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Behavioral Responses of Egg Parasitoid *Trissolcus semistriatus* (Nees) (Hymenoptera: Scelionidae) to Odors of Five Plant Species

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Abstract: The responses of the egg parasitoid *Trissolcus semistriatus* (Nees) (Hymenoptera: Scelionidae) females to chemical cues from the plants: bread wheat *Triticum aestivum* L. (Poaceae), *Lens culinaris* Medik. (Fabaceae), *Sinapis arvensis* L. (Brassicaceae), *Bifora radians* Bieb. (Apiaceae) and *Leontice leontopetalum* L. (Berberidaceae), were investigated in the laboratory. Studies were carried out to determine the first reaction, the residence time and linear speed of the parasitoid using Y-tube and five armed olfactometers. In the Y-tube olfactometer experiments, *T. semistriatus* showed the strongest responses to the odor of *T. aestivum* with 86.7%, followed by *L. leontopetalum* with 80%. The parasitoids showed the weakest responses to the odor of *B. radians* with 26.7%. The residence times of *T. semistriatus* were determined as 87.53, 66.16, 53.50, 46.20 and 38.25 for *T. aestivum*, *L. leontopetalum*, *L. culinaris*, *S. arvensis* and *B. radians,* respectively. The linear speed was determined as significantly lower (3.81) in *T. aestivum* than in odors of the other plants. The linear speeds of *L. leontopetalum*, *S. arvensis*, *L. culinaris* and *B. radians* were determined as 4.66, 5.30, 5.14 and 6.92, respectively. The statistical analysis showed significant differences in the residence time in the plants. While the parasitoid was attracted mostly by *T. aestivum*, the less attraction was caused by *B. radians*.

Key words: *Trissolcus semistriatus,* Scelionidae, olfactometer, odor response, plant, first reactions, residence time, linear speed

Introduction

Wheat (*Triticum aestivum* L.), the most common produced crop, has the advantages of being raised in different climatic and geographical ecosystems owing to its adaptation ability. It is the most important nutrient for the human beings, constituting approximately 20% of the calories obtained from the food all over the world (Akkaya 1994). *T. aestivum* is the Turkey's most important cereal crop in terms of both the area cultivated (8.9 million ha) and the amount of grain produced (18.8 million t) (Tuİ 2011).

The Sunn pests (SPs), *Eurygaster* spp. (Heteroptera: Scutelleridae), are the most important harmful insects on wheat in the Balkan Peninsula. They are distributed on 75% of the wheat fields across Turkey and their chemical control is carried out over an average of 1.2 million hectares every year (Koçak 2008). Both nymphs and adults of SPs cause plant damage, feeding on leaves, stems, and grains (Critchely 1998). Yield losses were estimated at 50 to 90% in wheat and 20 to 30% in barley (Lodos 1986). Apart from the direct yield reduction, during feeding the insect injects digestive enzymes which reduce the baking quality of the dough. If as little as 2 to 3% of the grain has been fed on, the entire grain batch may be rendered unacceptable for baking purposes because of poor-quality flour (Lodos 1986).

Various natural enemies attack SPs in Turkey. It was found that the natural enemies had the greatest effect among the factors restraining SPs population (Lodos 1986, Rosca *et al*. 1996). The egg parasitoid, *Trissolcus semistriatus* Nees (Hymenoptera: Scelionidae), is an important natural enemy of the SPs in Turkey (Koçak, Kilinçer 2001). Records suggest that seasonal dynamics, geographical and temporal distribution of egg parasitoids and their importance within the local natural enemy complex vary considerably in northern and southern parts of Turkey. For example, the activity of *T. semistriatus* in the field constituted 55 and 62% of the natural parasitism on the eggs of SPs in Southern Turkey in 2001 and 2002, respectively (Tarla, Kornoşor 2003). Many studies were concerned with the reproductive biology of *T. semistriatus* (Kivan, Kilic 2006a). There is growing interest in the biological control of this pest because of the concerns about adverse effects of the insecticides on the environment.

Owing to the fact that the pest chemicals lead to environmental pollution and disturbance of the natural equilibrium, they are not used as much as before and the importance of the biological control have increased recently. The agents for biological control have been used for different types of micro-and macro-organisms. The successful biological control will depend on the known biological properties of the host and parasitoid. However, the characteristics of the parasitoid behavior, as well as the physical, chemical and mechanical interactions with the host plant were also found to be important. In addition, the habitat and host selection behavior of parasitoids have been reported as a significant factor that influence the physical properties of the plant (Schmidt 1991, Vinson 1991).

