Seedling VOCs induce host preference in *Bagrada hilaris* Burmeister

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Abstract. The Painted bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is an invasive stink bug species native from Asia and Africa, recently reported in United States, Mexico and Chile. This pest attacks mainly cruciferous plants and results particularly aggressive to seedlings at cotyledon stage, causing severe tissue damage, and in some the death of the plant. In this study the role of VOCs emitted by seedlings of four cruciferous species in the host preference behaviour of B. hilaris was evaluated. Two choice experiments using the vertical open Y-shaped olfactometer were carried out testing the attraction of B. hilaris adults toward seedlings of *Raphanus sativus*, *Eruca sativa*, *Brassica rapa* and *B. carinata*. The VOCs emitted by seedlings of these species were also collected in headspace using solid phase micro-extraction (SPME) method and analyzed by gas chromatography mass spectrometry (GC-MS). The results of the behavioural experiment evidenced the *B. hilaris* preference for *R. sativus*, *E. sativa* and *B. rapa* over *B. carinata*. However, adults of *B. hilaris* did not elicit any significant preference among *R. sativus*, *E. sativa* and *B. rapa*. Results of the chemical analyses evidenced the VOCs of *R. sativus*, *E. sativa* and *B. rapa* have in common the same main compound identified as benzophenone, missing in *B. carinata*. These results suggest a role of this compound in the host preference elicited from *B. hilaris* and might be a possible candidate as attractant for this pest.

Keywords. *Raphanus sativus* - *Eruca sativa* - *Brassica campestris* - *Brassica carinata* - Olfactometer.

Plantules VOCs induisant préférence de l'hôte dans Bagrada hilaris Burmeister

Résumé. *La punaise peinte, Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae), est une espèce de punaise envahissante originaire d'Asie et d'Afrique, récemment signalée aux États-Unis, au Mexique et au Chili. Ce ravageur attaque principalement les plantes crucifères et est particulièrement agressif pour les plantules au stade cotylédon, causant de graves lésions tissulaires et parfois la mort de la plante. Dans cette étude, le rôle des composés organiques volatils (COV) émis par les semis de quatre espèces de crucifères dans le comportement des préférences de B. hilaris en tant qu'hôte a été évalué. Deux expériences de choix utilisant l'olfactomètre vertical en forme de Y ouvert ont été réalisées pour tester l'attraction des adultes de B. hilaris vers les semis de Raphanus sativus, Eruca sativa, Brassica rapa et B. carinata. Les COV émis par les plantules de ces espèces ont également été collectés à espace de tête par la méthode de micro-extraction en phase solide (SPME) et analysés par chromatographie en phase gazeuse-spectrométrie de masse (GC-MS). Les résultats de l'expérience comportementale ont mis en évidence la préférence de B. hilaris pour R. sativus, E. sativa et B. rapa par rapport à B. carinata. Cependant, les adultes de B. hilaris n'ont pas révélé de préférence significative entre R. sativus, E. sativa et B. rapa. Les résultats des analyses chimiques mettant en évidence les COV de R. sativus, E. sativa et B. rapa ont en commun le même composé principal identifié comme benzophénone, absent dans B. carinata. Ces résultats suggèrent un rôle de ce composé dans la préférence de l'hôte B. hilaris et peut être candidat possible comme attractant pour ce ravageur.*

Mots clés. *Raphanus sativus – Eruca sativa – Brassica campestris – Brassica carinata – Olfactomètre.*

I - Introduction

Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is a pest that feeds on the several crops belong to brassicaceous family (Huang *et al.,* 2014). *Bagrada hilaris* is native from Asia and Africa, its first report in Europe was 1978 in Pantelleria Island (Italy), where it feeds on caper bush *Capparis spinosa* L. (Colazza *et al.,* 2004). In 2008, *B. hilaris* was reported in USA in California, probably introduced by commercial trade, afterward it rapidly expanded its range

to the brassicaceous crops of coastal California and southwestern Arizona (Palumbo and Natwick, 2010). Successively it was found in Nevada, New Mexico, and Utah (Bundy *et al.,* 2012), and more recently in México (Sánchez-Peña, 2014), and Hawaii (Palumbo *et al.,* 2016).

Since its introduction in North America, *B. hilaris* had exerted a strong negative impact on agriculture, it has been estimated that about 90% of the broccoli acreage planted in USA has been infested by the painted bug, with yield losses often exceeding 10% of production (Huang *et al*., 2014). The economic impact caused by *B. hilaris* on this vegetable industry could be significant considering that the production of broccoli, cauliflower, cabbage, and other Brassicaceous crops in Arizona and California was collectively valued at over $1 billion in 2011 (Huang *et al.*, 2014).

In particular plant at seedling stages are highly susceptible to feeding from *B. hilaris* on cotyledons (Huang et al*.* 2014). several studies confirm that *B. hilaris* is strongly attracted by young seedlings of plants belonging to Brassicaceae, with marked preference for some species in particular as *Raphanus sativus* L. (Huang *et al*., 2014), *Eruca sativa* L. (Joseph *et al*., 2017), *Brassica oleracea* L and *Brassica napus* L (Guarino *et al*., 2018).

