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Effect of Olive Branch Aging and Extracts on the Attraction of Olive Neiroun Beetles *Phloeotribus Scarabeioides* (Bern)

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أثر تقاوم فروع الزيتون والمستخلصات على جذب خنافس نيرون الزيتون (فلوتريبس سكرابيورس "بيرن ")

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خلاصة: تمت دراسة استجابة خنفس نيرون الزيتون (فلوتريبس سكرابيورس) لتقادم فروع الزيتون المنزوعة في أوقات مختلفة في المختبر باستخدام مقياس الشم (أولفاكتوميتر) في الحقل. أوضحت النتائج أن الفروع التي مضى عليها عشرة أيام كانت الأكثر جذبا معنويا لنيرون الزيتون، مما يدل على أنها تفضل الفروع شبه الجافة على الفروع الرطبة أو الجافة. كما تمت دراسة كفاءة المواد المذيبة، بما في ذلك إن - هيكسين والكلوفورم والأسيتون والميثانول والمياه المقطرة، في استخلاص المواد الجاذبة من فروع الزيتون وذلك باستخدام مقياس الشم (أولفاكتوميتر). تبين أن مستخلص الأسيتون كان الأكثر كفاءة في جذب الخنافس كاملة النمو. كما أظهرت الدراسة أن خنافس الزيتون تستدل على الحاضنات المجهدة والفروع المنزوعة بالإدراك الشمي للمركبات المتطايرة من أنسجة الحاضنات المجهدة.

ABSTRACT : The response of the olive neiroun beetle *Phloeotribus scarabeioides* (Bern) to the aging of detached olive branches at different periods of time was investigated in the laboratory using an olfactometer and in the field. Results showed that ten-day aged branches were the most attractive to the olive neiroun, indicating that semi-dried branches were more preferred than dry or fresh ones. Furthermore, the efficiency of certain chemical solvents including n-hexane, chloroform, acetone, methanol and distilled water in extracting the attractive substances in olive branches was studied using an olfactometer. Acetone extract was found to be the most efficient in attracting adults *P. scarabeioides*. The study indicates that the olive neiroun may locate the stressed host trees or detached branches by olfactory perception of volatile compounds resulting from the stressed host tissues.

The scolytid bark beetles are among the main pests of forests and fruit trees (Mendel, 1986). Members of Scolytidae are able to attack a wide range of host trees, and to colonize different plant parts. For scolytids, it is generally recognized that the initiation of infestation is associated with attack on stressed trees (Wood, 1982). Finding the stressed host trees has been attributed to the efforts of those beetles that initially land upon and attack host trees, commonly referred to as "pioneer beetles" (Borden, 1974). How pioneer beetles find the stressed host is still unknown (Payne *et al.*,1986). Person (1931) proposed that primary attracting of beetles to the stressed host is due to olfactory perception stimuli from the stressed host tree. On the other hand, other workers (Borden, 1974, Wood, 1982) hypothesized that scolytid beetles land at random on both healthy and stressed host trees and on non-host trees alike. Then the beetles go through further stages of host selection in response to various gustatory stimuli after the initiation of biting behaviours (Wood, 1982).

The question about the quantitative and qualitative differences in release of volatile substances from healthy and stressed host trees is still not settled (Sjodin *et al.*,1989). The present study was initiated to investigate the presence of attractive substances in olive wood on the olive neiroun beetle *Phloeotribus scarabeioides* (Bern). This insect is an important pest attacking olives in the Mediterranean region (Mustafa and Al-Momani, 1990).

Materials and Methods

OLFACTOMETER : The olfactometer was composed of a hollow disc of glass, 5 cm in diameter by 3 cm in height. Six glass tubes were fixed at the periphery of the disc. The tubes were fixed on the disc so that one side of the tube opened to the inside of the disc while the other side opened to the outside. Five of the tubes were 5 cm in length and 1 cm in diameter. The sixth tube was 1 cm in diameter by 20 cm in length. This tube was used as an opening for introducing adult insects into the olfactometer. Transparent rubber tubes 1 cm in diameter by 15 cm in length were fixed at the outer end of each tube except the entrance and the control tubes.

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REARING OF ADULTS: The introduced adults were reared in the laboratory in a diurnal growth chamber (Forma scientific) at 25°C ± 1°C. Branches infested with the olive neiroun were collected from the field, cut in to small pieces (15 cm long) and placed inside glass jars (20 x 20 x 20 cm). The opening of each glass jar was covered by muslin and rubber bands to provide ventilation.

EXPERIMENTAL DETAILS: The effect of the aging of detached olive branches on the olive neiroun was investigated using the olfactometer. Five branch ages were used, i) fresh cut branch, ii) two-day aged branch, iii) five day aged branch, iv) ten day aged branch, v) fifteen day aged branch.