Although a lot of research showed the effects of the plant volatiles on the insect behavior (Kaiser, Carde 1991, Powell, Wright 1992), very little work has been done on *T. semistriatus*. In this study, we investigated the responses of *T. semistriatus,* the most common and most effective egg parasitoid of sunn pests in Turkey, towards two different cultivated plants (Bread wheat *Triticum aestivum* L. and lentil *Lens culinaris* Medik.) and three most common weed species (Wild mustard *Sinapsis arvensis* L., wild bishop *Bifora radians* Bieb. and rakaf *Leontice leontopetalum* L.) by Y-tube and five-armed olfactometers.

Materials and Methods

Plants

Five different plants were used for determining the odor response of *T. semistriatus,* of them *T. aestivum*, *L. culinaris, S. arvensis* and *L. leontopetalum* were obtained from Oguzeli district Gazianatep province (36 57 N; 37 30 E), while *B. radians* was obtained from Gölbaşı district of Ankara province 39 39 N; 32 56 E) The plants were collected with stem, leaves and flowers and brought in ice containers to the laboratory as an odor source.

Insects

After temperature reached 13°C in early spring, the scelionid parasitoids were collected by a sweeping net from newly planted wheat fields or from the plants around those fields at Adana Provinces (37º 08' N 37º 05' E). The parasitoid adults were transferred to the laboratory in plastic bags and then were separated by species. The parasitoids were placed in cotton-plugged glass tubes and streaked inside with a diluted honey (10% distilled water) as a food source. Sunn pest (SP) (*Eurygaster integriceps* Put.) egg-masses were introduced to *T. semistriatus* and kept in an incubator at 26±1 °C, 65±10% RH and L: D 14:10 hours. This way the parasitoid culture was established. When enough parasitoid numbers became available, nearly 100 SP eggs were introduced to 1 male and 3 females for parasitization. The males were easily separated from the females according to their antennal characteristics. The parasitized eggs were incubated up to the adult in their host eggs under the same conditions (Peri *et al.* 2011).

Y-tube olfactometer

The Y-tube olfactometer (stem 9 cm; arms 8 cm at 130° angle; ID 1.5 cm) used and the device adopted for the observations were similar to the ones described by Colazza *et al.* (1997). Compressed air flowed through both arms, thus creating an air stream of 144 ml/min per arm. A light source was positioned in such a way that each arm received equal amount of light. The temperature was maintained at 26°C at all times. One adult female was randomly selected and placed near the orifice of stem. The female wasps were tested individually and tests were repeated from 08:00 to 17:00. After each experimental trial, the whole system was cleaned with acetone. A single wasp was introduced into the Y-tube at the entrance of the stem and observed with the aid of a behavior recording program, "Laice S8". Each wasp was allowed 10 min to choose one of the arms of the olfactometer; then it was discarded whether or not it had made a choice. A choice was considered to have been made when a wasp passed a line 4 cm into any arm and remained there for 20 second (Peri *et al.* 2011, Salerno *et. al.* 2002).

Their behavior was measured in terms of: (1) response to odor (2) residence time. Wasps were tested only one time and discarded if they did not make a response over the observation period.

Residence time test in the five-armed olfactometer

The oriented responses to plant odor were repeated in a five-armed olfactometer (Vet *et al*. 1983). Pressurized air flowed through five arms (144 ml/min per arm), so that five arms of equal area were established. A source of light was positioned so that each arm may receive equal amount of light and the temperature was maintained at 26°C at all times. In the experiments, all the arms were odorized. The odor sources were provided by *T. aestivum, L. culinaris*, *S. arvensis*, *B. radians* and *L. leontopetalum*. To deliver the odor into each of the five arms of the olfactometer, each corresponding arm was connected to five air-tight glass jars (height: 15 cm; diameter: 10 cm), containing the odor source of different plant. A female parasitoid was introduced into a vial connected to the centre of the five-armed olfactometer, of which it could walk out freely. The observations were started on the female entering the chamber, and lasted for 1 min, which was sufficient to observe a perceptible attraction to the odor (Desneux *et al.* 2000). The position of the female (fields numbered clockwise from one to five) was recorded on a computer using event recorder software "Lice S8", allowing computing the overall time spent in each field. The experiment was repeated a total of 30 times. After observing approximately every 10 individuals, the olfactometer was carefully washed with acetone and the position of the odorized arena was changed. The effects of the five plant odors on the orientation responses of surviving females were examined according to Desneux *et al.* (2000).

