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Are trypsin inhibitors responsible for the suitability of different legumes for *Acanthocelides obtectus* development?

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Abstract: Legumes represent a valuable source of proteins in human and animal nutrition, as well as of different nutrients. Also, they enable diversification of agroecosystems and can be considered climate smart crops. Therefore, they are gaining importance in both developed and developing countries. The most important, and often limiting factor in legume production is the presence of the bean weevil (*Acanthoscelides obtectus*). This work tested the suitability of three different legume species, i. e. common bean, grass pea and faba bean (two accessions of each species) with growing interest in human nutrition, for the development and population growth of this pest, with special emphasis on the levels of anti-nutritive compounds – trypsin inhibitors. The suitability was assessed based on the progeny production after each month. The development and progeny production were significantly affected by the legume species, but also by the accessions. The highest number of emerged adults was on common bean, while the lowest regardless on observation period, was on faba bean, indicating its low suitability for weevil development. Correlation analysis detected significant influence of the level of trypsin inhibitors on progeny production.

Key words: bean weevil, common bean, faba bean, grass pea, progeny production, anti-nutritive compounds.

Introduction

Distributed worldwide, the legume family has a significant role in total agricultural turnover. Common bean (*Phaseolus vulgaris* L.) is among the most important crops for human consumption. For people in developing countries, it is usually the only source of proteins, fibres and other nutrients (Broughton et al., 2003). Besides common bean, two other legumes, faba bean (*Vicia faba* L.) and grass pea (*Lathyrus sativus* L.) were traditionally used by the local people. However, these two species are denoted as less cultivated and neglected. Some efforts are being made towards their reintroduction in cultivation and use because they meet the basic dietary needs of millions of people and animals around the world thanks to high protein, carbohydrate, fibre, and micronutrients contents. Also, these two species are getting more attention from producers and consumers due to their unimpaired nutritional and medicinal value, as well as a better tolerance to adverse environments (Multari et al., 2015; Ramya et al., 2022). Common bean, grass pea and faba bean enable diversification of agroecosystems and can be considered climate smart crops. Thus, they are important components in sustainable cropping systems (Pathania et al., 2014).

Legume production is often limited by insect pests, and major loses are caused by the bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchinae). This is a serious pest of several legumes worldwide that infests different hosts in the fields and storages (Vuts et al., 2018). Larvae feed inside kernels and cause quantitative damages by reducing seed weight, as well as qualitative losses by reducing the physiological quality and germination capacity. Also, their presence increases temperature and moisture content in a seed bulk and spoils the seeds purity with insect residues (Faroni and Sousa, 2006). Although the main host of this pest is *Phaseolus vulgaris* L., the bean weevil may adapt to different non-host legumes including grass pea (*Lathyrus* sp.) and faba bean (*Vicia faba*) (Labeyrie and Hossaert, 1985; Hamraoui and Regnault-Roger, 1995). Nonetheless, some leguminous species have developed a certain degree of resistance against insect pests. One of the "strategies" is the production of anti-nutritive compounds, such as protease inhibitors (PI) that may play defence role against insect pests (Alizadeh and Leung, 2011; Bhattacharjee et al., 2012). Production of PIs is one of the naturally occurring defence mechanisms in plants, which adversely affect protein digestion. PIs cause reduction in the availability of essential amino acids and exert physiologic stress on insects leading to growth retardation (War et al., 2012). PIs in legumes are one of the most promising weapons that confer resistance against insects by inhibiting proteases present in the gut of insect larvae (Jalalia et al., 2014). Also, they have the potential to be used in breeding programs to enhance the seed tolerance to storage pests.

There are some studies that are referring to the bean weevil bionomy, ecology and suppression. However, there is a lack of studies on its preference and biochemical bases for specific species tolerance to this pest. This work aimed to test the suitability of three different legumes with growing interest for human nutrition (common bean, faba bean, and grass pea), for the development of the bean weevil and progeny production, as well as the influence of trypsin inhibitors (PIs) level on legume preference and/or tolerance to this pest.

