

# How bean weevil (*Acanthoscelides obtectus*, Coleoptera, Bruchinae) larvae die on legume seeds

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## Research article

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# Abstract

## Background

The host specificity and the range of possible wild and cultivated hosts of the dry bean weevil, *Acanthoscelides obtectus* (Coleoptera, Chrysomelidae, Bruchinae), a seed predator of beans, is poorly known. In addition, female oviposition preference and larval performance relationship is complicated by the respective importance of seed coat and cotyledon, because, on the one hand, paradoxically, females lay eggs on the basis of stimuli of the seed coat alone, without directly being able to assess the quality of cotyledon's suitability for larval development. On the other, the thickness of seed coat may prevent first instar larvae from entering the seeds, even if cotyledons were suitable for development.

## Results

The seeds of 62 leguminous species and 82 accessions occurring in Hungary were tested in no-choice tests for egg-laying. The ability of first instar larvae to overcome seed coat, as a physical barrier, was measured with intact seed coat, whereas drilled seed coats allowed assessing the suitability of cotyledon for development. Seeds of 18 species (35% of them *Lathyrus*) supported larval development to adults, if the seed coat was drilled, however, only nine produced adults, if the seed coat was intact. Seed coats thicker than 0.1 mm could be an obstacle for first instar larvae. There was no overall positive correlation between oviposition preference and larval performance, with the exception of 16 so-called acceptable non-hosts (Kendall's  $\tau = 0.3088$ ). Bean weevil females also demonstrated an ovipositional hierarchy of legume species even in no-choice tests.

## Conclusions

Results demonstrate that whereas the use of some acceptable non-host species by the bean weevil is possible in seed stores, the same is unlikely under outdoor conditions, because it requires the recognition of basically different oviposition substrates (pods).

## Background

In a seminal paper entitled "How southern cowpea weevil larvae (Bruchidae: *Callosobruchus maculatus*) die on nonhost seeds" Janzen [1] discussed the importance of seed coats and cotyledons of tropical legume plant species in the interaction. Based on no-choice oviposition and larval development investigations in the laboratory, he found extreme selectivity of the bruchid species, which he attributed to the diversity of chemical compounds present in the hosts, and to physical traits, such as thickness, hardness and smoothness of the seed coat. However, more than forty years afterwards, knowledge concerning causes of host specificity of the same insect group is still poor.

Although only implicitly, Janzen [1] also became one of the pioneers of female preference-larval performance studies, testing whether insect mothers optimally select substrates for larval development and, whether oviposition on unusual or non-hosts, were attempts of host range expansions [2, 3]. The latter hypothesis is supported by the fact that the range of plant species supporting survival of larvae is frequently wider than the egg-laying preference of the mother [4, 5]. The preference-performance theory postulates that females select egg-laying substrates in order to maximize their progeny's fitness [3, 6, and references therein]. The female's selection behaviour in a choice situation, where several items are presented simultaneously, and the female encounters

substrates of different qualities in a sequential process, usually fits to the hierarchy of the genetically-based template of hosts [7]. Therefore, a positive correlation between the mother's preference and offspring performance (usually the yield of adult offspring from a particular substrate) can be expected. A meta-analysis of such studies [8], however, came to the conclusion that the results were contradictory: positive and negative correlations were equally abundant. Moreover, among the 23 studies of oviposition preference, there were only four no-choice tests. Females frequently make oviposition "mistakes", e.g., laying eggs on toxic or otherwise unsuitable substrates; however, it is possible that these merely reflect the neural limitations of insects [9]. The strength of the relationship seems dichotomous according to the degree of host specialization of herbivorous insects. Specialists show strong positive preference-performance relationships, whereas in generalists it may not exist at all [8, 10]. Several studies did not find genetic correlations between adult preference and larval performance either [11, 12]. The negative relationships refer not only to the lack of adaptation due to metabolic trade-offs, but to the importance of hitherto unconsidered life-history components and environmental factors [8, 13, 14].

The preference-performance relationship arises in a different form with seed predator bruchid species. The complicating factor is the seed coat (testa, seed integument) that may or may not provide information either for the egg-laying female or for the first instar larvae on the quality of cotyledon, but at the same time can impose formidable physical and chemical barriers for the larvae to enter the seed. The information on which the female bases her decision to oviposit may not adequately refer to the suitability of cotyledon, and the larva, following an exhausting period of drilling through a tough testa, may still die soon after making contact with it. Despite the enormous advances in chemical analysis and the wealth of information of seed chemical composition in Fabaceae [15], relatively little is known of the seed coat chemistry as it affects host selection by bruchids.

Preference-performance studies testing a wide range of hosts and non-hosts are missing due to the usually narrow host specialization of bruchids [1, 16]. Nevertheless, the bruchid species, *Acanthoscelides obtectus* (Say), *Callosobruchus* spp., and *Zabrotes subfasciatus* (Boheman) that are capable of propagating in stored conditions on leguminous seeds, provide a range of possibilities to study host specificity. On a limited number of hosts, for example, Fox [17] found negative genetic correlations between preference and performance components with *C. maculatus* (F.). Tucić et al. [18], then Milanović and Gliksman [19] showed that after rearing *A. obtectus* on chickpea (*Cicer arietinum* L.) for  $\geq 50$  generations, it had lower survival rate on the main host, *Phaseolus vulgaris* L. They concluded that there was a fitness cost for using an unusual host, and that whereas no genetic correlation was detected between preference and performance on *Phaseolus*, there was such a relationship on *Cicer*.

This paper investigates the potential host range and tests the preference-performance relations of the bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera, Chrysomelidae, Bruchinae). The bean weevil propagates on two species of cultivated beans (*Ph. vulgaris* and *Ph. coccineus* L.) in the temperate zone. In Europe, it can continuously reproduce on stored dry beans, and therefore can achieve a pest status on this commodity. It also seasonally infests beans outdoors, laying some eggs on nearly mature seeds within pods [20]. At harvest, infested seeds are collected, and in bean stores the cycle repeats. Females do not stick eggs to the seed surface, but rather place them among the seeds. First instar larvae possess legs and are able to move among seeds to select sites on seeds to enter, and can exercise independent selection behaviour, if a choice of seeds is available. While drilling through the seed coat, they avoid swallowing most of it [21], a behaviour observed with other

bruchid species also [1, 22, 23]. As a consequence, fine powder indicates the site of boring. Larvae tunnel into the cotyledon, develop through four instars and pupate within the seed.

