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FIRST STUDY OF HOST-PLANT PREFERENCES OF *SINOPLA PERPUNCTATUS* (HEMIPTERA: ACANTHOSOMATIDAE), A STINK BUG FROM THE ANDEAN-PATAGONIC FOREST

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ABSTRACT

Host plants of phytophagous insects are not equally attractive. The aim of this study was to evaluate the host-plant behavior of *Sinopla perpunctatus* Signoret (Hemiptera: Acanthosomatidae) and determine which parameters influence the insect's motivation. *Sinopla perpunctatus* is a green stink bug present in the Andean-Patagonian forest associated with various host species of *Nothofagus* (Fagales: Nothafagaceae) southern beech trees. For the assays, we collected adults at Quilanlahue forest (Neuquén, Argentina), an area affected by the deposition of volcanic ash, whose effects we decided to evaluate. A sequence of paired comparisons was used to evaluate the host-plant preferences. Branches from *N. antarctica, N. nervosa, N. obliqua* and plastic leaves (control), with or without ash were the stimuli offered. In total we performed 18 combinations of paired tests to evaluate the different combinations of treatments. We considered the choices of each individual by day as the response variable. The data were analyzed by several models that explain the probability of an individual choosing a particular option depending on host plant preferences and the effect of different parameters of motivation (including the age, ash, home effect and combinations).Thus a total of eight models were proposed to explain the data, from which we selected the model with the lowest value of the deviance information criterion (DIC). The selected model established that preferences were determined by the motivation for change after the organism has already made a choice (i.e., home effect). The preferred host plants of *S. perpunctatus* were found to be *N. antarctica* and *N. obliqua*, while its preference for *N. nervosa* occupied a second place. Physical or chemical defenses of the leaves among species could explain this rank. However, volcanic ash was found to have no effect on motivation or host preference. Finally, we concluded that such studies of behavior and of parameters that may affect insect motivation should allow us to reach greater understandings of insect-plant interactions.

Key Words: Acanthosomatidae, host-plant preferences, green stink bug, motivation, Nothofagaceae, volcanic ash

RESUMEN

Las plantas hospedadoras de los insectos fitófagos no les resultan igualmente atractivas. Poseen un gradiente de preferencias que está principalmente determinado por la motivación que posee el insecto durante el proceso de selección del hospedador. El objetivo de este estudio fue evaluar el comportamiento de *Sinopla perpunctatus* Signoret (Hemiptera: Acanthosomatidae) durante el proceso de elección de su planta hospedadora y determinar la influencia de la motivación del insecto en este proceso. *Sinopla perpunctatus* es una chinche verde que se encuentra presente en el bosque Andino-Patagónico asociada a diferentes especies de *Nothofagus* (Fagales: Nothafagaceae) como plantas hospedadoras. Para los ensayos recolectamos individuos adultos en el bosque de Quilanlahue (Neuquén, Argentina). En junio del 2011 esta área resultó afectada por la deposición de ceniza volcánica, por lo tanto también decidimos evaluar el efecto de la ceniza en la motivación. Para evaluar las preferencias, realizamos una secuencia de pruebas de comparaciones pareadas. Como estímulo les ofrecimos ramas de *N. antarctica, N. nervosa, N. obliqua* y hojas de plástico (control) con o sin ceniza. En total realizamos 18 combinaciones de pruebas pareadas para evaluar las diferentes combinaciones de tratamientos. Consideramos como variable respuesta las elecciones realizadas por cada individuo en cada día. Los datos fueron analizados mediante el desarrollo de varios modelos que explicaban la probabilidad de los individuos de elegir una opción determinada de acuerdo a sus preferencias y al efecto de diferentes parámetros (i.e. Efecto de la edad, ceniza, hogar y sus combinaciones) en la motivación del insecto. En consecuencia, se confeccionaron ocho modelos para explicar los datos obtenidos, de los cuales seleccionamos aquel con el menor valor de DIC. El modelo elegido establece que las preferencias están determinadas por la pérdida de motivación que posee el insecto a cambiar de planta hospedadora una vez que eligió. *S. perpunctatus* tiene una mayor preferencia por *N. antárctica* y *N. obliqua*, mientras que *N. nervosa* está como segunda opción. Consideramos

que las diferencias entre las defensas físicas y químicas de las distintas especies de plantas hospedadoras podrían explicar este gradiente de preferencias. En cuanto a la ceniza volcánica, no ejerce efecto alguno sobre las preferencias o motivación de estos insectos. Finalmente, consideramos que estudiar el comportamiento y los parámetros que podrían afectar la motivación de los insectos, nos permite alcanzar una mayor comprensión de las interacciones entre los insectos y las plantas.

