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Antibiosis levels of common bean genotypes toward *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) and its correlation with flavonoids

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ABSTRACT

Expression of the antibiosis-resistance category to weevils has been evaluated in several bean genotypes with very promising results. Among the several causes responsible for this resistance category the arcelin protein and trypsin inhibitors stand out. Other mechanisms may be associated with plant resistance to the attack of weevils; however, few studies seek to discover these possible causes. Thus, our research aimed at identifying bean genotypes resistant to *Zabrotes subfasciatus*, classify them into resistance levels, quantify the content of flavonoids, and correlate it with data obtained from the genotypes. An antibiosis test was performed with beans of 43 genotypes and 40 replications (bean grains) under a completely randomized design. The biological parameters recorded from *Z. subfasciatus* were the periods from egg to larvae, larvae to adult, egg to adult, longevity and life cycle, in addition to egg viability, adults emerged, susceptibility relative index, adult weight, sex ratio, and the dry mass consumed by larvae. The chemical profile of flavonoids from each genotype was assessed by means of LC-MS. Based on the results of the weevil biological parameters and dry mass consumed provided by univariate and multivariate statistical analyses, the bean genotypes were classified into four levels of resistance (antibiosis): highly resistant, moderately resistant, susceptible and highly susceptible. Among the evaluated flavonoids, we could identify isoquercitrin; however, it has no correlation with the expression of resistance. From the results obtained in this work, more studies will be conducted with the genotypes that stood out as resistant, evaluating other resistance categories and defense mechanisms of these materials against the attack of *Z. subfasciatus* and other pests, as well as studies of other important agronomic characteristics aiming at future commercialization of the bean genotypes.

1. Introduction

Plant resistance through antibiosis occurs when the mechanism responsible for the resistance is of chemical nature, negatively affecting insect biology, without interfering with their feeding or oviposition behavior (Boiça Júnior et al., 2015). These negative effects can be acute, usually affecting insect immature forms such as young larvae and eggs, and chronic, which can cause mortality in older larvae or even prevent adult emergence, in addition to debilitating effects, such as reductions in size and weight, increased life cycle, and reduced fertility (Smith, 2005). Antibiosis resistance against weevils has been reported in several bean genotypes, with very promising results. Schoonhoven and Cardona (1983), Baldin and Pereira (2010), Costa et al. (2013), among others, found decreased adult emergence, increased development period, and reduced weight of adults of weevils fed some resistant bean

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genotypes.

Many can be the causes underlying the expression of this resistance category to weevils, highlighting defense proteins. One of the most famous and important defense proteins is arcelin due to its insecticidal properties, and in particular, because of its inhibitory activity on larval development of pests of stored grains (Cardona et al., 1990; Janarthanan and Suresh, 2003; Janarthanan et al., 2008). Resistance in bean genotypes to weevils caused by the presence of arcelin has been found by several authors (Osborn et al., 1988; Wanderley et al., 1997; Barbosa et al., 1999; Miranda et al., 2002; Mazzonetto and Vendramim, 2002). Another important group of defense proteins that has been reported as the cause of antibiosis resistance to weevils are trypsin inhibitors and vicilin globulins (Gatehouse et al., 1979; Macedo et al., 1993; Sales et al., 2005). The α-amylase inhibitors found in wild beans can also cause antibiotic effects to weevils by inhibiting the amylase activity in the insect midgut (Suzuki et al., 1993).

Several other chemical mechanisms may be responsible for the expression of antibiosis; however, few studies seek to discover new potential resistance causes in common beans. In a study conducted by Lima et al. (2014), the authors found isoflavonoids in common bean genotypes. These polyphenolic compounds are known to be important components of plant secondary metabolism by reducing the stress caused by abiotic and biotic factors (Taiz and Zeiger, 2009). In addition, isoflavonoids can interfere negatively with insect feeding, oviposition, and development (Harborne and Willians, 2000; Simmonds, 2001, 2003). Associating the presence of determined flavonoids in the expression of plant resistance can be valuable for genetic breeding programs aiming to incorporate these secondary compounds into bean genotypes to obtain commercial cultivars resistant to pest-insect attack.

With genotypes obtained from the genetic breeding program developed by the Agronomic Institute of Campinas (IAC) that associated high-yield disease resistant cultivars (IAC Alvorada, IAC Diplomata and IAC Una) (Iac, 2013) and genotypes with resistance to *Z. subfasciatus* (Boheman, 1833) (Coleoptera: Bruchidae) (Arc 2, Raz 49, Raz 55 and Raz 59) (Ribeiro-Costa et al., 2007; Baldin and Pereira, 2010; Moraes et al., 2011), to obtain genotypes with weevils resistance and high yields. We carried out an antibiosis test together with analysis of eight flavonoids by means of liquid chromatogram and mass chromatogram with tandem mass spectrometry (LC–MS/MS), to identify genotypes resistant to *Z. subfasciatus*, sort them into resistance levels, and verify possible correlations between flavonoids and genotype data.

