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Resistance categories to *Acanthoscelides obtectus* (Coleoptera: Bruchidae) in tepary bean (*Phaseolus acutifolius*), new sources of resistance for dry bean (*Phaseolus vulgaris*) breeding

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ABSTRACT

Acanthoscelides obtectus (Coleoptera: Bruchidae) is one of the most important post-harvest pests of dry bean *Phaseolus vulgaris* L. Tepary bean (*Phaseolus acutifolius*) could be a novel source of resistance against *A. obtectus* to improve dry bean. We studied the resistance of tepary bean varieties to *A. obtectus*. We focused on three categories of defense: antixenosis, antibiosis, and tolerance. Tepary pinto amarillo (T-amarillo) and Tepary pinto negro (T-negro) beans show high antixenosis to adults, increased numbers paralyzed per hour and per day, and increased duration of development in winter; reduced oviposition, number of emerged adults and adult weight. Due to the small number of emerged adults, there was very little grain weight loss. Volatile compounds were discarded as the cause of paralysis in adults. Adults in T-amarillo and T-negro were paralyzed until day 10 and 14 and then, they die at an exponentially increasing rate. In addition T-negro and T-amarillo produced the highest mortality in larvae before entering the seeds. T-amarillo and T-negro showed antixenoxis, resistance and tolerance to *A. obtectus* infestation and they can be used as sources of resistance for *P. vulgaris* breeding. The compound that causes the antixenosis and paralysis in adults is in the testa of the resistant varieties and is not likely to be a volatile compound, whereas the mortality and antixenosis in larvae is caused more probably by a volatile compound present in the testa.

1. Introduction

The area planted with bean in 2013, globally, exceed 29.5 million of hectares, with a total production of 22.8 million of tones (FAOSTAT, 2017). *Acanthoscelides obtectus* (Coleoptera: Bruchidae) (Say) is one of the most important post harvest pests of dry bean *Phaseolus vulgaris* L. around the world. Some studies have reported losses around the 7–40% caused by weevil damage (Mbogo et al., 2009). This equates to a loss of 1.59–9.12 million of tonnes each year in the world caused by bruchids.

Dry bean production is affected around the world by biotic and abiotic factors. Some of the major biotic constraints involving post-harvest losses are caused by the bruchid species *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Boheman. The common bean weevil *A. obtectus* is a devastating insect pest capable of causing severe bean crop losses in widespread regions of Latin America, Africa and Europe (Schmale et al., 2003; Silva et al., 2007). Larvae burrow into the seed to feed and metamorphose from larva to adult within the seed. Adults cause no direct damage to the beans in storage due because their consumption is imperceptible, but females can lay up to 60 eggs (Parsons and Credland, 2003).

Large warehouses use chemical controls to reduce and eliminate infestations. Small holder farmers in developing countries usually pursue low-cost, low-technology strategies for control of bruchids. Farmers reduce their losses by limiting the time that they keep their harvest and by not producing large quantities of the crop in any growing season (Mbogo et al., 2009).

Breeding for insect resistance can increase yield stability contributing to a stable supply of dry beans in developing countries

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(Mbogo et al., 2009). The use of resistant varieties against storage insect pests, when successful, has a number of comparative advantages over other control measures, particularly the use of chemical insecticides. Controls based on chemical insecticides mostly need to be repeated periodically and hence are more expensive in comparison with genetic manipulation of the crops themselves for resistance to storage pests (Keneni et al., 2011). The best approach for this pest is to develop resistant lines to *A. obtectus*, the most difficult bruchid to control. The number of adults present after 182 days of seed storage was low for all tepary accessions and was high for most common bean accessions; in fact tepary beans may provide a useful germplasm source for *Phaseolus vulgaris* breeding (Shade et al., 1987). Seed storage protein "arcelin" has been previously described as causing antibiosis against *Z. subfasciatus*, without providing protection against *A. obtectus* (Cardona and Kornegay, 1999; Osborn et al., 1986, 1988; Minney et al., 1990). Resistance is also associated with lectin-like seed storage proteins (LLPs) (Sales et al., 2000), and α-amylase inhibitor (Fory et al., 1996). In particular, moderate resistance to *A. obtectus* has been found by Cardona et al. (1990), Kornegay and Cardona (1991) and Kornegay et al. (1993).

In the highly resistant accession G12952, resistance was expressed as antibiosis causing delayed and reduced adult emergence, high mortality of late first instar larvae, reduced female fecundity, and negative rates of population growth. The factors responsible for resistance are present in the cotyledons of seeds and are chemical in nature (Cardona et al., 1989). The natural storage protein arcelin, in G12952, causes sub-lethal effects to the bruchid *A. obtectus* (Kornegay and Cardona, 1991; Schoonhoven et al., 1983), prolonging immature development of young bruchid larvae, extending the period of adult emergence, and reducing adult weight (Velten et al., 2007; Osborn et al., 1988).