Palabras Clave: Acanthosomatidae, ceniza volcánica, chinches verdes, Nothofagaceae, preferencias de plantas hospedadoras.

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Phytophagous insects have the ability to affect plants both at the individual and population levels. They can cause stunted growth or even death of the plant and influence population dynamics by feeding on flowers, fruits or seeds (Dajoz 2001). The ability of phytophagous insects to select a host depends on the integration of extrinsic and intrinsic factors (Bell 1990). Chemical and physical properties of plants, the insect's internal state, environmental characteristics, interactions (competition, predation) in which they are involved and the range of host options are some of the factors that influence host selection (Perez-Contreras 1999).Therefore host plants are not equally attractive for all insects of a species, which may establish a ranked order of host preferences (Singer et al. 1992). The gradient of host preferences is mainly determined by the motivation during the host- selection process, which may result in differences between acceptance and preference of a given host plant species (i.e., being attracted to the host versus selecting and using it for its benefit). Motivation can be influenced by environmental factors, but mainly by the internal state and the age of insect (Visser 1988; Singer et al. 1992).

In the Andean-Patagonian forest located in the mountainous regions of southern Chile and southwestern Argentina, there is a great diversity of phytophagous insect species associated with forest species of the genus *Nothofagus* (Gentili & Gentili 1988; McQuillan 1993; Grandon 1996). These native trees, in addition to its intrinsic importance, possess characteristics that make them of great economic and silvicultural importance for the quality of its wood and its ability to accept various methods of forest management and industrial use (Barbero et al. 2011).

Sinopla perpunctatus Signoret (Hemiptera: Acanthosomatidae) it is a native green stink bug is one of the most representative species present in the Andean-Patagonian forest (Osorio 2009; Faúndez & Osorio 2010). It was described in association with different host species of *Nothofagus*(Fagales: Nothafagaceae): *N. antarctica* (G. Forster) Oersted "ñire", *N. nervosa* (Phil.) *Krasser* "raulí" and *N. obliqua* (Mirb.) Oersted "roble pellín" (Faúndez 2007).

The aim of this study was to evaluate the host-plant behavior of *S. perpunctatus* individuals and determine the factors that influence of their motivation.

MATERIALS AND METHODS

Experimental Material

During the summer season 2011-2012, we collected adults of *S. perpunctatus* from *N. antarctica*, *N. nervosa,* and *N. obliqua* trees at Quilanlahue forest in Neuquén (S 40° 8' 10.92 " W 71 ° 28'14 .75") which subsequently were kept in the laboratory without food for 24 h.

This area was affected by the deposition of volcanic ash from a major volcanic eruption that had occurred in Chile at the "Cordón - Caulle" volcanic complex on 4 Jun 2011 (Gaitán et al. 2011). On the basis of work that has shown the erosive and abrasive power of volcanic ash, its potential effect on the integument, digestive system and survival of insects, and because it caused massive destruction of the vegetation that insects use for feeding and/or habitats (Wille & Fuentes 1975; Buteler et al. 2011; Fernández-Arhex et al. 2013), we decided to evaluate it effect on host preference by means of a preference test.

Preference tests were conducted under laboratory conditions with controlled temperature and humidity (20.43 °C ± 0.01, 39.57 ± 0.09% RH).

Experimental Design

A sequence of paired comparisons was used to evaluate the host-plant preferences (David 1988; Bruzzone & Corley 2011). For this purpose, we offered to *S. perpunctatus* adults branches from *N. antarctica, N. nervosa, N. obliqua* and plastic leaves of approximately 10 cm in length. These artificial leaves were used as a control because they had the same color, size, provided shelter as natural leaves and were odorless. The branches were or were not sprinkled with 0.5 g of volcanic ash of 2 particle sizes. Consequently, each type of leaves was subjected to 3 treatments: clean leaves, leaves with fine ash (< 500 μm) and leaves with coarse ash (> 500 μm).

In total there we performed 18 combinations of paired tests to evaluate the different combinations of treatments. Each test group, which consisted of 7 individuals, $n = 126$ was introduced into plastic

containers (12 × 16 × 25 cm). The offered stimuli were placed at the end corners of the container. We observed each individual in its respective container at the same time each day, this procedure allowed us to evaluate the selection made by the insect and to determine whether motivation and preferences were modified over time (Singer et al. 1992; Fernández-Arhex et al. 2013).