The bean weevil is an oligophagous species within the tribe Phaseoleae (Fabaceae) that comprises *Glycine*, *Lablab*, *Vigna* and *Phaseolus* genera [24]. Spontaneous occurrence in some cultivars of all these genera are known, but it is a rare event and poorly documented, along with occasional observations concerning infestations in other legume genera such as *Lupinus* (tribe Genisteae), *Cicer* (Cicereae), *Vicia*, *Lens* and *Lathyrus* (Fabeae) [5, 25, 26, 27, 28]. In seed storage facilities, only dry seeds are available, so selection for seeds for oviposition is dependent on the number of species being stored. Therefore, while selection choices for females can be limited, her oviposition decisions may be important for offspring survival.

Several questions are pending. How wide is the host range? What factors associated with seeds influence successful survival of larvae to adults? How do females rank oviposition substrates in a no-choice situation? And, is there any relationship between the female's oviposition and the subsequent performance of larvae? Females rank seeds by size [29], but are they able to judge the suitability for larval development, based on information provided solely by the seed coat? The following hypotheses were also tested: (1) that as the seed coat increases in thickness, the ability of larvae to enter the seed is reduced, and (2) that in the absence of the seed coat, the suitability of the cotyledon determines survival of larvae.

## Results

### *Female oviposition preference*

Bruchid females deposited significantly decreasing number of eggs/female on seeds in the order of host (H), acceptable non-host (ANH) and non-host (NH) species groups (see definitions of these in the Materials and methods). Females laid 38.6 eggs on H species (22 SCA), 18.6 eggs on ANH species (55 SCA), and 13.5 eggs on NH species (63 SCA), and significantly more eggs on larger seeds (Table 1). Mean mass of H seeds was 340.8 mg, that of ANH 235.6 mg, and that of NH 70.3 mg (Table 1). All 66 plant species (62 leguminous and 4 outgroup species) received eggs, but females laid less than 15 eggs on 55%, less than 30 eggs on 35%, and between 30-45 eggs on 10% of plant species. Fig. 1 shows the distribution of eggs among leguminous tribes. Not surprisingly, *A. obtectus* females laid the highest number of eggs on members of the tribe Phaseoleae, where the main hosts are also found. Within Phaseoleae, soybeans (*Glycine*) were the least preferred (ESM Table 1). Comparable responses were noted to some species within Caesalpinieae, Genisteae, Robinieae, Cicereae and Fabeae (ESM Table 1). Although non-host species for the bean weevil, seeds of species such as *Gleditsia delavayi* Franch. (Caesalpinieae), *Laburnum alpinum* (Mill.) Bercht. & J. Presl, *L. anagyroides* Medik. (Genisteae), and *Robinia viscosa* Vent. (Robinieae) received high numbers of eggs. *C. arietinum* (Cicereae) and *Vicia faba* L. (Fabeae) are known as occasional hosts. The number of eggs laid/female on them fell into the medium and high categories, respectively. A mean number of less than 10 eggs/female were laid on *Vicia tenuifolia* Roth, *Robinia pseudoacacia* L. and *Amorpha fruticosa* L., and on some other members of the Fabeae tribe (ESM Table 1).

Egg-laying on artificial beans. Females accepted the artificial seeds incorporated with seed coat as an oviposition substrate and laid comparable number of eggs to the control (Table 2).

### *Larval performance*

Mortality of larvae. The major stages of larval performance were: (a) entering the seeds that was constrained by the thickness of seed coat, resulting in L1 mortality outside seeds, and (b) within-seed mortality of various developmental stages. Both were modulated by the intact or drilled status of seeds. The seed coats of host (H) and acceptable non-host (ANH) species were significantly thinner (Table 1). L1 mortality outside intact seeds was different among plant species groups (Table 1). It was significantly lower for the H (24.75%) and ANH groups (93.3%), in comparison with the NH group (100%, all are medians). For the two H and 16 ANH species Table 3 provides data of seed coat thickness, whereas ESM Tables 2 and 3 give similar information for NH, and SCA of H and ANH groups. Remarkably, seed coat constitutes a barrier even on the primary host, beans (*Ph. vulgaris*), for the bean weevil. Contrary to the very low larval mortality inside seeds, there was substantial mortality outside intact seeds of bean cultivars (27.3%, 13.6-53.3, median, lower and upper quartiles, N=21), but only 4.4% (0-11.4) for drilled seeds. Similar values can be given for other legume species from which adults emerged, with the difference that the upper level of mortality usually reached 100% with intact seeds, with some exceptions such as *Vigna unguiculata* (L.) Walp. and *V. angularis* (Willd.) Ohwi & H. Ohashi, where seed coats were extremely thin (Tables 3 and 4). A remarkable case is the runner beans (*Ph. coccineus*) with 0% (intact seed) and 2.2% (drilled seed) larval mortality outside seeds.

Mortalities of various developmental stages inside intact seeds were substantially different from those of drilled ones, however, the critical event for development in the seeds is invariably the survival of first instar larva. Tables 1 and 4 provide results for host (H) and acceptable non-host (ANH) species, and further data are available in ESM Tables 2 and 3 for bean, pea and soybean varieties, as well as for non-host (NH) species, showing differences among host-types. Mortality inside intact NH seeds was practically nil due to the inability of L1s to penetrate the seed coat, whereas for drilled seeds it was 75% (44-93%, median and quartiles). Many L1s first entered the seeds, then left them again, and died outside of starvation or by toxins taken up from the cotyledon. Seed testa frequently bore several shallow pits, where L1 attempted to bore in (e.g. all *Gleditsia japonica* Miq. seeds, both intact and drilled, had such traces). It is worth remarking that ca. 10% of L1 entered the intact *G. delavayi* seeds, but inside the seeds many died by various manners: on the surface of the cotyledon, or after burrowing in the radicle by various lengths. Although it is well documented that additional *A. obtectus* larvae may enter through the hole made by a pioneer larva [30], the number of L1-made and artificial holes was 1.5:1 on the most preferred bean seeds (*Ph. coccineus*), i.e., many L1 larvae did not use the pre-prepared holes on this host. In some instances (e.g., *Caragana* or *Onobrychis* genera) L1s entered the seeds through the hilum. First instar larvae entering *Gleditsia* seeds through an artificial hole made at the embryo area invariably died within the embryo. In cases where the cotyledons were soft (several *Glycine* cultivars/accessions and *Caragana*), larvae made longer tunnels before dying.

Development in artificial seeds. This experiment proved that, besides being a physical barrier, seed coat also inhibited larval development at the lowest concentration incorporated into artificial seeds (Table 2). To the contrary, controls (without any seed coat content) were fully suitable for development.