No other sources of bruchid resistance have been detected in over 17,000 bean genotypes originating elsewhere in Latin America (Valencia, 2006). Few genotypes with high resistance to *A. obtectus* were found: G40199 (*Phaseolus acutifolius* A. Gray) (Kusolwa and Myers, 2011), G02770 (*P. vulgaris*), QUESS (*P. vulgaris*) (Zaugg et al., 2013), G12952 (*P. vulgaris*) (Schoonhoven et al., 1983).

The variables that have been mainly used for characterizing resistance were: number of emerged adults, days to adult emergence, and adult weight, and how these traits depend of on the cotyledons. In fact the genes for resistance against *A. obtectus* were based on days to adult emergence. Few studies had been conducted to study the antixenosis, antibiosis and tolerance in larvae and adults to determine where the highest mortality occurs, if before or after entering the seed. Few researchers measured mortality of larvae before entering the seed. We want to determine if the presumable compounds are in the testa or in the cotyledon. Besides, few studies were conducted with mixed seed (susceptible and resistant varieties) to see the preference of larvae, larval mortality before entering the seed, larvae antixenosis, and adults emerged.

The novelty of this manuscript is that we focus on every category and at all stages of the biological cycle of *A. obtectus* to visualize where is the highest level of antibiosis seems to use these traits in the phenotyping of genetic mapping populations to discover the genomic regions involved in *A. obtectus* resistance.

Therefore the objective was to study the resistance of tepary bean varieties to *A. obtectus*. We focus on three categories of defense: antixenosis, antibiosis, and tolerance and how the different bean varieties affect the adults, larvae and biological cycle of *A. obtectus*.

2. Materials and methods

Eleven bioassays were carried out (Table 1) to check for antixenosis, antibiosis and tolerance to *A. obtectus* of five dry bean varieties (Table 2). The insects used in the experiments were obtained from stock cultures reared in Pinto Rojo variety at the Misión Biológica de Galicia (CSIC), Pontevedra, Spain.

2.1. Antixenosis in adults

Two bioassays (1 and 6) were performed on 4 July 2015 and 7 August 2015. Antixenosis percentage was analyzed over the time as the percentage of adults that were not on or below the seed.

2.2. Antibiosis in adults

2.2.1. Paralyzed adults per hour and day

Bioassays 3 and 4 were performed on 6 August 2015. Bioassay 5 was performed on 10 August 2015. Paralyzed adults were those on their backs with legs raised and unable to rise. Then they were accounted at different times after exposure. In bioassay 5, paralyzed adults per day and adult mortality per day were recorded. In addition, in this bioassay we used five varieties and one control without seeds.

2.2.2. Adults paralyzed to check volatile compounds

Bioassay 7 was performed on 7 August 2015 without scarified seeds, and Bioassay 8 was performed on 14 August 2015 with scarified seeds. In Bioassay 7 we used complete seeds and a paper barrier with holes and in the Bioassay 8 we used scarified seed and a paper barrier that was lernished with holes to avoid direct contact of the adults with the seed but to allow the circulation of the volatile compounds. Numbers of paralyzed adults were recorded at lowly intervals in both bioassays.

2.2.3. Number of eggs and adult mortality

Three bioassays (1, 2 and 5) were performed for checking the number of eggs in each variety on 4 July 2015, 30 June 2015 and 10 August 2015, respectively. Numbers of eggs were counted at 20 days after infestation. Bioassay 5 was also used for checking adult mortality.

2.2.4. Antixenosis in larvae

Bioassay 11 was performed to determine the percentage of larvae that died before entering the seed. It was performed on 19 May 2016. From 19 to 23 May the conditions were at room temperature (20 °C). From 24 to 31 May 2016 an experiment was carried out under controlled conditions (for optimal conditions at 27 ± 2 °C and 70 ± 2 relative humidity, alternating 12-hr with illumination and 12-hr in the darkness). From June 1, 2016 to adult emergences were at room temperature (22 °C). In general, 4 repetitions and 4 seeds of each variety per repetition were used. Besides, a bottle with mixed seed of the 5 varieties (one seed of each variety) and a bottle without seed as a control were added in each repetition. The variables measured were: total larvae, dead larvae, antixenosis, adults emerged and days to adult emergence.

2.2.5. Antibiosis in larvae

Bioassay 9 was carried out on 15 February 2016. The dead adults were removed on 8 March 2016 to expect the first generation of adults. The bioassay was designed for counting the number of adults emerged from each seed.

Bioassay 10 was performed on 12 December 2015. We used 40 infested seeds of Pinto Rojo bean per repetition to achieve uniform infestation, and 15 days after, Pinto Rojo was removed from the experiment. Was counted the number of adult emerged and duration of the biological cycle (which was estimated from the date of the experiment until the first adult emerged).