Olive branches 60 - 65 cm in length and about 2 cm in diameter were cut 15, 10, 5, 2 and 0 days before the beginning of the experiment. All these branches were laid on the ground in the north - south direction and under the shade of a tree canopy. The branches were wrapped by muslin to prevent infestation with the olive neiroun. These branches was brought to the laboratory in polyethylene plastic bags and cut into small pieces.

Twenty grams from each branch age-group were placed inside a 25 ml conical flask. The flasks were fixed at the outer end of each rubber tubes on the olfactometer so that the opening of the rubber tube located inside the flask. The point of attachment between the flask and the rubber was tied with parafilm. Seventy adults were introduced into the olfactometer via the entrance tube using a fine brush. The entrance tube was closed with parafilm. Then the olfactometer was placed inside a box made of white carton. After 3 hours the carton was opened and the flasks were removed from the olfactometer. The number of adult insects present in the tunnel leading to each flask and the number of adults inside each flask were recorded.

The experiment was repeated four times at room temperature (23°C). Each time the arrangement of the flasks were altered according to the olfactometer rubber tubes.

The effect of aging of detached olive branches on the olive neiroun response was also studied in the field. The experiment was set up in a randomized complete block design with four blocks. Each block consisted of 10 Nabali olive trees. Spacing between the blocks and also, between the treatments within a block was 16 meters. Five branch ages were assigned randomly to five olive trees by locating one branch inside an olive tree and leaving the next tree without any branch. The branch description and ages used were the same as in the first experiment. The number of saw-dust infestations on the main stem was recorded after two days.

A third experiment was conducted using the olfactometer to detect the presence of attractive substances in olive branch extracts. The ten-day aged branch was used in the experiment depending on previous results. The branches were obtained from the field by cutting a branch and leaving it under the tree canopy below muslin for a period of 10 days. The chemical extractants used were n-hexane, chloroform, acetone, methanol and distilled water.

The semi-dried ten-day aged olive branches were brought to the laboratory in polyethylene plastic bags. The procedure used in the extraction process was as follows: The branches were cut into 10 g pieces which were extracted with 50 ml of the extractant using a blender. Blending was carried out for two min at low speed. The homogenate was then shaken at 200 RPM for half an h and then passed through filter paper (Ederol No. 4, finely pored 12.5 cm). The solvent was evaporated using a cold air current to a final volume of 20 ml.

Three ml of the extract were taken and spread on a disc of sponge measuring 1 cm in height by 5 cm in diameter. The chemical extract was thoroughly evaporated from the spongy disc using a cold air current from a hair dryer. This procedure was applied to all the five extractants under investigation. Each spongy disc represented a separate extractant. Each of the five spongy discs was placed inside a 25 cm conical flask. The flasks were fixed to the olfactometer rubber tubes. Seventy adults were introduced as mentioned previously. The experiment was repeated five times, each time changing the arrangement of the flasks according to the tubes.

Statistical analysis involved determination of means with standard errors. The differences between means were compared for significance using Duncan's multiple range test. Probability of 0.05 was taken as significant.

Results

The effect of aging of detached olive branches on the attraction of olive neiroun beetles in the laboratory is shown in Table 1. There were differences ($P < 0.05$) in both the flask readings and the flask and tunnel readings. With regards to the flask readings, the flask with ten-day aged branches attracted a higher ($P < 0.05$) number of beetles among all the treatments. The results for the two-day aged branch was significantly higher than that of the five-day aged branch and the control. There were no significant differences between the two-day aged branch, the fresh cut branch and the fifteen-day aged branch readings. Also, there were no differences ($P > 0.05$) between the fresh cut branch, the five-day aged branch and the control.

TABLE 1

Effect of the aging of detached olive branches on the olive neiroun response in th laboratory

Age of branch	Number of insects attracted	
	In flask	In flask and tunnel
Fresh cut	4.3 ± 1.5^{bc}	6.8 ± 1.3^{bc}
Two-day	7.0 ± 1.5^{b}	10.6 ± 1.6^{b}
Five-day	1.5 ± 0.9^{c}	3.8 ± 1.5^{c}
Ten-day	16.0 ± 2.0^{a}	22.8 ± 1.7^{a}
Fifteen-day	2.5 ± 1.3^{bc}	3.8 ± 1.9^{c}
Control	1.3 ± 0.3^{c}	2.0 ± 0.7^{c}

^{abc} Means in the same column without common superscripts differ ($P<0.05$). Mean ±SE.

However, in the flask and tunnel reading, the ten-day aged branch attracted a significantly higher number of beetles. The two-day aged branch was significantly higher than the fifteen-day aged branch, five-day aged branch and the control, but it was not significantly different from the fresh cut branch. There were no significant differences among the fresh cut branch, the five-day aged branch, fifteen-day aged branch and control.

Field results showed that the ten-day aged branch attracted a significantly higher number of beetles than the other treatments. The fifteen-day aged branch was significantly higher than the five-day aged branch, fresh cut-branch, the two-day aged branch and the control. There were no differences ($P>0.05$) between the five-day aged branch, the fresh cut branch and the two-day aged branch. Also, there were no differences ($P>0.05$) between the two day aged branch and the control (Table 2).