Adult emergence. Significantly more adults emerged from H species/cultivars than either from ANH species/cultivars or NH species, and whether intact or drilled (Table 1). Of the 62 legume species *A. obtectus* larvae developed into adults in 18 (29%) species in four tribes, if the seed coat was drilled, and from only nine species, if they had intact seed coat (Table 4). However, the picture varied considerably concerning SCA (ESM Table 3). Although adult emergence in *Ph. vulgaris* was generally high, at the cultivar level it ranged between 51 and 100%. In *Ph. coccineus* all larvae developed into adults. Whereas cowpea (*Vigna*) species supported larval

development to adults, white lupin (*Lupinus*), soybean (*Glycine*) varieties and hyacinth bean (*Lablab*) did so only sporadically, and especially in drilled seeds. This is also paralleled by the length of developmental time needed until adult emergence (Table 4). In some cases (*G. max* (L.) Merr., *V. angularis* and *P. sativum* L.) it was two to three times longer in comparison with beans. Of the 27 cultivars of garden peas, adults emerged from 24 (88.9%), however, only from 13 of these, if the testa was intact. Similar values occurred for 17 *G. max* cultivars/accessions: adult emerged from six (35.3%), but only from four of these with an intact seed coat. A surprising feature is the asymmetric distribution of adult emergence within the Fabaeae tribe. Whereas there was adult emergence from six *Lathyrus* species (one, *L. sativus* L., from intact, the rest from drilled seeds), there was only one such case among vetches: the faba bean (*Vicia faba*). Both intact and drilled seeds yielded adults from faba beans, although it had a relatively thick testa. Although larval development proceeded in some NH reaching as far as L3, no adult emerged from these (ESM Table 2).

In the 18 adult-yielding legume species five (28%) produced malformations and some adults could not leave the seed in 10 of these. Typical malformation was a substantial decrease in elytra width and length: the elytra became shorter and triangular in shape. There were 0.1% malformed adults in beans, 33.0% in peas, 31.6% in *L. tuberosus* L., 6.7% in *L. sativus*, and 0.7% in *V. faba*.

#### *Female preference versus larval performance*

Correlations. ESM Table 5 provides the most important nonparametric correlation coefficients referring to the overall relationship between plant traits and insect responses. Accordingly, only in the ANH group there was a significant positive relationship between preference and performance, i.e., between the number of eggs laid/female and the adult emergence (Kendall's  $\tau=0.3088$ , intact seeds, N=55). The correlations between seed mass and the number of eggs laid were extremely low in all three plant groups, but were significant with ANH and NH species. The thicker the seed coat, the higher was the first instar larval mortality outside on ANH and NH seeds, but seed coat thickness did not affect L1 larval mortality on H seeds.

Results of logistic regressions. The logit-regression provided evidence that L1 mortality outside seeds was due to different seed coat thicknesses. Significantly (15:1) higher number of cases indicated <50% L1 mortality outside seeds, if seed coat was thin ( $0.08 \pm 0.001$  mm, mean $\pm$ SE, N=62), in comparison with thick seed coat ( $0.15 \pm 0.005$  mm, N=78; Wald test: 8.2, df=1, p=0.0043; log-likelihood: -48.4; goodness of fit  $\chi^2$ : 17.9, df=1, p<0.001). Approaching the same hypothesis from another angle, i.e., if seed coat was assigned as 'penetrable' or 'impenetrable', significantly higher number of L1 entered seeds with 'penetrable' seed coat (Wald test: 27.3, df=1, p<0.001; log-likelihood: -81.4;  $\chi^2$ = 31.1, df=1, p<0.001). Here only those cases were taken into account where L1 larvae entered a seed, then died immediately after this. This result is interesting, because 'penetrable' seed coat thickness was  $0.0998 \pm 0.004$  mm (N=64), whereas 'impenetrable' showed  $0.119 \pm 0.005$  mm (N=76), a mere 0.02 mm difference.

As for the second hypothesis, larval performance and adult emergence did depend on the 'quality' or 'suitability' of cotyledons of seeds. Significantly higher number of cases showed <30% larval mortality inside seeds, if cotyledon was 'suitable' (4.7%, 2.2-43.2, N=124) in comparison with 'unsuitable' cotyledon (61.3%, 12.9-90.7, medians and quartiles, N=88; Wald test: 27.6, df=1, p<0.001; log-likelihood= -131.5;  $\chi^2$ =29.8, df=1, p<0.001). Adult emergence from 'suitable' seeds was 37.8% (10-84.1, median and quartiles, N=123) vs. from 'unsuitable' cotyledons: 0%

(N=89). Logistic regression for adult emergence could not be performed, because one cell of the  $\chi^2$  table contained zero. Nevertheless effect sizes could be computed.

Effect sizes and risk analyses. The first phase of the preference-performance relationship refers to the larval ability to overcome seed coat thickness. Odds ratio (OR) provided 19 times larger chance for L1 larvae to have <50% mortality, and risk difference (RD) indicated 45% higher survival for larvae, if they happened to bore in a seed with seed coat thickness of <0.1 mm, in comparison with seeds having thicker testa (ESM Table 6). However, the regression coefficient ( $\varphi^2$ ) explained only 10% of variance of seed coat effect, referring to other important factors affecting larval entry. On the other hand, when facing penetrable/impenetrable seed coat (results are not shown in table), L1 larvae had three times higher risk to die with 'impenetrable' seed coat (RR =  $3.01 \pm 1.3$ , mean  $\pm$  SE,  $CI_{95} = 1.9$  & 4.8), whereas chances for larvae to enter a seed with penetrable testa was 7.5 times higher (OR =  $7.5 \pm 1.5$ ,  $CI_{95} = 3.5$  & 16.1), even if they died after the first bites from the cotyledon. Here, the regression coefficient explained a relatively high level (21%) of the variance.

The second phase of the preference-performance relationship is manifested in the adult progeny production as related to the number of eggs laid/female on legume species. The risk of >30% mortality inside seeds for larvae increased ca. 2 times (RR =  $1.95 \pm 1.13$ ,  $CI_{95} = 1.52$  & 2.49) in cotyledons unsuitable for reaching later developmental stages. The odds for such an outcome was high (OR =  $4.85 \pm 1.28$ ,  $CI_{95} = 2.96$  & 7.94, results are not shown in table). The chance for reaching adulthood in seeds from which >10% adults emerged was ca. 4 times higher (RR in ESM Table 7), than in cotyledons allowing only 1-2 weevils to successfully complete their development. The odds for adulthood in suitable seeds were extremely high (OR =  $523.42 \pm 1.23$ ,  $CI_{95} = 348.74$  & 785.60) due to the asymmetry caused by the NH seeds. The regression coefficient ( $\varphi^2$ ) explained a high portion (54%) of the variance (ESM Table 7).

As expected, there was an interaction between penetrable/impenetrable and intact/drilled states of seed coats: the joint effect (OR<sub>11</sub>=0.6718) was larger than the multiplied value (0.4820) of their respective effects (OR<sub>10</sub>=0.7179 and OR<sub>01</sub>=0.6714). The joint effect of both variables is 1.4 times higher than the combined effect of the variable acting separately.