The individual's choice was considered as the response variable, specifically when the insect was found to rest on the branch or leaf that had been offered; which suggested a degree of host acceptance. Elections and movements made by the insect were monitored for 1 wk and leaves were renewed daily to assure their freshness.

Statistical Analysis

The data were analyzed by a Bayesian approach, i.e., updating the probability estimate for a hypothesis as additional data is acquired. Therefore; several models were employed to explain the probability of an individual choosing a particular option depending on host plant preferences. The preference scale was based on the Thurstone model of comparative judgments case V (Thurstone 1927; Bradley & Terry 1952), which is the most common way to generate a preference scale from a series of paired comparisons (David 1988). On this scale the preference for *N. nervosa* was arbitrarily assigned the value 0 in the Thurstone scale with 1 as the standard deviation; the value 0 was used as a coordinate origin to reference the preference values of all the other options and parameters. Higher values on it indicate stronger preferences, and minor/negative values indicate rejection.

We used the developed models to evaluate the host plant preferences and the effect of the addition of new parameters. Since we did not have a priori information for the studied variables, we used as an uninformative a priori distribution for all the parameters, i.e., a normal distribution with a mean of 0 and a deviance of 10 and a normal likelihood to measure the function fit. For each model we performed 2 million iterations from which were discarded the first million as a burn-in. From the remainder we chose one in 1,000 to avoid autocorrelation. Geweke plots were used to test convergence (Geweke 1992) and perform visual inspection of variable traces. Analyzes were performed by the PyMC library for Bayesian estimation (Patil et al. 2010) in the Python programming language. Thus the following total of 8 models were proposed to explain the data:

Simplest model (Thurstone case V simple preference model),

Age effect model (the preferences of host plant change linearly as a function of age),

Home effect model (the preference is affected by the motivation of the insect to change its selection),

Age and home effect model (an additive combination of the 2 previous models),

Simplest model plus an effect of the presence of volcanic ash,

Age effect model plus effect of volcanic ash,

Home effect model plus effect of volcanic ash, and

Age plus home model and volcanic ash effect.

Finally, we selected the model with the lowest value of the deviance information criterion (DIC) (Gelman 2003).

RESULTS AND DISCUSSION

The analysis of the proposed models showed that not all host-plant options are equally attractive for *S. perpunctatus*. The selected model with a minimum DIC value corresponded to the home effect, where the host scale was determined by the motivation for change after the insect had made a choice (Table 1; model 3). The other 7 models that included the effect of age, volcanic ash or the combination between the home effect and age, did not explain the host preferences of this insect. Visser (1988) argued that when a phytophagous insect selects a host, it decreases its locomotion due to the loss of motivation.

The preferred host plants of *S. perpunctatus* were found to be *N. antarctica* and *N. obliqua*, while its preference for *N. nervosa* occupied a second place, but within the acceptance range - unlike the plastic leaves which were less preferred (Fig. 1). *Nothofagus nervosa* was characterized by greater leaf thickness, hardness, and hairiness than the other 2 species of southern beech (Barrera et al. 1993). Patterns of host plant volatiles

TABLE 1. VALUES OF DIC (DEVIANCE INFORMATION CRITERION) FOR THE MODELS PROPOSED TO MEASURE THE EFFECT OF VOLCANIC ASH ON FEEDING PREFERENCES OF *SINOPLA PERPUNCTATUS*. THE ASTERISK (*) INDICATES THE SELECTED MODEL.

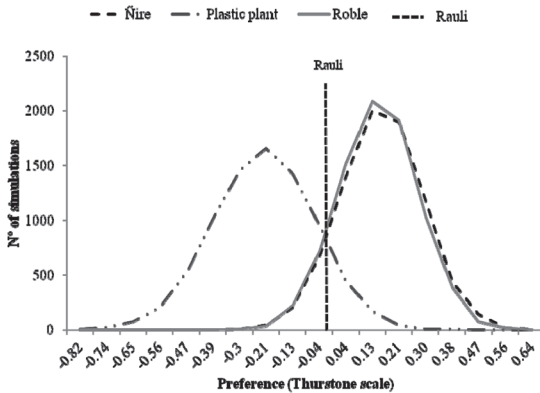| N°	Model	DIC
1	Preferences model	420.28
2	Age effect model	419.94
3	*Home effect model	415.84
4	Home effect and age model	427.28
5	Preferences model + ashes effect	418.17
6	Age effect model + ash effect	422.05
7	Home effect + ash effect	417.94
8	Home effect + age effect + ash effect	422.55