Statistical analysis

In the Y-tube olfactometer experiments, the number of wasp females that made a response was analyzed by a χ^2 test. The residence time and linear speed of the female wasps in the plant odors were statistically calculated with one-way ANOVA, followed by Tukey's HSD post-hoc test for multiple comparisons between the means.

In the five-armed olfactometer experiments, the wasp's arena resident time was calculated with one-way ANOVA, followed by Tukey's HSD post-hoc test for multiple comparisons between the means. All the data were analyzed using SPSS 16.0 program (Salerno *et al.* 2002, Peri *et al.* 2011).

Results

Y-tube olfactometer bioassays

Most of *T. semistriatus* females made a response in the Y-tube olfactometer bioassays in similar proportion among the different tests. The response of *T. semistriatus* females towards the different plant volatiles is presented in Fig. 1.

When the parasitoid females were exposed to different plant volatiles, they chose one of equally tested. *T. semistriatus* showed the strongest responses to the odor of *T. aestivum* with 86.7%, followed by the odor of *L. leontopetalum* with 80%. The parasitoids showed the weakest response to the odor of *B. radians* with 26.7%. The respenses to the odors of *L. culinaris* and *S. arvensis* were determined as 40% and 66.6%, respectively. In the statistical analysis, while the responses to *T. aestivum* x Air (χ^2 = 16.13; df = 1; P = 0.000) and *L. leontopetalum* x Air ((χ^2 = 10.80; df = 1; P = 0.003) were positive, those to *S. arvensis* x Air (χ^2 = 3.33; df = 1; P = 0.068) *L. culınaris* x Air (χ^2 = 1.20; df = 1; P = 0.466) were not significant. The response to *B. radians* x Air (χ^2 = 6.53; df = 1; P = 0.027) was determined as negative. In the control (air) arm and choosing amount of in both arms were found as insignificant (χ^2 = 1.200; df = 1; P = 0.233).

Comparison was made over the residence times of *T. semistriatus*, related to the odors of *T. aestivum*, *L. culinaris*, *L. leontopetalum*, *B. radians* and *S. arvensis*. The parasitoid females exhibited a clear preference in Y-tube olfactometer. The residence time in the odor of *T. aestivum* was significantly higher than that in the other plant odors (87.53±6.46). The residence times for other plants *L. leontopetalum*, *L.culinaris, S.arvensis* and *B.radians* were determined to be 66.16±3.53, 53.50±3.51, 46.20±7.40 and 38.25±5.85, respectively. The statistical analysis showed significant differences between the five plant odors (F = 15.020; df = 4; P = 0.000) (Fig. 2).

Inside the olfactometer the linear speed of the wasps was significantly affected by the volatiles from different plant treatments. The linear speeds of *T. semistriatus*, which are related to the different plant odors, were compared. The linear speed was significantly lower in the odor of *T. aestivum* than in the other plant odors (3.81±0.15). The linear speeds for *L. leontop-*