## Discussion

This study could not prove an *overall* positive correspondence between host preference and host suitability. However, 16 leguminous plant species (Table 4) did show positive significant relationship between oviposition preference and larval performance (ESM Table 5), and these are called *acceptable non-hosts* (ANH) [31]. Nine of these legume species supported development into adult stage even if the seed coats were intact. Besides the two *Phaseolus* species, rearing in laboratories is possible on chickpea through generations [18, 33], and occasional infestation is present in stores on garden peas or faba beans (Szentesi unpubl. results). How females assess host suitability of ANH members through the seed coat, remains an intriguing aspect of the positive relationship between female choice and larval success. The distribution of larval mortality and adult emergence (Tables 1 and 4, ESM Tables 2 and 3) among plant groups, and the results of logistic regression (ESM Table 6) overwhelmingly emphasize the importance of seed coat in allowing or blocking the use of a seed. For instance, regression coefficients ( $\varphi^2$ ) explained 10% and 21% of the variance, respectively of the effect of seed coat thickness and 'penetrability', supporting the hypothesis that several yet unknown physical and chemical factors are also

important. In spite of the fact that a preference for larger H seeds has been proven in bruchids in choice-tests [29, 32], the seed mass of plant groups had negligible effects on the number of eggs laid/female (ESM Table 5), and the significant positive preference relationship in ANH and NH species might be attributable to physical traits, e.g. shape.

In the present study only a limited number of variables that may affect preference-performance relationships (seed mass, number of eggs laid/female and seed coat thickness), among those possible, was taken into consideration. Other important factors (chemicals, further physical, environmental, life-history traits, mobility, predators, host genotype, etc.) that influence host selection [8, 10, 34, 35, and others] were not investigated. In addition, experience may promote acceptance of ANH species [36, Szentesi unpubl. results], and may even lead to host-race formation [37], but neurological constraints can impose limitations to perception and integration of stimuli [9]. In the light of many factors influencing preference-performance relationship, and specifically egg-laying, it is doubtful whether the oviposition response of bean weevil females on ANH and NH species was “erroneous” or “defective”, as there are possible alternative explanations of the behaviour [38].

#### *Is there a hierarchy in host selection in no-choice tests?*

The widely known hierarchy-threshold model [7] for individual host selection in choice situations explains that specificity is bound to genetic and physiological status, and that the rank-order of hosts is invariable. Oviposition occurs when acceptability of a substrate exceeds the motivational threshold, and the different acceptabilities create a rank-order. An alternative hypothesis [39] states that it is the actual set of eggs available for laying that determines acceptance: higher number of eggs motivate acceptance of low quality hosts. In this study however *no-choice* tests were applied. Bean weevil females did produce a rank-order of ANH plants, however, with the exception of the primary host's position, beans, this was different from those obtainable in choice tests, and even more different concerning larval development (Szentesi unpubl. results, ESM Table 8). In choice tests *A. obtectus* visits items in a sequential way, and lays the overwhelming majority of eggs on beans. However, it never totally ignores other substrates (even glass beads), because variance in egg-laying is also modulated by factors, such as egg-load and/or in most cases unknown physical and chemical stimuli. In this study females laid ca. 1/3 eggs on NH species of those deposited on H plants. This means that elements of acceptability of different NH plants could induce the motivational state to accept a lowered egg-laying threshold as time passed.

#### *The host-range of the bean weevil*

Pest status, width of geographic distribution and host range can be interconnected [40]. The bean weevil is narrowly specialized on some wild and cultivated members of the genus *Phaseolus* [41], but it is a widespread and important pest, whose host affiliation may also include some species of pantropical leguminous tribes [42]. In the present study, *Ph. vulgaris* and *Ph. coccineus* represented the hosts, the second providing better preference/performance ratio as also confirmed by Leroi and Jarry [25]. Some species and cultivars of the following genera belong to the ANH group: *Lupinus*, *Glycine*, *Lablab*, *Vigna*, *Cicer*, *Vicia*, *Lens*, *Lathyrus*, and *Pisum* (Table 4), largely agreeing with findings of [5, 16, 26, 27]. Differences depend on whether the authors applied a sufficiently wide spectrum of cultivars, because differences in acceptance among cultivars can be as large as or larger than those between species (ESM Tables 2 and 3).

Drilled seeds contributed to the understanding of host-range by demonstrating how important chemical constituents of the cotyledons were, when seed coats did not function as barriers. The surprisingly high ratio of

*Lathyrus* species supporting development to adulthood (Table 4) was possible only with drilled seed coats. It is noteworthy that most of those plant species that allowed some larval development were members of the tribe Fabaeae, and specifically of the genus *Lathyrus* and much less of *Vicia* (ESM Table 2). Outside this circle, all other plants were non-hosts, even if some eggs were laid on them and larval development advanced to L2 or L3, if the seed coat was drilled.

### *The importance of seed coat*

The legume seed coat is a formidable barrier in itself. In Janzen's [1] work no bruchid larva was able to drill through >0.4 mm thick seed coat. From another perspective, Thiery [43] demonstrated that seed coat hardness caused L1 mortality with *A. obtectus* on *Ph. vulgaris*, and that it was in inverse correlation with seed water content. The high L1 mortality with intact seed testa in case of plants grown in nature in this study (ESM Table 2) calls attention to further factors. These complementary findings indicate that both physical traits (thickness, smoothness and hardness) and chemical traits of the seed coat contribute, and it seems that no study has attempted to separate the respective effects.

### *Chemical factors in the seed coat*

One obvious factor that should direct the attention to phytochemicals in seed coats is the observation with several bruchid species [1, 22, 23, 44], bean weevil included (Szentesi pers. obs.), that L1 larvae consume little or none of the seed coat. It was also demonstrated by Stamopoulos and Huignard [45] and in this study (Table 2) that the consumption of bean seed testa was toxic to *A. obtectus* larvae. A multitude of chemical factors have been identified in legume seeds coats [15]. For example, bean weevil L1s did not even attempt to penetrate the intact seed coat in the Genisteae tribe, due probably to quinolizidine alkaloids that were present in the testa [46].

### *Chemical factors in the cotyledon*

The cotyledon ultimately determines whether larval development can reach the adult stage. A wide spectrum of secondary plant substances occur in leguminous tribes, but one of the most characteristic groups is non-protein (also called toxic) amino acids [47]. Not only are the remarkable amounts inside seeds (up to 8% of dry weight [48]) important, but so also is their taxonomic distribution in Fabaeae. The cotyledon of most *Lathyrus* species contains a diverse array of toxic amino acids, most frequently homoarginine and lathyrine, whereas *Vicia* species can be characterized by the dominance of canavanine [49, 50]. In this study, within the Fabaeae tribe, *V. faba* was the only species that allowed development of *A. obtectus* to adulthood (Table 4). Canavanine can be a significant factor influencing survival of *A. obtectus* in NH *Vicia* seeds, in spite of the fact that bean weevil larvae show a remarkable tolerance to this compound: at 2.0% w/w concentration some adults (4% of the control) still emerged from artificial seeds (Szentesi unpubl. results). However, toxic amino acids occur in many other legume species and they act in combination with several other chemical groups to form the chemical resistance profile of a seed [51]. Indicators of the effects of this complex milieu are malformations and slower development, presumably consequences of the higher metabolic costs of handling substances in the cotyledon.

