004, 2007). Several insect species of different orders feed and damage on or within poplar leaves, stems, trunks, roots, etc. One of the most economically important pests of poplar trees in Iran is the poplar and willow lace-bug. This pest has probably existed since past decades in the country (Babmorad & Askari, 2004) and has been reported with different scientific names in Persian literatures. For example, it has been recorded as *Monosteira inermis* Horvàth (Farahbakhsh, 1961), and *M. discoidalis* (Jakovlev) (Abaii & Adeli, 1984; Khial & Sadraei, 1984; Babmorad, 1993). In the latest literatures, it has been reported on many poplar and some willow species as *M. unicostata* (Mulsant and Rey, 1852) with severe damage (Babalmorad, 1998; Abaii, 2000). The recent species occurs on poplar and willow trees in Iran and on the same and also other ornamental and fruit trees in some parts of the World (Önder & Lodos, 1983; Péricart, 1983; Schaefer & Panizzi, 2000), and seriously damages often to the inferior and sometimes superior surfaces of host leaves and causes yellow spots on their upper parts. Also, insect minute black excrements remain under leaf surfaces. Early leaf falling occurs in spring and summer seasons and causing tree weakness and preparing xylophagous pests attacking to the infested trees. This pest is found on different poplar species in some parts of Iran, including Isfahān and Chahārmahāl-o-Bakhtiyārī provinces and north parts of the country (Abaii, 2000; Jafari et al., 2002; Sadeghi et al., 2002; Haghighian & Sadeghi, 2006). Also, severe feeding activity and damage of the pest have been reported on poplar species and clones in Karaj region, Tehran province (Babmorad et al., 2002; Babmorad & Sadeghi, 2004). Babmorad & Sadeghi (2004) considered the pest as a specific pest of plants of the willow family (Salicaceae) in Iran, and totally reported 18 species and 50 clones of poplar as the tree hosts of the bug in Karaj region.

Using resistant plant species and varieties is a beneficial method for controlling pest damage in IPM programs with many advantages. Among the most important are effectiveness, selectivity against the pest, relatively long stability, compatibility with other tactics (such as pesticides), human and environmental safety (Pedigo, 1996). Antixenosis is a category in resistance in which the plant is a poor host, deterring any insect feeding (Gullan & Cranston, 2005). Literature reviews of national and international publications show that there is more probably no article on resistance mechanisms of poplar species and clones against *M. unicostata*, other than a few studies being accomplished on host preference of the pest in natural conditions. For instance, Babmorad et al. (2002) in a primary study in Karaj just reported near 20 poplar species and clones and two willow species as the pest hosts. Ghasemi & Modir-Rahmati (2004) observed the pest activity on poplar clones in natural conditions in Karaj, and indicated the pest' damage on different clones of *Populus* x *euramericana* and the clones *P. alba nivea*, *P. deltoides* 69.55, *P. deltoides* 73.51, *P. deltoides* 77.51, and *P. nigra betulifolia*, but *P. deltoides* clones show less susceptibility. Babmorad et al. (2007) evaluated its damage on 15 clones of 5 poplar species in Karaj region. Zargaran et al. (2008) studied the pest population density on 10 poplar clones and observed the most densities on two clones *P. euramericana* 561.41 and *P. nigra* 62.154. Babmorad et al. (2008) studied the pest damage on different poplar species and clones, and showed that *P. alba* 44.9 and *P. alba* 58.57 have been the most susceptible clones, while *P. deltoides* 77.51 and *P. deltoides* 73.51 were considered as the most resistant. Up to our knowledge, the international researches on *M. unicostata* damage were often preformed on resistance and susceptibility of almond trees (Egea et al., 1984; Russo et al., 1994) and other fruit trees (Roversi & Monteforte, 2005), not on poplar species and clones, just Serafimovski (1973) observed this pest with high density on *P. euramericana robusta* and *P. simonii* Carrière, and with low density on *P. tremula* Linnaeus and willow species in Macedonia.