Materials and methods

Commodities and tests

The development of the bean weevil (*A. obtectus*) and progeny production was assessed on different legume species and accessions common bean (KP114 and KP139), faba bean (KVF4 and KVF19) and grass pea (KL4 and KL12), in a "no-choice" test. The legume accessions were chosen as the most typical representatives of the legume species (typical seed weights, shapes, and colours within the respective species). Seeds were not treated with pesticides and were exposed to -80 °C for 30 min to eliminate potential pests and fungi.

Experimental protocol

The laboratory population of the bean weevil was reared under laboratory conditions in glass jars (5L), on the common bean variety "Belko", at a constant temperature of 26 ± 2 °C, 50 % air humidity and at light regime 16:8 (day/night), as described by Szentesi (1972). Newly emerged adults (60 specimens per jar) were placed in separate jars on each legume (500 g) and were let to develop for four months (120 days). The jars with weevils were incubated in a climatic chamber during the entire experiment, under the same conditions as for the rearing of the parenteral population. After each month, the number of newly emerged weevils (progeny production) was counted and let further to develop. The experiment was set in four replicates.

The trypsin inhibitor activity (TIA)
TIA assay was based on the hydrolysis of N-α-benzoyl-DL-arginine-p-nitroaniline by trypsin as described by Kakade et al. (1974). Procedure for TIA testing was carried out using microtiter plate method with assay conditions described by Liu and Markakis (1989) and modified by Župunski et al. (2018). Absorbance of reaction mixtures were measured using photometer, at a wavelength of 410 nm (Multiskan Ascent microplate photometer, Thermo Fisher Scientific). Trypsin inhibitor activity has been defined as trypsin units inhibited per mg of sample.

Statistical analysis
The differences in progeny production (number of emerged adults) were analysed using one way ANOVA and Bonferroni test (95% confidence interval). The dependence of progeny production on trypsin inhibitors activity was analysed using correlation analysis in statistical software SPSS 21 (2018).

Results

The progeny production of the bean weevil was significantly affected by the legume species on which the development occurred (Table 1), as well as by the accession. The number of emerged adults after each month, varied significantly among legume species and accessions ($F_{3,12}$ = 1087,10**; 2321,91**; 5433,61** and 7034,35**, $P < 0.000$; respectively; Figure 1). Significantly higher number of weevils emerged on the common bean and grass pea, regardless on the observed periods. The recorded average number of weevils on KP139 was 81.5 after the first month, 265 after the second, 748 after the third and 1236 after fourth month, while on KP192 it was 84.6, 199, 705 and 1067, respectively. On the grass pea, variety KL12, the average number of weevil specimens was 95.7, after the first month, 289 after the second, 783.5 after the third and 1312.5 specimens after fourth month. For variety KL4, the number of emerged adults was 80, 187, 657 and 843.5, respectively. Nevertheless, the total number of emerged adults did not significantly differ between the common bean and grass pea after the first month, but it was significantly different after two, three and four months (Figure 1).

Table 1. Activity of trypsin inhibitors.

Seed species	Variety	TIA (TU/mg)
common bean	KP139	38.01 d
	KP192	47.02 c
grass pea	KL12	32.00 e
	KL4	52.17 c
faba bean	**KVF4**	**63.34 b**
	KVF19	**71.01 a**
F value		156.08**