### *Evolutionary considerations*

The seed coat not only excludes potential exploiters; it also effectively divides the "perception space" of egg-laying insects. Whereas in cases of leaves and fruits, insects can directly perceive plant quality by probing, with seeds females are excluded from judging suitability of cotyledons by the seed coat. The possibility exists that the

seed coat can mediate information on the inner quality of the seed, however, the stimulus complex leading to egg-laying on an unsuitable seed is not known. The outcome of preference-performance conditions is further complicated in the field. Although the bean weevil was able to lay eggs into *Vigna unguiculata* pods in the laboratory [28], this would not necessarily happen in nature. These findings also direct attention to the need of more tests with pods of different ANH plant species, as this must be the first step to host-range expansion.

The intriguing result of this study was the surprisingly high frequency of development of *A. obtectus* in meadow peas (*Lathyrus* species) into adults, when seed coats were drilled. There are no reasons for assuming any evolutionary connection in the relationship: (1) larvae cannot enter *Lathyrus* seeds having intact testa, (2) some *Lathyrus* species do not contain toxic substances to *A. obtectus* larvae by chance or domestication, and (3) *A. obtectus* females most likely do not recognise the suitability of *Lathyrus* seeds for larvae when only pods are available in nature. (During 30 years of collecting legume seeds from *Lathyrus* species from the field, *A. obtectus* has never been reared, Szentesi and T. Jermy unpubl. obs.)

As in several other instances [52], the egg-laying by bean weevil females onto ANH seeds also raises the question as to whether host range expansion has occurred. The first steps in host-shifts are behavioural events [53, 54, 55], viz. the ultimate conditions were recognition and acceptance of the new plant species. Although oviposition preference and larval performance likely are governed by different gene complexes [4, 56, 57], there must be genetic covariance between preference and performance to adapt to a new plant species [58, 59]. Egg-laying should be accompanied by physiological adaptation of larvae to handle compounds metabolically once they are inside seeds [60, 61]. Unless preadaptation [62] played a role, as was recently suggested with a related species (*A. macrophthalmus*) [63, 64], host-shift with the bean weevil is less likely.

## Conclusions

The following traits maintain bean weevil in the current host specialization: (a) first instar larvae are not constrained to enter an acceptable non-host seed in a no-choice environment, because they have legs, and as long as their energy reserves allow, they can actively seek suitable hosts; (b) as data of this paper demonstrate, first instar larvae rarely enter acceptable non-host seeds having intact seed testa; and (c) the life cycle of the *A. obtectus* takes place both in stores and in the field, and this spatial segregation regularly interrupts possible breeding and selection on potential additional hosts. Even if generations of beetles were produced on acceptable non-host seeds, the recognition of these plants as suitable oviposition sites must happen based on traits of pods (not seed coats) in the field that, as a first step, would require substantial genetic changes.

## Materials And Methods

### *The experimental insect*

The test insects originated from a continuous laboratory rearing maintained on commercial beans for at least five years at 26-28 °C and 18/6-light/dark regime as described by Szentesi [65]. Following emergence several hundred adults were placed into 10x15 cm size jars on corrugated paper, fed honey-water and allowed to mate. Adult are sexually mature at emergence from beans, and by the third day females are ready to lay eggs. One to three days old males and females were separated by the shape and coloration of pygidium and used for the experiments. To obtain eggs for the experiments, several hundred beetles were placed into an 18 cm high and 20 cm diameter glass cylinder, whose lower mouth was closed with a 1x1 mm mesh screen. The weevils laid eggs for days on a

single layer of dry beans placed above the screen, and the eggs fell down into a dish when the cylinder was shaken (see more details in Szentesi [65]). Daily collection of eggs allowed precise timing of large numbers of the suitable (“black-headed”) stage of the first instar larva. At this stage of development the egg-shell becomes transparent, and the head of larva turns black in the egg, indicating that hatching would commence within several hours.

### *Plant species used in the tests*

In this study, 62 species from the Fabaceae tribes Cercideae, Caesalpinieae, Genisteae, Amorphaeae, Phaseoleae, Robinieae, Galegeae, Cicereae, Trifolieae and Fabeae, and 82 varieties, cultivars or accessions of six legume and four non-legume species (the latter as an outgroup) were tested for egg-laying and larval development (see Electronic Supplementary Materials [ESM] Tables 1, 2 and 3 for species and cultivars/accessions [SCA] names). These species occur naturally in Hungary, are naturalized, or are cultivated as food or fodder. Those selected were only seeds which had masses large enough to support development of a bean weevil larva [66]. Seed mass was determined at room temperature with the help of an electronic balance (Sartorius A210P, Germany) operating with 0.1 mg accuracy.

As to developmental suitability of the plant species de Boer and Hanson’s [31] classification was accepted. *Hosts* (H) are those plant species that fully support development through generations and regularly harbour natural infestations. There are only two such species, the common bean (*Ph. vulgaris*) and the scarlet runner bean (*Ph. coccineus*) that fulfil these conditions in Hungary. *Acceptable non-hosts* (ANH) are not recognised and used as suitable substrates for larval development in nature. They grow usually asynchronously with bruchid phenology in space and time, and thus bruchid females are only occasionally constrained to use them, e.g., in seed stores. Although some adults may emerge, developmental time is typically long and larval mortality high. Chickpea is an example that belongs to this group. The third group, *non-hosts* (NH), comprises plants that are nutritionally inadequate or toxic, and never supports development, although occasional egg-laying cannot be excluded in seed stores. The seed coat, the primary interface to cotyledon, was defined as *penetrable*, if a first instar larva of *A. obtectus* was able to bore through it in case of an *intact* seed, and the cotyledon was considered *suitable* for development, if an adult emerged from a seed regardless of whether the seed coat was intact or drilled.

The seed samples originated from field or cultivated collections, and some were obtained from plant breeding companies. The samples were stored at a dry, cool place until use. Plant identification was carried out by the author.