Starting with the flask reading, acetone extract was significantly higher than any other extract. There were differences between methanol, chloroform, distilled water, n-hexane-extractables and the control.

TABLE 2

Effect of the aging of detached olive branches on the olive neiroun response in the field

Age of branch	Number of infestations/branch
Fresh cut	3.5 ± 1.0^{c}
Two-day	2.8 ± 0.9^{cd}
Five-day	5.8 ± 0.5^{c}
Ten-day	18.0 ± 1.7^{a}
Fifteen-day	12.3 ± 1.7^{b}
Control	0.0^{d}

^{abcd} Means in the same column without common superscripts differ ($P<0.05$). Mean ± SE.

The n-Hexane extract attracted the least number of *P. Scarabeioides* (Table 3).

However, concerning the flask and tunnel readings, acetone extract was also more attractive ($P<0.05$) to *P. scarabeioides* adults than chloroform, distilled water and n-hexane extracts. There were no differences between extracts in acetone and methanol. There were also no differences among methanol-, chloroform, distilled water, and n-hexane extracts and the control.

Discussion

Investigations on the effect of detached olive branch aging on attractiveness to the olive neiroun showed that the ten-day aged branches had the highest attractiveness to the olive neiroun. This indicates that the semi-dried branches were more preferred by the olive neiroun, than dry or fresh branches. These beetles are known to prefer semi-dried branches for reproduction in Jordan (Mustafa and Al-Momani, 1990).

The effective response of the olive neiroun, to the ten-day aged branches but not to the other ages of cutting might indicate the presence of olfactory stimuli substances resulting from the deterioration of the host tissues.

These substances reach their maximum concentration ten day after the removal of the branches. Neuenschwander and Alexandrakis (1982) conducted an olfactory experiment with the olive neiroun and found that females of the scolytid were highly attracted to semi-burned olive branches but not to semi-burned pine branches. The results found on the laboratory using the olfactometer were supported by results taken in the field, where ten-day aged branches showed the highest attractiveness to the olive neiroun. The fresh cut branch and two-day aged

TABLE 3

Efficiency of extractants with various solvents in attracting the olive neiroun adults in the laboratory

Chemical extractant	Number of insects attracted	
	In flask	In flask and tunnel
n-Hexane	1.0 ± 0.6^{b}	2.6 ± 0.7^{b}
Chloroform	1.8 ± 0.6^{b}	4.2 ± 0.5^{b}
Acetone	5.0 ± 1.8^{a}	8.4 ± 2.4^{a}
Methanol	1.8 ± 0.5^{b}	5.8 ± 1.0^{ab}
Distilled Water	1.4 ± 0.9^{b}	2.8 ± 1.5^{b}
Control	1.4 ± 0.4^{b}	3.8 ± 0.7^{b}

^{ab} Means in the same column without common superscripts differ ($P<0.05$). Mean ± SE.

branch were very poor in attracting the olive neiroun in the field in contrast with results obtained in the laboratory. This might be due to the availability of adequate feeding branches in the field compared with the experiments in the laboratory where the fresh cut branch and the two-day aged branch were the only branches available for adult feeding.

Person (1931) hypothesized that bark beetles in general find the stressed host trees by means of olfactory stimuli in response to primary attraction from the stressed host. Heikkenen (1977) proposed that the primary attraction could be volatile compounds resulting from deterioration of host tissues. Depending upon the above results and this hypothesis, the efficiency of different chemical solvents in extracting the attractive compounds was investigated. Since the ten-day aged branch was the most attractive branch to the olive neiroun, it was concluded that this branch had the highest amount of attractive compounds. So, ten-day aged branches were tested for the presence of attractants using a series of chemical solvents. The extracts used were chosen according to their polarity; n-hexane as a non-polar extractant, then moving from chloroform to acetone to methanol and finally distilled water, the polarity increases. Acetone extract was found the most efficient in attracting the olive neiroun adults using the olfactometer. However, with regards to the number of adults entering the flask and its tunnel, no significant differences were found between acetone and methanol. This indicated that the attractive substances present in the ten-day aged branch were polar in nature.

Although our results indicated that attractive compounds to the olive neiroun in the detached branches can be extracted using acetone, the number of insects attracted was relatively small compared with that attracted to the ten-day aged branch in the laboratory experiment. This suggests that acetone and methanol did not extract all of the compounds. Another suggestion is that some of these may have been lost during the evaporation and concentration of the solvents. Procedures to extract and collect all attractants in the olive branches are being investigation in this laboratory at the present time.

Based on the results obtained from the olfactometer experiment, it can be concluded that the principal way in which olive neiroun beetles locate the stressed host trees, or detached branches, is via olfactory perception of polar volatile compounds present in and released from the stressed host tissues.

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