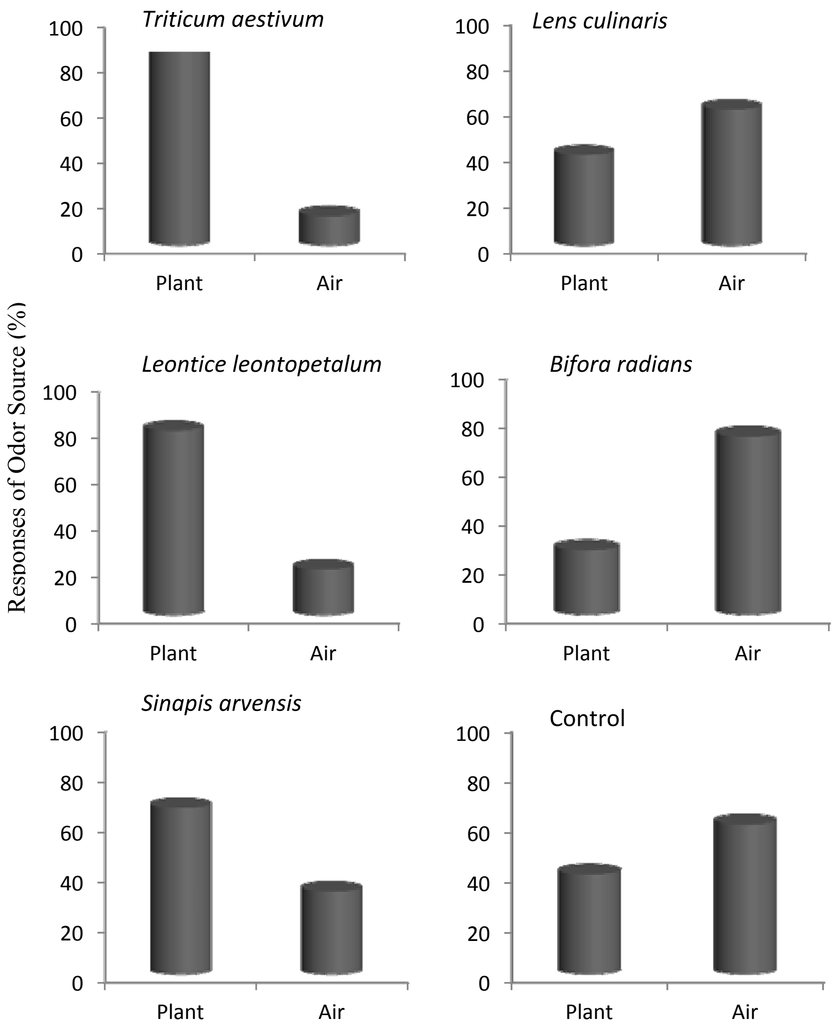

Fig. 1. The first reaction of *Trissolcus semistriatus* against odor of some plants in Y-tube olfactometer

etalum, *S. arvensis, L. culinaris* and *B. radians* were determined as 4.66±0.12, 5.14±0.10, 05.30±0.30 and 6.92±0.20, respectively. The statistical analysis showed significant differences among the five plant odors ($F = 33.346$ $df = 4$; $P = 0.000$) (Fig. 3).

Behavioral test in the five-armed olfactometer

The oriented responses towards the plant odors were repeated in a five-armed olfactometer. The response of *T. semistriatus* females towards the different plant volatiles is presented in Table 1.

The responses of *T. semistriatus* females to volatiles from different plants: *T. aestivum, L. leontopetalum, L. culinaris, B. radians* and *S. arvensis* were determined in the five armed olfactometer. *T. semistriatus* showed the strongest response to the odor of *T. aestivum* with 24.16±1.19, followed by the odor of *L. leontopetalum* with 16.26±0.69. At the same time the parasitoids showed the weakest response towards the odor of *B. radians* with 3.80±0.58. The responses to the odors of *L. culinaris* and *S. arvensis* were determined to be 8.56±0.64 and 7.20±0.62, respectively. The statistical analysis showed significant differences among the volatiles ($F = 109.320$ $df = 4$; $P = 0.000$).

Discussion

Our results demonstrated that *T. semistriatus* females responded to the volatiles emitted by *T. aestivum, L. leontopetalum, L. culinaris, B. radians* and *S. arvensis* both in the Y-tube and the five-armed olfactometer. It was reported that other measures recommended for the control of SP were the removal and destruction of weeds, particularly of *L. leontopetalum,* which was preferred for oviposition, as well as the arrangement of artificial hibernation quarters consisting of ditches filled with trap plants, from where the bugs could be collected and destroyed in February (HIBRAUOL 1930). *B. radians* is distibuted in the eastern Mediterranean region (DAVIS 1972). It is

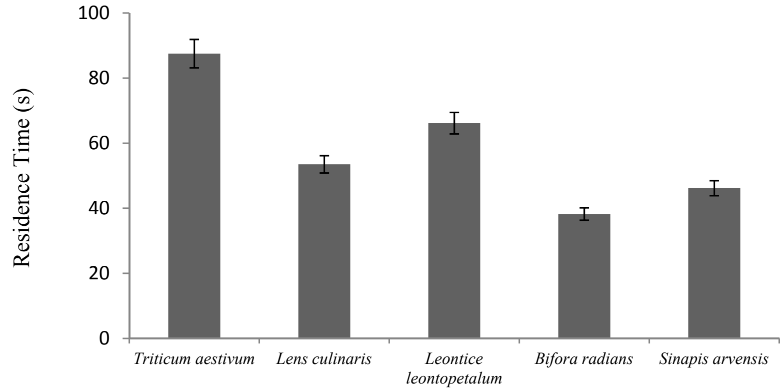

Fig. 2. The residence time of Trissolcus semistriatus against odor of some plants in Y-tube olfactometer