### *Egg-laying tests*

Because no-choice egg-laying tests were carried out, “preference” is not used in the sense of choice tests, where selection by an insect is based on free movement among items offered, but instead refers to a position in the hierarchy of host-range. The measure of such preference for a plant species was the number of eggs laid/female on seeds. For the no-choice tests, the seeds of plant species were kept at 26-28 °C and 70-80% RH for a week before test to allow them to take up humidity. Three seeds of the same SCA were put into a 2x5 cm glass vial, and three one-to-two-day old females and three males were introduced into each vial. A piece of white linen cloth closed the vials. The experiment ran in darkness until all insects died, and then the lifetime number of eggs laid was counted. Seven replicates were used per plant SCA.

### *Larval performance test*

Success of larval development on the different hosts was assessed by the yield of adult offspring. Seeds of all SCA were handled as described in the previous section.

Two parallel treatments, with 45 replicates each, were set up. In one treatment the seeds were left intact, in the other they were drilled with a high speed electric drill (Triplex Miniplex, France) bit 0.14 mm in diameter, corresponding to the diameter of the first instar bean weevil larva. One or two holes per seed were made at the ends and in the middle, respectively (depending on the size of seed) under a binocular microscope. Efforts were taken to drill through the seed testa only. Seeds were placed individually into glass vials (1x6 or 2x8 cm size) corresponding to seed size, and a single “black-headed” bean weevil egg was placed onto the inner wall of the vial, ca. 1-2 cm above the seed, with the help of a fine wet brush, then the vial was capped with a cotton stopper. The vials were placed at 26-28 °C and 60-80% RH for development in darkness. To calculate the duration of time needed for development, the time of egg hatching and L1s’ boring into the seeds were recorded by daily inspection from the beginning of the experiment. The observation of adult emergence was started after ca. 30 days. At least four months were allowed for development, then the seeds were opened under a dissecting microscope and the dead life stages recorded.

The bean weevil develops through four larval stadia in beans in Hungary. After establishing egg mortality, the remaining number of instars, such as L1 dead *outside* beans, plus dead L1, L2, etc. to pupae *inside* seeds, were taken as 100%; this value was then divided up among instars recorded. The instar of each larva was verified by head capsule measurement. All developmental malformations were also recorded. During dissection of the seeds, seed coat thickness was measured on  $\geq 10$  randomly selected seeds, using a micrometre under a microscope.

### *Effect of seed coat on larval development*

In order to investigate the hypothesis that the bean seed coat is not only a physical barrier against entering the cotyledon, but contains chemicals detrimental to the L1s, artificial beans (balls) were prepared. The ca. 150 mg size balls were prepared from finely pulverized cotyledons (seed coat removed) of *Ph. vulgaris* cv. Valja, and seed coat powder of the same cultivar was added in 2.5, 5 and 10% w/w concentrations. (The seed mass of this bean variety is  $240.7 \pm 5.4$  mg [mean  $\pm$  SE, N=35], and the seed coat is <10% of the mass.) The cotyledon was milled by a water-cooled grinder (Tekmar A-10, IKA, Germany) to avoid chemical deterioration from heat. The balls consisted of a standard amount of bean cotyledon powder (80%) mixed with 20% water-soluble potato starch. These balls served as controls. Adequate portions of the potato starch were substituted with seed coat to create the above concentrations, e.g., in case of balls containing 10% seed coat powder, there were 80% cotyledon and 10% starch powders. Addition of distilled water created a pastry from which 6 mm diam. balls were made by hand, and they were dried at 40 °C for a day. The acceptability of the artificial beans for egg-laying was tested with three males and three females and three balls for each treatment. For the balls’ suitability for larval development one egg having “black-headed” larva was placed on individual balls in vials with 13 respective replicates/treatment. The number of eggs laid, the percentage of larval stages dead without or after boring into the balls, as well as that of emerging adults were recorded.

### *Statistical procedures*

Variance homogeneity of variables (larval mortality outside and inside seeds, and adult emergence by intact or drilled seeds, etc.), notwithstanding various transformations, did not meet conditions of parametric tests, therefore nonparametric tests were used. Between-groups comparisons were performed for three variables (e.g., plant groups) with Kruskal-Wallis ANOVA, and for two variables (e.g., intact and drilled seed coat) with Wald-Wolfowitz runs test. The results are presented as medians and quartiles, if data were obtained in percentages.

As a nonparametric version of discriminant function analysis, logistic regressions of the generalized linear models [67, 68] were applied to reject or accept two major null hypotheses: (a) seed coat thickness does not influence L1 mortality, and (b) the suitability of seed cotyledon does not affect larval development. The continuous dependent variables indicated dichotomous distributions, therefore they were transformed to categorized variables at biologically reasonable cutpoints, and the coding of  $\chi^2$ -table cells was performed accordingly: independent variables ( $x_i$ ), such as seed coat thickness, was assigned 1, if  $<0.1$  mm, and 0, if  $>0.1$  mm; cotyledon supporting larval development and adult emergence was given 1, if 'suitable', and 0, if 'unsuitable'. Dependent variables ( $y_j$ ), such as L1 mortality outside seeds (N=140), successful penetration of the seed coat (N=140), larval development inside seeds (N=212), and adult emergence (N=212) were coded 1, if L1 mortality was  $<50\%$ , if L1 entered the seeds, if larval mortality inside seeds was  $<30\%$  and, if adult emergence from seeds was  $>10\%$ . Zero codes were assigned to the opposite outcomes (ESM Tables 6 and 7). Such zero-one "reference cell" coding [69] produced  $\chi^2$ -tables, where the left upper cells (A) were 11, and the right lower ones (D) were 00.

For logistic regression STATISTICA ver. 6's [70] GLZ module with binomial distribution, logit link function and sigma-restricted parameter estimation was used. The respective analyses used only a single categorical predictor variable and one response variable. The program provided the estimate of the categorical predictor that maximized the probability of the dependent variable. For goodness of fit Wald- and  $\chi^2$ -statistics of log-likelihood were calculated.

With the categorical data, effect sizes (risk difference, risk ratio, odds ratio and confidence intervals, CI) for L1 mortality and adult emergence were estimated by the methods described in [71, 72]. The calculations were based on 2x2  $\chi^2$ -tables (ESM Tables 6 and 7). The odds ratio (OR) of the outcome was computed by the probabilities of cells:  $[p(1)/(1-p(1))]/[p(0)/(1-p(0))]$ , where in  $p(1)$  the categorical independent predictor is 1, and in  $p(0)$  it is 0. In order to be able to calculate effect size for adult emergence, 0.5 was added to all values in cells, because one element (adult emergence from NH plants) of the frequency table was zero. Interactions between categorical variables (e.g., 'penetrable'/'impenetrable' and intact/drilled seed coat) were computed by multiplying two odds ratios ( $OR_{10} \times OR_{01}$ ) assuming  $H_0$  (that there is no interaction) if the odds ratio cell  $OR_{11}$  equalled the result [73]. One-way ANOVA was used to evaluate the effects of concentrations of seed coat powder on larval mortality. All statistical procedures were performed with STATISTICA 6 [70].