The above mentioned researches showed that there is not specific study on resistance mechanisms of poplar species and clones against *M. unicostata*, and because of high damage of the bug on poplar trees in Iran, the purpose of this paper is to evaluate the antixenosis mechanism of poplar species and clones to the willow and poplar lace-bug, and finding the poplar clones resistant to the pest for providing a successful integrated pest management program in the future in susceptible areas of poplar-growing. Up to our knowledge, the present research is the first study on antixenosis resistance of poplar trees against this destructive pest.

MATERIALS AND METHODS

1) Sampling site:

Field samplings were conducted in a poplar farm in the Alborz Research Station of the Research Institute of Forests and Rangelands (RIFR) in south of Karaj, Tehran province. Experimental researches were performed at the Insect Laboratory of the Conservation and Protection of Forest and Rangeland Research Group at the RIFR, Tehran.

2) Poplar species and clones:

Fifteen clones belonging to four poplar species including *Populus alba* (*P.a.*), *P. deltoides* (*P.d.*), *P. euramericana* (*P.e.*) and *P. nigra* (*P.n.*) were selected for the study. Names and origins of the studied species and clones are listed in Table 1. Origin countries of the imported slips in the table were mostly extracted from Ghasemi & Modir-Rahmati (2004). Age of the examined poplar trees was five years old at time of the study.

3) Antixenosis mechanism of poplar species and clones:

This experiment was carried out with 10 replications for both female and male bugs, separately. Adult bugs reared on a poplar species in natural conditions, were collected and separated.

In order to perform this research, an olfactometer, designed as follows, was used:

The set was organized with a central cylindrical container, 21.7 cm in length and 14 cm in diameter, with 15 white glasses around the main central container. The connections of the main container and lateral glasses were prepared with 15 colorless elastic tubes. One or two frail and short cuts bearing 3-5 uniform and similar leaves were placed in each glass. Afterwards, the glass openings were closed by cloth net. Then, 50 adult females/males were released in the main container of each olfactometer and its opening was closed by net. The olfactometers were placed in a germinator at temperature 24±0.1 °C, 50 % relative humidity, and a photoperiod of 16L:8D hours. This experiment was performed at the indicated conditions with 10 replications, separately for adult females and males. The numbers of adults attracted to each poplar clones were counted and recorded after 24 hours.

4) Statistical analysis:

Recorded data were analyzed using the SAS 9.1 software program and the average of attracted female and male bugs were compared by Tukey's test. Table 8 shows the mean comparison of attraction of adult bugs to poplar clones, in which the letters "a" and "ab" have been considered for susceptible clones, "abc" for semi-susceptible, "bcd" for semi-resistant, "cd" and "d" for resistant clones.

RESULTS

Multiway analysis of variance showed that numbers of adult bugs attracted to cuts significantly differ ($P<0.001$) among poplar species and clones (Table 2). Statistical analysis also revealed significant differences in numbers of attracted male bugs (Tables 3 and 4) and female bugs (Tables 5 and 6) to the poplar species and clones. The averages of attracted males and females and total adults, which attracted to the poplar species, have been presented in Table 7. The table shows that the highest numbers of attracted males were counted on *P. nigra*, significantly differed with other three species, while *P. nigra*, *P. alba* and *P. deltoides* attracted more females with no significant differences, compared with *P. euramericana*, on which the lowest attracted females were observed. The most attracted adults (total females and males) were observed on *P. nigra* and *P. alba*, respectively, and the lowest attracted ones were counted on *P. euramericana* and *P. deltoides*.

Table 8 shows the averages of attracted female and male bugs to poplar clones. The comparison of means indicated that the clones *P. nigra* 56.53 and *P. n.* 63.135 showed the highest attraction of adult males among all 15 clones, while the numbers of males attracted to *P. deltoides missouriensis* were less than other clones with no significant differences. The highest attractions of females were observed on *P. n.* 42.78 and *P. n. betulifolia* with significant differences with most clones, while *P. euramericana vernirubensis* didn't attract any female. The comparison of means showed that the clones *P. n.* 42.78, *P. n.* 56.53 and *P. n. betulifolia* attracted the most adult bugs and were considered as the most susceptible clones, and *P. e.* 561.41, *P. d.* 73.51, *P. e. marilandica* and *P. alba* 44.9 were considered as semi-susceptible clones. Three clones including *P. d. missouriensis*, *P. e. vernirubensis* and *P. e. triplo* attracted the lowest numbers of adult bugs, and were considered as the most resistant clones. Other 5 poplar clones were considered as semi-resistant clones.