2. Materials and methods

2.1. Site of experiment conduction and insect rearing

The experiment was performed at the College of Agricultural and Veterinary Sciences of the University of São Paulo State (UNESP), Campus in Jaboticabal. Trials were carried out in the Department of Crop Protection at the Laboratory of Plant Resistance to Insects. It was used a acclimatized room at 25 ± 1 °C, relative humidity of 70 ± 10% and 12 h controlled photophase. The insects were obtained from the laboratory rearing stock, which remain for more than 60 generations in common beans of “Bolinha” cultivar.

2.2. Development of the bean genotypes tested

Grains from 43 bean genotypes were used for antibiosis testing. The Agronomic Institute of Campinas (IAC), in Campinas – SP, Brazil, provided the genotypes. These genotypes are derived from parental crosses performed in CIAT (International Center for Tropical Agriculture), located in Calina, Columbia, owning resistance to bean weevil, such as Arcelina 2 (ARC 2) and Raz (abbreviation of resistance to *Z. subfasciatus*) (Table 1). The genotype used as the susceptibility standard was IAC Una and as the resistance standard was Raz 49, classified as highly susceptible and highly resistant, respectively, by Costa et al. (2013).

2.3. Methodology for evaluating antibiosis resistance in bean genotypes

Testing began by packing grains, 40 of each genotype, into cylindrical plastic containers with 3.9 cm high and 3.8 cm in diameter, in which 10 couples of *Z. subfasciatus* were released per container. The number of eggs on each grain was observed daily; then the ones containing two eggs were selected, and for grains with more than two eggs, surplus was removed. Eggs were removed with the aid of a stylet, and when there was only one egg on the grain, it was similarly removed and grain was inserted back into a container.

Selected grains were set apart, weighed (obtaining the grain mass before infestation (IGM)) and left into 2-mL Eppendorf® vials for 10 days. Larvae hatching was daily accounted with the aid of a 40× stereomicroscope to establish the period from egg to larvae hatching (incubation time) and egg viability (%). Larvae were given as hatched when started feeding, releasing excreta inside eggs, which became whitish, being different from eggs of non-hatched larvae (unviable eggs) that remained translucent.

After 25 days from oviposition onset, grains remained under daily observation to check adult emergence, for further determining the period between larvae hatching and adult emergency (larva to adult), as well as the percentage of larvae that reached adulthood (percentage of adults emerged). They were sexed and confined in 2-mL Eppendorf® for adult weight ratings (mg), which was measured in an analytical precision balance model AR2140 four digits (0.0001 g) after 24 h of emergence, longevity and sex ratio of weevils, using the formula: sex ratio = females number/total number of weevils emerged.

Based on the number of emerged insects and the average development time we calculated the susceptibility relative index (SRI), adapted from Dobie (1977), using the formula $IS = (Ln \sum x/T) \times 100$; SI = susceptibility index, Ln = logarithm neperian, x = number of weevils emerged, T = Average time to development of weevils, this latter calculated by the formula $T = \sum (xy)/\sum x$, begin y = number of days from egg posture to adult emergence. And finally the susceptibility relative index of (SRI), using the formula SRI = (SI of genotype tested × 100)/SI of standard susceptible genotype (IAC Una).

By the end of adult emergence (five consecutive days without emergence), beans were placed in sealed circulating oven at 60 °C for 48 h, and again weighed to measure dry mass after infestation (DMI). It was also used an aliquot (non-infested grains) containing 10 grains of each treatment, which remained in the same place and for the same period of experiment conduction.

This aliquot was weighed obtaining the average grain mass before (beginning of infestation) and after drying in oven, similarly to the infested grains, being named as aliquot grain mass (AGM) and aliquot dry mass (ADM), respectively. By these results, it was possible to calculate the consumed dry mass of grains (CDM) by larvae using the following formula: CDM = (IGM – DMI) – (AGM – ADM).

2.4. Analysis of flavonoids

Qualitative and quantitative chromatographic analysis of flavonoids were carried out at the Chemistry Department of the Federal University of São Carlos – UFSCar. One-hundred grams of grain of each genotype were singly dried in a sealed circulation oven at