It is important to understand the olfaction cues that elicit host plant preferences of *B. hilaris* at seedling stage, in order to expand our knowledge on its chemical ecology and possibly for developing useful tools for integrated pest management programs (Huang *et al*., 2014). *Volatile organic compounds (VOCs) that mediate B. hilaris attraction toward host plants at seedling stage are still to be characterized.* In this study the role of VOCs in the host preference of *B. hilaris* adults was evaluated by using four cruciferous species at seedling stage (7-days old): *Raphanus sativus, Eruca sativa, Brassica rapa* and *B. carinata*.

II - Materials and Methods

1. Rearing

The colony of *B. hilaris* was established and restocked regularly with individuals collected from caper (*Capparis spinosa* L.) fields on the island of Pantelleria (Italy). The colony was fed with cauliflower and cabbage plants, depending on seasonal availability. Insects were reared in an environmentally controlled room (30 ± 2 °C, 70 ± 10% RH, photoperiod 16L: 8D). Seedling of *R. sativus, E. sativa, B. rapa* and *B. carinata* used in this experiment was grown in cotton wool (10 g) soaked with distilled water and held in glass containers with a distance of circa 0.5 cm between seeds, then placed in an environmentally controlled growth chamber (25 ± 1 °C, 70 ± 10% RH, photoperiod 16L: 8D).

2. Bioassays

Bioassays were carried out with an open vertical Y-shaped olfactometer consisting of a brass rod (left and right arms 20 cm long, central arm 25 cm long, 1.5 cm diameter). The left and right arms were covered with two glass tubes (18 cm long, 5 cm diameter) terminating in hose nipples connected by tygon tubes to a high–purity air source, and air flow was controlled with a flow-meter at a rate of 0.2 l/min. The air flowed through two glass chambers (125 ml each) which held the test stimuli. Light was provided with a halogen lamp (Osram, 12V–35W, Münich, Germany) hanging 30 cm above the olfactometer. Experiments were carried out under ambient laboratory temperature and humidity conditions (25 ± 3 °C, and 50 ± 15% RH). For each replicate, a single adult was gently placed at the bottom of the central arm of the olfactometer with a paint brush and allowed 10 min to respond. The bugs moved from the bottom upward toward the light source and upon arriving at the Y junction, chose between the two different volatile stimuli. The criterion for a response was that the test bug walked in the test arm or the control arm (left–right), for at least 5 cm past the Y junction (first choice). In this experiment, volatiles from *R. sativus, E. sativa, B. rapa* and *B. carinata* were tested according to the

following design: 1) *B. carinata* vs *R. sativus*, 2) *B. carinata* vs *E. sativa*, 3) *B. carinata* vs *B. rapa*, 4) *B. rapa* vs *E. sativa*, 5) *B. rapa* vs *R. sativus,* 6) *R. sativus* vs *E. sativa.* The number of replicates carried out for each experiment was from 82 to 113.

3. VOCs collection

The plant volatiles were collected separately from each of the four species using clusters of fifty seedlings (seven days old). The VOCs collection was carried out for twenty hours by headspace solid phase micro-extraction (SPME) using a polydimethylsiloxane (PDMS) fiber. Chemical analysis of VOCs headspace was carried out using a GC-MS Agilent 6850 GC system equipped with a DB5-MS column, interfaced with an MS5973 quadruple mass spectrometer.

1. Statistical analysis

Data from open vertical Y-shaped olfactometer experiments were analyzed with χ^2 tests. Statistical analyses were performed using Statistica 7.0 for Window (Statsoft 2001, Vigonza, PD, Italy).

III - Results and discussion

In open vertical Y-shaped olfactometer bioassays *B. hilaris* adults were more attracted to *R. sativus* rather than to *B. carinata* VOC (χ^2 = 6.45, df = 1, P < 0.01, N = 113), to *E. sativa* rather than to *B. carinata* VOC (χ^2 = 7.71, df = 1, P < 0.005, N = 109) and to *B. rapa* rather than to *B. carinata* VOC (χ^2 = 7.36, df = 1, P < 0.006, N = 99) (Figure 1). In the same experiment bugs did not show preference for the *B. rapa* over *E. sativa* (χ^2 = 0.72, df = 1, P < 0.39, N = 88), for *B. rapa* over *R. sativus* (χ^2 = 0.04, df = 1, P < 0.82, N = 82) and for *R. sativus* over *E. sativa* (χ^2 = 1.41, df = 1, P < 0.23, N = 102).