DISCUSSION

The results of this research showed that there were significant differences among poplar species and clones for attracting adult bugs, but no differences were found between females and males (Table 2). Therefore, both sexes were relatively equally attracted to tree species (Table 7).

According to the results shown in Table 7, it could be concluded that *P. nigra* and *P. alba* contain a low level of antixenosis resistance, while *P. deltoides* and *P. euramericana* showed the less attraction of bugs without any significant differences between themselves. These results indicated that *P. nigra* and *P. alba* could be considered as two suitable hosts for *M. unicostata*. Our field observations during the years 2007-2008, confirm the laboratory examination results. Figs. 1-4 show extensive damage of the pest on *P. nigra* and *P. alba* in natural conditions.

This study confirms the results of researches performed by Ghasemi & Modir-Rahmati (2004), Sadeghi et al. (2006), and Babmorad et al. (2008). Ghasemi & Modir-Rahmati (2004) showed that *P. deltoides* clones demonstrated the lowest invasion of *M. unicostata*. Sadeghi et al. (2006) observed the most invasion rate of the pest on *P. nigra*, *P. alba* and *P. euramericana*, while *P. deltoides* clones showed the lowest infestation. Babmorad et al. (2008) also showed that *M. unicostata* caused severe damage on *P. alba* and *P. nigra*, but *P. euramericana* and *P. deltoides* were sustained the lowest damage in natural conditions.

The results of mean comparison of adult bugs attraction to poplar clones partly agree with those taken by Sadeghi et al. (2006) and Babmorad et al. (2007, 2008). In all mentioned studies, *P. a.* 44.9 and *P. n.* 42.78 were respectively considered as susceptible and resistant clones, our results almost confirm the former, but refuse the later one. The differences between these results are apparently related to their experimental performance methods. The three mentioned researches were performed by evaluating pest damage rates on poplar leaves in natural conditions, while the present research was accomplished under laboratory conditions in order to clarify the pest host preference and poplar antixenosis mechanism. Abrahamson et al. (2001) in an extensive research on insect resistance evaluation of willow and poplar clones indicated that insects could choose their preferences between clones in the field, whereas, in the lab bioassay the insects were not given a choice. Also, the plants which insects choose in the field may not necessarily be the ones they prefer in the lab (Abrahamson et al., 2001). This opinion can be considered for *M. unicostata* preference on the poplar clones, during the different results of the indicated researches and the present work, concerning *P. n.* 42.78 responses. The other reason for the differences can be related to the age of examined poplar trees which could influence the experimental results. Babmorad et al. (2007) evaluated the poplar and willow lace-bug damage on one year and two years old seedlings, while the poplar trees, by which the cuts were taken, were five years old at the time of our antixenosis study. Whereas physiological responses in plants vary with plant age, and these can lead to change in the expression of cultivar resistance (Pedigo, 1996), more probably the differences among poplar clone ages tested in Babmorad et al. (2007) and the present work, can be the other reason for different results taken in these two studies.

According to Babmorad et al. (2008), two poplar clones *P. d.* 77.51 and *P. d.* 73.51 showed the lowest damage of the pest under natural conditions. Our results showed that these trees could be considered as semi-resistant and semi-susceptible, respectively. The most invasions of the pest were observed on *P. a.* 44.9 and *P. e. marilandica* by Sadeghi et al. (2006). The present work indicated that the later one was considered as a semi-susceptible clone with relatively high attraction of adult bugs. Zargaran et al. (2008) observed the most density of *M. unicostata* on *P. e.* 561.41 and *P. n.* 62.154, among 10 native and exotic clones. Our results showed that *P. e.* 561.41 is a semi-susceptible clone with relatively high attraction of adult bugs and confirm the mentioned research.

Among the examined poplar clones, some are native to Iran, and others are exotic clones. All resistant and semi-resistant clones originally belonged to abroad countries, including Italy, USA, and Turkey, while two susceptible and semi-susceptible clones (*P. n.* 42.78 and *P. a.* 44.9, respectively), are native to Iran. Probably it could be one of the reasons that *M. unicostata* has not yet been established on the exotic clones.