Fig. 1. Host-plant preferences of *Sinopla perpunctatus* for plastic and 3 beech tree species, i.e., *Nothofagus nervosa* ("raulí"), *N. obliqua* ("roble pellín") and *N. antarctica* ("ñire") according to the home effect (selected model). On the X axis is the scale of preferences according to the Thurstone model, while on the Y axis are plotted the numbers of iterations. The curves represent the probability distributions that the insects chose a certain stimulus. Curves located at the right side of the vertical line represent preferred stimuli, whereas negative values (or at left of the vertical line) represent rejected stimuli. The vertical dotted line, which represents the response to «raulí» (*N. nervosa*), was arbitrarily selected as a reference. The data show that *S. perpunctatus* adults prefered "roble" and "ñire" leaves as their food.

emitted from *Nothofagus* leaves could have also played a significant role in host orientation like in the case of *Neuquenaphis* sap-suckers (Hemiptera: Aphididae) (Quiroz et al. 1999). We suggest that the host selection rank of *S. perpunctatus* can be explained by the physical or chemical defenses of the leaves of the *Nothofagus* species (Quiroz et al. 1999; Russel et al. 2000). These defenses would cause a certain degree of antixenosis (i.e., a change in the behavior of the insect caused by the presence of morphological and/or chemical plant defenses).

Even though the volcanic ash could exert a negative effect on insects (Buteler et al. 2011; Fernández-Arhex et al. 2013; Masciocchi et al. 2013). However, this did not occur in the case of *S. perpunctatus* probably because of the tendency of this insect to be located on the underside of the leaves or between folds of the bark, which act as protective barriers (Dajoz 2001; Faúndez & Osorio 2010). This behavior may have decreased the exposure and contact with the ash, and its potentially abrasive effect on the insects integument. The possible impact of the volcanic ash is not the same for all insect species, depending mainly on their habits and behavior (Marske et al. 2007).

The study of the behavior and motivation of insects allows us to determine the gradient of host preferences and reach a greater understanding

of the process whereby host preferences are established (Singer et al. 1992). The host searching behavior - as well as the interaction with plants and ecosystems - determines the success of the individual and its progeny (Bell 1990).

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<center>APPENDIX</center>

Description of the models

A total of 8 models were proposed to explain the results, departing from the simplest model, where $P_{ab} = \text{probit}^{-1}(S_a - S_b)$, where P_{ab} is the probability to choose the host plant a over host plant b, and S_a, S_b are respectively the positions of each offered host in the Thurstone scale of preferences. Each new variable added was expected to cause a change in preferences; expressed as ΔS (or delta in preference).

1) Thurstone case V simple preference model.

2) Age effect model, in which the preference value of the plants changes linearly as a function of age so $P_{ab} = \text{probit}^{-1}((S_{a(t=0)} + s_a T) - (S_{b(t=0)} + s_b T))$, where s is the rate of change in preference as a function of time (T).

3) Home effect model, where the estimated preference is affected by the motivation of the insect to change the host plant: $P_{ab} = \text{probit}^{-1}(S_a + \Delta S_{a} - S_b)$ where the preference S_a is corrected by the motivation to change the plant (ΔS) given that the insect is already in the plant a.

4) Age and home effect, an additive combination of the two previous models:

$$P_{ab} = \text{probit}^{-1}((S_{a(t=0)} + s_a T) + \Delta S_a - (S_{b(t=0)} + s_b T))$$

Finally 5), 6), 7), 8) models plus an effect caused by the presence of volcanic ash:

5) $P_{ab} = \text{probit}^{-1}(S_a + \Delta S_{ah} - S_b)$, where ΔS_{ah} is the factor of correction in the preference by the presence of ash over the plant a.

Therefore the remaining models are:

6) $P_{ab} = \text{probit}^{-1}((S_{a(t=0)} + s_a T) + \Delta S_{ah} - (S_{b(t=0)} + s_b T))$.

7) $P_{ab} = \text{probit}^{-1}(S_a + \Delta S_a + \Delta S_{ah} - S_b)$.

8) $P_{ab} = \text{probit}^{-1}((S_{a(t=0)} + s_a T) + \Delta S_a + \Delta S_{ah} - (S_{b(t=0)} + s_b T))$.