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Research paper

Ecological morphology of the sugar beet weevil Croatian populations: Evaluating the role of environmental conditions on body shape

Darija Lemic[a], Hugo A. Benítez[b,c,*], Thomas A. Püschel[b], Helena Virić Gašparić[a], Mihaela Šatvar[a], Renata Bažok[a]

[a] University of Zagreb Faculty of Agriculture, Department for Agricultural Zoology, Svetošimunska, Zagreb, Croatia
[b] Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, United Kingdom
[c] Instituto de Alta Investigación, Universidad de Tarapacá, Casilla, Arica, Chile

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ABSTRACT

Different rearing environments produce dissimilar stress level in insects, which can be reflected on the ability of an individual to overcome these pressures and spread further. Sugar beet weevil, *Bothynoderes punctivnetris* Germar. (Coleoptera: Curculionidae) is one of the most harmful pests of sugar beet, infesting agricultural fields in different parts of Croatia. It is highly invasive being able to inhabit diverse environments across the country. The aim of this study was to assess the association between different environmental and rearing conditions and the morphological variation observed in the sugar beet weevil body shape. The present results showed significantly different eco-morphs related to different environmental conditions, thus suggesting that the observed ecomorphological changes could be associated to the ability of this pest to spread further.

1. Introduction

The sugar-beet weevil *Bothynoderes punctiventris*, Germar Germar (Coleoptera: Curculionidae) is one of the most devastating pests of sugar beet occupying the southwestern part of Europe and North Africa (EPPO, 2015; Hoffmann, 1966; Manninger, 1990; Toth et al., 2007a,b). In Croatia sugar beet presence has been reported on 20.245,00 ha with the total root yield of 51.9 t/ha (Statistical Yearbook of Croatia, 2013). Due to climate change and insufficient crop rotation, sugar beet weevil populations have caused serious economic damage in the agricultural industry (Čamprag, 1954, 1956, 1973). This pest can be very destructive both in adult and larval stages (Čamprag, 1959). Weevils, just after overwintering, feed on young sugar-beet plants and reduce crop stand (at high population densities re-sowing may be required) (Sekulic et al., 1997). During their larval stages, they live underground damaging the sugar-beet root system mainly during spring and summer, consequently reducing the quantity and quality of the yield (Čamprag, 1959; Toth et al., 2007a). The first sugar beet weevil mass attack recorded in the eastern part of Croatia occurred in 1922 (Kovačević, 1929). Since then sugar beet weevil has been an important pest for agricultural sugar beet producers from eastern Croatia. However, throughout the last decade several attacks have been recorded in the western areas of the country as well (Maceljski, 2002). The areas where sugar beet is produced in Croatia differ in terms of soil type and climate conditions. Southeast Croatia is characterized by chernozemic soils, while the climate is continental with hot summers and low rainfall. The northeast area is described as exhibiting a leached or pseudogley soil type with a continental climate with characteristic hot summers. The west portion of the country has been described as showing gley soils and alluvial soils, whilst the climate is continental with slightly lower summer temperatures, and a greater amount of total annual rainfall (Bogunović, 1987; Bogunović et al., 1996; Gračanin, 1942; Zaninović et al., 2000).

Soil type and climatic conditions are important both for cultivation and production of sugar beet as well as for the infesting sugar beet weevil population. According to Čamprag (1986) the most favorable soil type for the development of sugar beet weevil is the chernozem type. It has been pointed out as well, that the most suitable climate conditions for sugar beet weevil development are temperatures >10 °C (Čamprag, 1973, 1986; Maceljski,

* Corresponding author at: Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester M139PT, United Kingdom.
E-mail address: hugo.benitez@postgrad.manchester.ac.uk (H.A. Benítez).

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Table 1
Localities with the corresponding geographical coordinates and environmental factors.

Location	Specimens (n)	Coordinates	Average annual temperatures	Total annual rainfall	Soil type
Lukač	37	45°52′26,21″N; 17°25′8,732″E	12.5 °C	816.2 mm	Alluvial
Karanac	100	45°45′37,257″N; 18°41′9,275″	12.6 °C	1261.9 mm	Luvic and pseudogley ground
Tovarnik	100	45°9′53,576″N; 19°9′7,94″E	13.1 °C	822.6 mm	Chernozem

2002). According to Maceljski (2002) future warming will benefit further population expansions, thus increasing the possible level of weevil harmfulness both worldwide and in Croatia. In addition to climate change the emergence of a more invasive pest population is affected by the fact that newly applied insecticides have much lower secondary effect on sugar beet weevils as compared with previously used insecticides (Bažok, 2010; Bažok et al., 2012).