Based on the similar results observed in the field conditions, it is led to a conclusion that antixenosis mechanism of poplar species and clones can play a major role on the plant resistance against the willow and poplar lace-bug. Knowing susceptibility and resistance capacities of poplar clones in antixenosis mechanism, substantiating their probable conditions in antibiosis mechanism in future researches, and considering the climatic conditions as major factors influencing poplar resistance, can help the farmers to choose and plant the suitable clones in those areas in which *M. unicostata* is an important pest. Among the resistant clones, *P. e. missouriensis*, *P. e. vernirubensis* and *P. e. triplo* showed the highest resistance to the pest. Extracting resistance produced genes of poplar trees and using transgenic high-quality clones can help to approach a suitable control for the pest according the integrated pest management programs. Also, all environmental factors, including climatic conditions, weather, soil, etc., and proper cultural practices, such as irrigation, fertilization, and weed control, which can influence the plant growth vigor and pest damage to the plant (Pedigo, 1996), should be evaluated as well as possible in those regions in which poplar clones are used to decrease the pest damage. Other examinations on resistance ability of the tested clones against other poplar key pests should be studied.

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Table 1. Name and origin of examined poplar clones.

Row	Clone	Origin
1	*Populus alba* 44. 9	Iran (Isfahan)
2	*P. a.* 58.57	Italy
3	*P. deltoides* 69.55	USA
4	*P. d.* 73.51	USA
5	*P. d.* 77.51	USA
6	*P. d. missouriensis*	USA
7	*P. euramericana* 561.41	Italy
8	*P. e. grandis*	bridUSA hy
9	*P. e. marilandica*	Probably USA
10	*P. e. triplo*	Italy
11	*P. e. vernirubensis*	Italy
12	*P. nigra* 42.78	anIr (Karaj)
13	*P. n.* 56.53	Turkey
14	*P. n.* 63.135	Turkey
15	*P. n. betulifolia*	Italy

Table 2. Three way analysis of variance (poplar species/ poplar clones/ bug sex) of willow and poplar lace-bug attraction to *Populus* species and clones.

Sources	DF	Sum of Squares	Mean Square	F-value	P-value
Poplar species	3	395.198	131.733	11.02	<0.0001
Poplar clone	14	1071.825	76.559	6.40	<0.0001
Sex	1	1.949	1.949	0.16	0.687
Poplar species * Sex	3	17.571	5.857	0.49	0.690
Poplar clone * Sex	14	465.241	33.232	2.78	0.001
Error	264	3156.584	11.957		
Total	299	5108.368			

Table 3. One way analysis of variance (poplar species) of male willow and poplar lace-bug attracted to 4 poplar species.

Sources	DF	Sum of Squares	Mean Square	F-value	P-value
Poplar species	3	221.487	73.829	5.40	0.002
Error	146	1997.532	13.682		
Total	149	2219.020			

Table 4. One way analysis of variance (poplar clones) of male willow and poplar lace-bug attracted to poplar clones.

Sources	DF	Sum of Squares	Mean Square	F-value	P-value
Poplar clone	14	421.824	30.130	2.26	0.008
Error	135	1797.195	13.313		
Total	149	2219.020			

Table 5. One way analysis of variance (poplar species) of female willow and poplar lace-bug attracted to 4 poplar species.

Sources	DF	Sum of Squares	Mean Square	F-value	P-value
Poplar species	3	191.282	63.761	3.45	0.018
Error	146	2696.118	18.467		
Total	149	2887.4			

Table 6. One way analysis of variance (poplar clones) of female willow and poplar lace-bug attracted to poplar clones.

Sources	DF	Sum of Squares	Mean Square	F-value	P-value
Poplar clone	14	1115.242	79.660	6.07	<0.0001
Error	135	1772.157	13.127		
Total	149	2887.4			

Table 7. Mean comparison of attraction of male, female and total adults of willow and poplar lace-bug to poplar species.