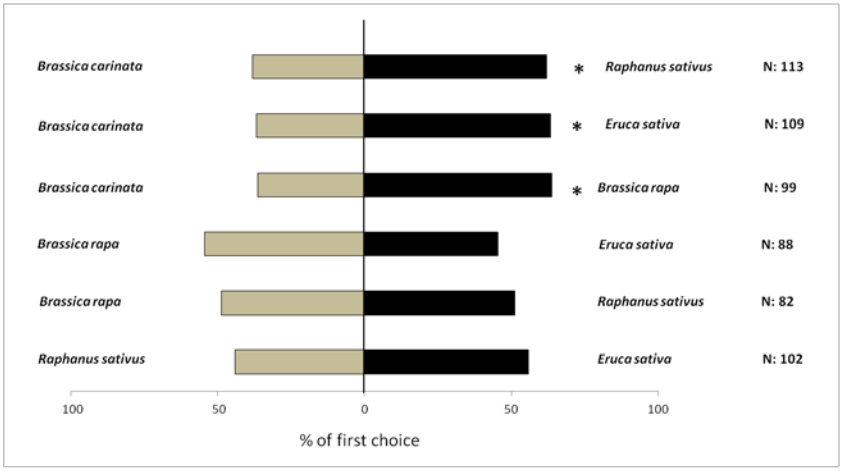

Figure 1. **Host preference responses (% first choice) in open vertical Y-shaped olfactometer bioassays of *B. hilaris* adults to seedlings of *R. sativus, E. sativa, B. rapa* and *B. carinata*. N = number of replicates; * = P < 0.01; χ^2.**

These results provide primary information about host plant VOCs are exploited by adults of *B. hilaris* in location and recognition of their host plants. In bioassay by vertical Y shape olfactometer indicated that some *Brassica* species are preferred over others, with *B. hilaris* adults preferentially orientating toward seedlings of *R. sativus, E. sativa, B. rapa* rather than *B. carinata,* while no difference was observed between *R. sativus, E. sativa* and *B. rapa* which suggested that attraction to preferred hosts is primarily mediated by olfactory rather than visual cues.

Chemical analyses by GC-MS (Figure 2) of VOCs collected from *R. sativus, E. sativa, B. rapa* and *B. carinata* seedlings showed that the VOCs emitted by *R. sativus, E. sativa* and *B. rapa* seedlings were quite similar, whereas the VOCs of *B. carinata* seedlings were markedly different. Unexpectedly, the common green leaf volatiles and monoterpenes observed in other studies of *B. oleracea* var. botrytis volatiles using plants 4-5 weeks old or older (Guarino et al. 2017) were not observed in the VOCs from seedlings. Rather, the VOCs from seedlings of *R. sativus, E. sativa* and *B. rapa* were dominated by a single organic compound, with minor amounts of other unknown compounds. The mass spectra of this organic compound were matched in the NIST mass spectral database to Benzophenone with the formula$C_{13}H_{10}O$. Because of the benzophenone detected in this study constitute >90% of the VOCs of *R. sativus, E. sativa* and *B. rapa* seedlings that attracted *B. hilaris* in our study, and no difference was observed between *R. sativus, E. sativa* and *B. rapa*, it seems likely that they are the key compounds exploited by *B. hilaris* in its strong attraction to seedlings of its host plants. However, at present, it is not possible to exclude the possibility that other compounds present in minor amounts may be partly or wholly responsible for the activity seen. The possibility that *B. hilaris* may exploit benzophenone for host location is interesting, given that these types of compounds have generally a defensive role (Curtze *et al*., 1998; Schmitt *et al.,* 2006).

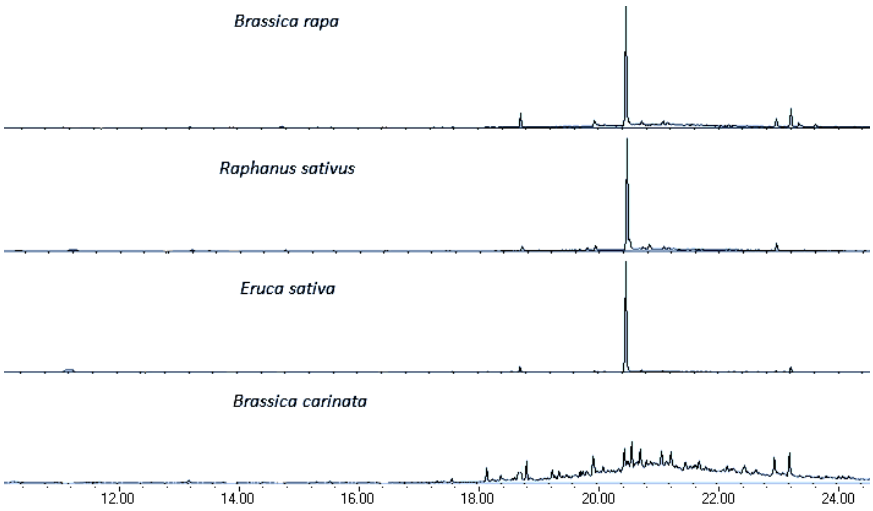

Figure 2. Representative gas chromatograms of VOCs from *R. sativus, E. sativa, B. rapa* and *B. carinata.*

IV - Conclusions

This study provides important ecological information about the possibility that host plant VOCs mediate the host preference behaviour of *B. hilaris* toward some specific cruciferous seedlings. As the main compound present in all the preferred species is benzophenone, it is likely that this compound could be a primary mediator of these insect-plat interactions. Moreover, in consideration that at the moment suitable lures for monitoring this pest in the field are missing, benzophenone might be considered a promising candidate for further trials to test it as attractant in laboratory and field.

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