## Abbreviations

**H** = host

**ANH** = acceptable non-host

**NH** = non-host

**SCA** = species/cultivar/accession

**KW** = Kruskal-Wallis

**ESM** = electronic supplementary material

**OR** = odds ratio

**RD** = risk difference

**RR** = risk ratio

**CI** = confidence interval

**GLZ** = generalized linear model

## Declarations

**Ethics approval:** This article does not contain any work with protected animal species, therefore, no ethical approval/permission was necessary from an appropriate ethics committee/review board local, regional or national board, to carry out sampling and experiments described in the Methods.

**Consent for publication:** Not applicable.

**Availability of data and material:** The datasets used and/or analysed during the current study are available from the author on reasonable request. Additional information is available in Electronic Supplementary Materials.

**Competing interests:** Not applicable.

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## Tables

**Table 1** Plant traits and responses of the bean weevil (*Acanthoscelides obtectus*) to host, acceptable non-host, and non-host leguminous plant species

Insect responses and plant traits	Hosts		Acceptable non-hosts		Non-hosts	
	Seed coat					
	Intact	Drilled	Intact	Drilled	Intact	Drilled
L1-pupal mortality inside seeds (%) <sup>1</sup>	0a (0-2.2) (N=22)	2.2A (0-2.3) (N=22)	2.3b (0-7.7) (N=55)	48.0B (24.4-82.8) (N=55)	0c (0-0) (N=63)	75.0C (44.0-93.0) (N=63)
Adult emergence (%) <sup>2</sup>	75.3a (46.7-90.5) (N=22)	93.3A (86.1-97.7) (N=22)	0b (0-5.0) (N=55)	26.7B (8.9-48.8) (N=55)	0c (N=63)	0C (N=63)
L1 mortality outside seeds (%) <sup>3</sup>	24.7c (9.1-53.3) (N=22)		93.3b (80.5-100) (N=55)		100a (100-100) (N=63)	
Number of eggs laid/female <sup>4</sup>	38.6±0.8a (N=154)		18.6±0.7b (N=418)		13.5±0.5c (N=630)	
Seed mass (mg) <sup>5</sup>	340.8±4.3a (N=773)		235.6±3.9b (N=1823)		70.3±1.6c (N=2052)	
Seed coat thickness (mm) <sup>6</sup>	0.09±0.001c (N=374)		0.10±0.001b (N=933)		0.13±0.002a (N=1098)	

Kruskal-Wallis ANOVAs: <sup>1</sup>Intact seed coat: KW  $H'_{2,140} = 28.4$ ,  $p < 0.001$ , drilled seed coat: KW  $H'_{2,140} = 60.6$ ,  $p < 0.001$ . <sup>2</sup>Intact seed coat:  $H'_{2,140} = 93.6$ ,  $p < 0.001$ , drilled seed coat:  $H'_{2,140} = 120.9$ ,  $p < 0.001$ ; <sup>3</sup> $H'_{2,140} = 77.9$ ,  $p < 0.001$ ; <sup>4</sup> $H'_{2,1202} = 283.0$ ,  $p < 0.001$ ; <sup>5</sup> $H'_{2,4648} = 2515.2$ ,  $p < 0.001$ ; <sup>6</sup> $H'_{2,2405} = 216.2$ ,  $p < 0.001$ . In comparisons among intact or drilled seeds of plant groups, medians or means signed with different lower or upper case letters in the same row, are significantly different, resp. <sup>1-</sup><sup>3</sup>Medians and quartiles; <sup>4-6</sup>Means  $\pm$  SEs. A comparison of adult emergence between intact and drilled seeds of H plants was not significant ( $Z_{adj}=0.7627$ ,  $p=0.4456$ ), whereas comparisons between  $H_{intact}$  and  $ANH_{intact}$  ( $Z_{adj}=6.1818$ ,  $p < 0.001$ ), between  $H_{drilled}$  and  $ANH_{drilled}$  ( $Z_{adj}=5.6180$ ,  $p < 0.001$ ), and between  $ANH_{intact}$  and  $ANH_{drilled}$  ( $Z_{adj}=3.3525$ ,  $p < 0.001$ ) were significant (Wald-Wolfowitz Runs tests)

**Table 2** Egg-laying and larval survival of the bean weevil (*Acanthoscelides obtectus*) on artificial seeds incorporated with bean seed coat powder

Type of artificial seed <sup>1</sup>	Number of eggs laid/female (mean±SE)	Dead L1 outside (%)	Dead larvae inside (%)	Adults emerged (%)
2.5% seed coat	8.6 ± 2.1a	14.3	85.7	0
5.0% seed coat	4.5 ± 1.6a	28.9	71.1	0
10.0% seed coat	6.6 ± 1.2a	12.9	87.1	0
Control	7.6 ± 2.3a	2.7	2.7	94.6

<sup>1</sup>Artificial seeds (balls) consisted of 80% cotyledon powder plus 20% water soluble potato starch powder. A portion of the latter was substituted with 2.5-10% seed coat powder. Control balls contained only cotyledon and starch powders. The Brown-Forsythe test indicated homogeneity of variances of the number of eggs laid/female:  $F_{3,48}=1.5344$ ,  $p=0.2176$ , and one-way ANOVA ( $F_{3,48}=0.8445$ ,  $p=0.4763$ ), as-well-as the Scheffé post-hoc test were not significant at  $p<0.05$

**Table 3** Mean seed mass and seed coat thickness of leguminous plants from which bean weevil (*Acanthoscelides obtectus*) adults emerged