Species	Mean ± SE		
	Male	Female	Total
P. alba	4.151 ± 1.054 b	4.906 ± 1.177 ab	4.528 ± 1.116 ab
P. deltoides	3.714 ± 1.095 b	3.917 ± 1.116 ab	3.815 ± 1.105 b
P. euramericana	4.369 ± 1.254 b	3.682 ± 0.982 b	4.026 ± 1.118 b
P. nigra	6.708 ± 1.021 a	6.405 ± 1.145 a	6.557 ± 1.083 a

Means followed by the same letter in each column are not significantly different using Tukey's test at $P<0.05$.

Table 8. Mean comparison of attraction of male, female and total adults of willow and poplar lace-bug to poplar clones.

Clone*	Mean ± SE			Susceptibility/ Resistance
	Male	Female	Total	
P. a. 44. 9	4.953 ± 0.958 ab	5.315 ± 1.328 abc	5.134 ± 1.143 abc	Semi-susceptible
P. a. 58.57	3.348 ± 1.15 ab	4.496 ± 1.026 bcd	3.922 ± 1.088 bcd	Semi-resistant
P. d. 69.55	4.257 ± 0.958 ab	4.345 ± 1.255 bcd	4.301 ± 1.107 bcd	Semi-resistant
P. d. 73.51	6.343 ± 1.596 ab	5.104 ± 1.231 abcd	5.724 ± 1.414 abc	Semi-susceptible
P. d. 77.51	3.109 ± 1.06 ab	4.257 ± 0.958 bcd	3.683 ± 1.009 bcd	Semi-resistant
P. d. missouriensis	1.148 ± 0.765 b	1.961 ± 1.109 cd	1.554 ± 0.892 d	Resistant
P. e. 561.41	5.104 ± 1.231 ab	6.886 ± 0.992 abc	5.995 ± 1.112 abc	Semi-susceptible
P. e. grandis	5.493 ± 1.025 ab	2.145 ± 1.152 bcd	3.819 ± 1.089 bcd	Semi-resistant
P. e. marilandica	4.68 ± 1.111 ab	6.653 ± 1.309 abc	5.666 ± 1.21 abc	Semi-susceptible
P. e. triplo	3.455 ± 1.537 ab	2.725 ± 1.458 bcd	3.090 ± 1.498 cd	Resistant
P. e. vernirubensis	3.115 ± 1.367 ab	0 d	1.557 ± 0.684 d	Resistant
P. n. 42.78	6.251 ± 0.894 ab	10.271 ± 1.267 a	8.261 ± 1.081 a	Susceptible
P. n. 56.53	7.417 ± 1.415 a	7.214 ± 1.401 abc	7.316 ± 1.408 ab	Susceptible
P. n. 63.135	7.036 ± 0.614 a	0.574 ± 0. 574 d	3.805 ± 0.594 bcd	Semi-resistant
P. n. betulifolia	6.127 ± 1.159 ab	7.561 ± 1.336 ab	6.844 ± 1.248 ab	Susceptible

Means followed by the same letter in each column are not significantly different using Tukey's test at $P<0.05$.
* *P. a.= Populus alba*; *P. d.= P. deltoides*; *P. e.= P. euramericana*; *P. n.= P. nigra*.

Figure 1. Willow and poplar lace-bug damage to *P. nigra* leaf (May 2008).

Figure 2. Damage and adults of the willow and poplar lace-bug on *P. nigra* leaf (June 2008)

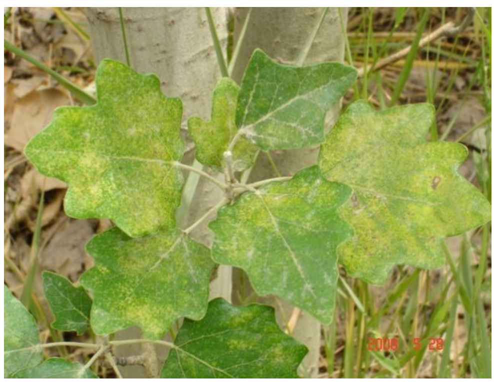

Figure 3. Willow and poplar lace-bug damage and on *P. alba* 44.9 leaves (May 2008).

Figure 4. Damage and nymphs of the willow and poplar lace-bug under *P. alba* 44.9 leaf (May 2008).