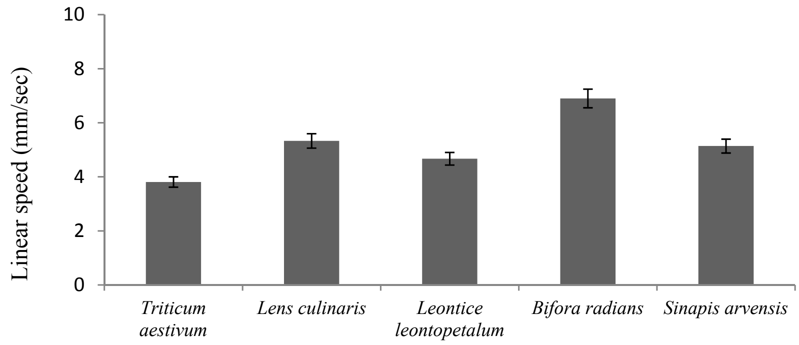

Fig. 3. The linear speed of Trissolcus semistriatus against odor of some plants in Y-tube olfactometer

Table 1. The first reaction of *Trissolcus semistriatus* against odor of some plants in five armed olfactometer. Different letters indicate significant differences determined by Anova test

Plant	mean±se	df	F	P
Triticum aestivum	24.16±1.19 **a**	4	109.32	0.000
Leontice leontopetalum	16.26±0.69 **b**			
Lens culinaris	8.56±0.64 **c**			
Sinapis arvensis	7.20±0.62 **c**			
Bifora radians	3.80±0.58 **d**			

a thermophilic plant that adapts well to nitrogen-rich and alkaline soils. Adaptation of the plant to cultivation has not been pursued, but the plant grows well as a weed in some spring crops and would be adaptable to cultivation. *B. radians* contains many terpenoids mainly (E)-2-tridecenal and (E)-2-tetradecenal and alkaloids (Baser *et al.* 1998). The methanol extract of *B. radians* repels many important pest species belonging to Lepidoptera and Coleoptera. Furthermore, the identification of the bioactive components in *B. radians* may allow the development of botanical insecticides with greater potency than the crude plant extract evaluated here (Gökçe *et al.* 2010). After wheat harvest, Pentatomidae feed on *S. arvensis* and in the collected eggs on these plants *T. semistriatus* were observed (Tarla, Doğanlar 1999)

The parasitoid females chose for egg-laying a host plant that provides the appropriate conditions for the development of their offspring (Courtney, Kibota, 1990). This choice is determined by the characteristics of the plants, such as, among others, chemical defenses, physical defenses, maturity and hardness of tissue (Coley *et al.* 2006). Kivan, Kiliç (2006b) tested the effects of the wheat (*Triticum vulgare*), cow cockle (*Vaccaria pyramidata* var. *grandiflora*), wild bishop (*Bifora radians*), common vetch (*Vicia sativa*) and turnipweed (*Rapistrum rugosum*) on the parasitism rate of *T. semistriatus* on *E. integriceps* Put. (Heteroptera; Scutelleridae). The highest rate of parasitism (94.9%), was found in the common vetch, while the lowest (68.9%) in the cow cockle; the emergence ratio was also the lowest (89.9%) in the cow cockle. The presence of plants significantly influenced the parasitism rate and adult emergence, while the development periods of males and females were not affected from plants; for this reason several plants appear to be slightly repellant (Kivan, Kiliç 2006b). In the choice test (first bioassay with the Y-olfactometer), in darkness and with distilled water as a control, the females chose the odor source of the quinoa plant. Under that condition (darkness), the preference for a plant is based on the olfactory stimulus and depends on the metabolites and chemical compounds emitted by the host plant (Coracini *et al.* 2004). The response to an olfactory stimulus in insect-plant interactions is an ecological process that is difficult to quantify and analyze. This is owing to the complex factors that interact in this relationship (Keller 1999).

According to our results, some weeds, in particular *B. radians* which have a repellant effect in the edges of wheat fields, can negatively affect either the parasitism rate or the biological control. Their control should be done very carefully. But some other weeds that show no such effect could be even more useful because of the possibility of carrying the pentatomids and playing the role of a very good host for them.

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