It is known that various environmental conditions result in different levels of stress in insects, which consequently reflect the ability of individuals to adapt and spread (Clarke, 1998; Lalouette et al., 2011). For instance, in warm areas with dry climate, the sugar beet weevil can cause severe damage by intense feeding (Bažok et al., 2012; Maceljski, 2002). The assumption that the expansion and population increase are caused by warming, especially in the past 10 years, which have been hot and dry, is justified since the number of sugar beet weevil population is higher in eastern Croatia where conditions for growth, development and reproduction are the most suitable (Čamprag, 1973, 1986; Maceljski, 2002). On this area massive attacks and damages on sugar beet are extremely abundant (Kovačević, 1929; Maceljski, 2002). In recent years sugar beet weevil has spread in other sugar beet cultivation areas in Croatia, but to the date there are no severe damages recorded. Since climatic and soil conditions of sugar beet cultivation areas differ between different parts of Croatia, it is logical to assume that they are significant factors influencing population growth and expansion to new areas.

The overtime adaptation of species to a specific environment is the result of environmental influence, pressure and geographic distance (Alibert et al., 2001). Previous studies showed that temperatures, adverse nutritional stress, chemical presence in soils, population density and many other factors generate stress during development and can lead to increased morphological differences (Benítez et al., 2008; Benítez, 2013; Clarke, 1998). Therefore, it is expected that when environmental conditions change, organisms and populations should adapt to the new conditions (Benítez et al., 2014a; Clarke, 1998). Adaptive variation in insects reflects historical evolution and determines the populations phenotypic response (Ghalambor et al., 2007; Parsons and Joern, 2014; Pfennig et al., 2010). Moreover, Bouyer et al. (2007) found that the environmental impact on the organism's genotype takes more time to manifest than on the phenotype, and therefore they propose to investigate the impact of environmental factors primarily on phenotypic characteristics. Morphology can be studied by analyzing morphometric measurements (i.e., shape and size) and its changes in relation to other factors (e.g., soil, temperatures, rainfall, growing conditions) (Adams and Rohlf, 2000; Henderson, 2006; O'Higgins, 2000; Rohlf and Marcus, 1993). Studies have analyzed the influence of environmental conditions on insect morphology (Benítez et al., 2014a,b). For instance, it is possible to obtain important information about the influence of different ecological factors on morphology (e.g., anti-predator defenses, behavior and sexual dimorphism, physiology and environmental adaptations), by using geometric morphometric methods. These studies have provided evidence for the detection of developmental instability, as well as differences between resistant and susceptible variants (Benítez et al., 2014a,b; Lemic et al., 2014). GM methods can also be really useful as a tool to assess the occurrence and expansion of certain insect species (Benítez et al., 2014a,b). Therefore our aim was to analyze the association between environmental factors such as soil type, climate factors and weevil morphology, because understanding this relationship can provide better insight regarding how this species morphologically adapts to new conditions. This evidence could provide in turn more information that could be associated with the invasive process of this pest species.

2. Materials and methods

2.1. Samples collection

Adult specimens of *Bothynoderes punctivnetris* were collected on sugar beet fields in April and May 2015 on three locations in continental Croatia. The research was conducted in Lukač, Virovitica-Podravina County, Karanac, Osijek-Baranja County and in Tovarnik, Vukovar-Sirmium County.

At each of these locations aggregation pheromones with hunting containers type TAL for sugar beet weevil (CSALOMON®) were entrenched in the ground on peripheral part of the fields. Traps were checked weekly and collected specimens were preserved in 96% ethanol. Fifty males and fifty females from the site Tovarnik and Karanac and 19 females and 18 males from the site Lukač were used in further analysis. The lower number of specimens from the Lukač site is the result of the smaller population size in that area.

2.2. Climate conditions

Average and total monthly temperatures and annual amount of rainfall for vegetation season 2014 were provided from the Croatian Meteorological and Hydrological Service (Table S1). Soil type was defined based on study of Bogunović et al. (1996). In the area of Tovarnik, chernozem soils are dominant, while in Lukač gley soils are more abundant. Finally in Karanac, aluvic and pseudogley soils are characteristic (Table 1).

From each field 1.5 kg of soil was sampled. Samples were taken by a pedological probe from five locations in each considered field site, to the depth of a plow layer (30 cm). Soil testing and analyses were conducted in the pedology laboratory of the Department of Soil Science, Faculty of Agriculture, University of Zagreb and Josip Juraj Strossmayer University of Osijek for sediment grain size, humus content and pH. Soil texture was determined by following standard methods (ISO 11277, 2004a). The humus content of soil was analysed using Tjurin's method (Škorić, 1991). According to international standards and Croatian Standards (ISO 10390, 2004b) soil pH in H_2O and KCl were determined using a Beckman's electrometrical pH meter. All the values of the environmental conditions used in this study are explained at the Table 2.

2.3. Shape analysis

Geometric morphometric analyses were performed using an image of the ventral body side of individuals taken by Leica DFC295 digital camera on a trinocular mount of a Leica MZ16a stereomicroscope and saved as JPEG files using Leica Application Suite v3.8.0 (Leica Microsystems Limited, Switzerland).

16 landmarks were digitized (LMs: anatomical homologous coordinates) using the tpsDIG v2.17 software (Rohlf, 2013) (Fig. 1). X–Y coordinates were obtained for all LMs and the shape informa-