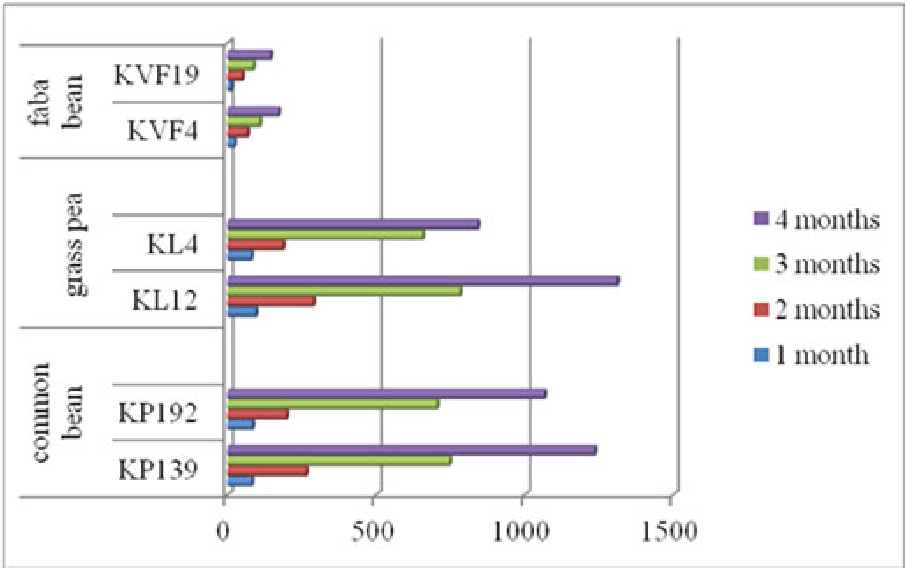

Figure 1. Bean weevil development on different legumes after one, two, three and four months.

The lowest total number of emerged bean weevils, regardless the observation period was on both accessions of faba bean. On KVF4 the average number of weevils was 8 after the first month, 67 after the second, 109 after the third and 172.3 after fourth month. On KVF19 the average number of specimens was 5, 50, 87 and 145.5, respectively.

According to Szentesi (2020) the bean weevil is an oligophagous species feeding on plants within the tribe Phaseoleae (Fabaceae) that comprises *Phaseolus, Glycine, Lablab* and *Vigna* genera. Occasional observations were made on other legume genera such as *Lupinus*, *Cicer, Vicia, Lens* and *Lathyrus* (Jarry and Bonet, 1982; Hamraoui and Regnault-Roger, 1995). On seeds of preferred hosts, under favourable conditions, practically every larva becomes an adult, but on non-host and acceptable non-host seeds, such as the faba bean, only a very small percentage of the young larvae reach maturity (Larson and Fisher, 1938). Our results support these findings since the lowest number of adults emerged from the faba bean seeds, regardless on the observation period, and the population growth was the lowest thorough the entire experiment. Several authors (Cronin and Abrahamson, 2001; Zhang and Liu, 2006) noted that some species belonging to the acceptable non host group like *Vicia* sp. (faba bean included) and *Lathyrus* sp. (grass pea included), allowed larval development at some extent. However, the seeds of genus *Lathyrus* were preferent and more suitable for the bean weevil development, while the development was much lower on seeds of *Vicia* species, as also proven in our work.

The highest activity of TI was detected in both faba bean varieties (Table 1), while the lowest was on grass pea variety KL12 (32.00) and common bean KP139 (38.01).

The correlation analysis indicates a strong negative correlation between the activity of TI and the number of emerged adults after all four months. Pearson's correlation coefficient is significant after the first month (-0.919*), and highly significant after the second (-0.985**), third (-0.923**) and forth months (-0.968**). Results indicate that the increase in the activity of TI causes reduced progeny production, deduced from the number of emerged adults after each observation period. The correlation is more expressed after the second, third and fourth month, which implies reduced fitness of the bean weevil population.

This is the first report on the influence of trypsin inhibitors activity in legume varieties and species on the bean weevil progeny production. We can speculate that a lower bean weevil development and progeny production on faba bean was due to biochemical characteristics including level of trypsin inhibitors. Other important factors (chemicals, further physical, environmental, life-history traits, mobility, predators, host genotype, etc.) that influence host selection (de la Masselière et al., 2017) were not investigated in this work, and will be studied in further investigations. Mickel and Standish (1947) reported that the larvae of certain insect species were unable to normally develop on soybean products, due to the presence of TIs, while Lipke et al. (1954) reported the same for larvae of the flour beetle, *Tribolium confusum*. Following these previous studies, there have been many examples of protease inhibitors activity against different insect species (Koiwa et al., 1998). Opposite results were presented by Guzmán Maldonado (1996). Namely, when testing seeds of 17 common bean varieties for resistance to *A. obtectus* under no-choice and free-choice conditions, no correlations were found with seed hardness, coat thickness, tannins, protein content or trypsin inhibitor activity.

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