Plant tribe and species <sup>1</sup>	Seed mass <sup>2</sup> (mg) ±SE (N)	Seed coat thickness <sup>3</sup> (mm) ±SE (N)
<b>Genisteae</b>		
<i>Lupinus albus</i> L.	197.2 ± 4.1 (46)	0.20 ± 0.007 (25)
<b>Phaseoleae</b>		
<i>Glycine max</i> (L.) Merr. <sup>4</sup>	167.9 ± 0.7 (910)	0.09 ± 0.001 (265)
<i>Lablab purpureus</i> (L.) Sweet	142.2 ± 3.5 (298)	0.15 ± 0.004 (24)
<i>Vigna unguiculata</i> (L.) Walp. <sup>4</sup>	109.6 ± 5.2 (70)	0.04 ± 0.002 (36)
<i>Vigna angularis</i> (Willd.) Ohwi & H. Ohashi	114.1 ± 1.7 (35)	0.07 ± 0.004 (20)
<i>Vigna radiata</i> (L.) R. Wilczek	69.1 ± 1.0 (35)	0.05 ± 0.001 (15)
<i>Phaseolus vulgaris</i> L. <sup>4</sup>	334.8 ± 4.4 (738)	0.09 ± 0.001 (359)
<i>Phaseolus coccineus</i> L.	467.9 ± 8.3 (35)	0.09 ± 0.006 (15)
<b>Cicereae</b>		
<i>Cicer arietinum</i> L.	189.6 ± 4.3 (35)	0.17 ± 0.004 (15)
<b>Fabeae</b>		
<i>Vicia faba</i> L. <sup>4</sup>	613.9 ± 10.1 (210)	0.18 ± 0.004 (110)
<i>Lens culinaris</i> Medik. <sup>4</sup>	48.7 ± 1.3 (105)	0.05 ± 0.001 (45)
<i>Lathyrus hirsutus</i> L.	26.8 ± 0.6 (35)	0.15 ± 0.003 (12)
<i>Lathyrus latifolius</i> L.	57.3 ± 1.8 (35)	0.14 ± 0.006 (20)
<i>Lathyrus odoratus</i> L.	54.0 ± 0.9 (33)	0.12 ± 0.006 (16)
<i>Lathyrus pratensis</i> L.	15.0 ± 0.4 (35)	0.10 ± 0.002 (17)
<i>Lathyrus sativus</i> L.	248.7 ± 7.0 (35)	0.09 ± 0.007 (15)
<i>Lathyrus tuberosus</i> L. <sup>4</sup>	37.0 ± 0.8 (70)	0.15 ± 0.003 (63)
<i>Pisum sativum</i> L. <sup>4</sup>	243.2 ± 1.6 (975)	0.09 ± 0.001 (489)

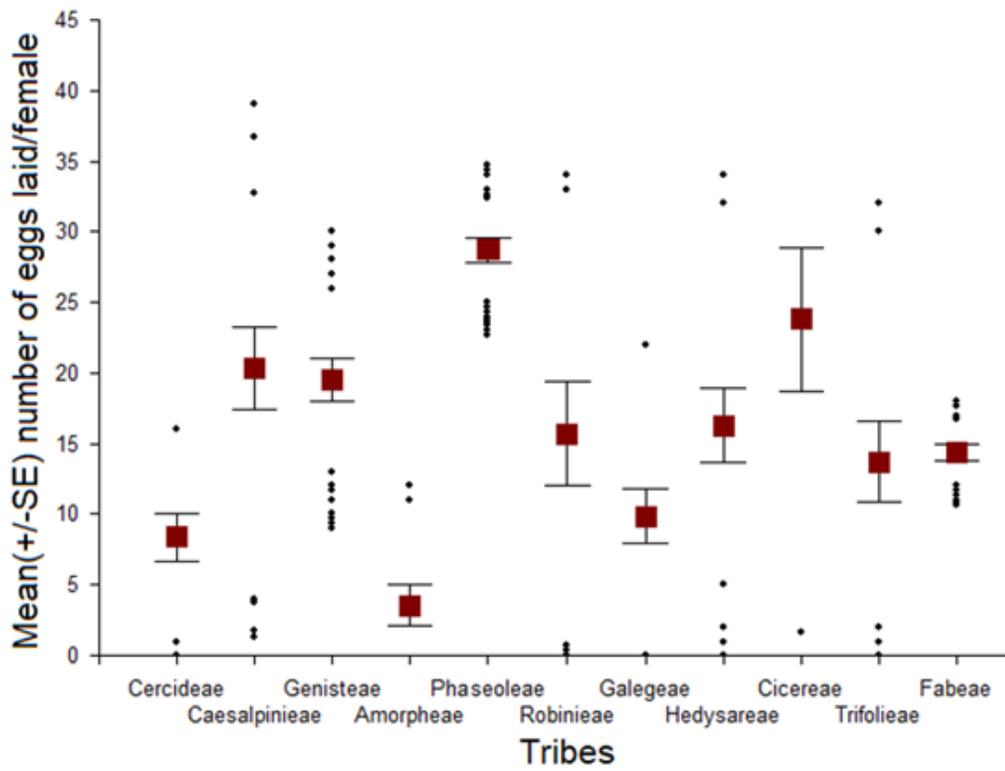
<sup>1</sup>Species' and authorities' names are according to ILDIS (International Legume Database & Information Service) <https://ildis.org/index.shtml> [74]. Accessed Oct. 2020; <sup>2</sup>Measured at room temperature; <sup>3</sup>Measured under the dissection microscope; <sup>4</sup>The number of cultivars/accessions included in the measurements was 17, 2, 21, 6, 2, 2 and 27 in the order of plant species from above

**Table 4** Emergence and development time of bean weevil (*Acanthoscelides obtectus*) adults and mortality of stages in host and acceptable non-host plant species. Percentage data are presented medians (see quartiles in ESM Table 4)

Plant tribe and species <sup>1</sup>	Adult emergence (%) from seeds		Development time (days) <sup>2</sup>		L1 mortality outside seeds (%)		L1-pupal mortality inside seeds (%)	
	Intact	Drilled	Intact	Drilled	Intact	Drilled	Intact	Drilled
<b>Genisteae</b>								
<i>Vicia albus</i>	0	3.5			100	18.8	0	77.7
<b>Haseoleae</b>								
<i>Vicia max</i>	0*	0	68-101	67-92	97.7	2.3	2.2	95.5
<i>Vicia ablab</i>	0	7.0			100	10.3	0	82.7
<i>Vicia purpureus</i>								
<i>Vicia igna</i>	65.2	93.3	30-40	28-51	32.5	3.3	2.3	3.4
<i>Vicia unguiculata</i>								
<i>Vicia angularis</i>	11.4	63.6	51-154	41-68	34.1	4.6	54.6	31.8
<i>Vicia radiata</i>	0	86.7		30-40	100	2.2	0	11.1
<i>Vicia haseolus</i>	72.7	93.2	31-59	31-86	27.3	4.4	0	2.2
<i>Vicia vulgaris</i>								
<i>Vicia coccineus</i>	100	97.8	37-44	37-44	0	2.22	0	0
<b>Ciceraceae</b>								
<i>Cicer arietinum</i>	2.2	97.8		33-58	97.8	2.2	0	0
<b>Fabeae</b>								
<i>Vicia faba</i>	6.7	38.4	32-68	35-79	83.9	35.6	4.7	19.2
<i>Vicia ens culinaris</i>	0	2.3			100	51.2	0	48.8
<i>Vicia athyrus</i>	0	20.0		84	100	32.0	0	48.0
<i>Vicia hirsutus</i>								
<i>Vicia latifolius</i>	0	4.2		85	100	87.5	0	8.3
<i>Vicia odoratus</i>	0	12.0		85	100	32.0	0	56.0
<i>Vicia pratensis</i>	0	46.7		84	100	6.7	0	46.7
<i>Vicia sativus</i>	57.8	75.6	33-90	33-47	40.0	11.1	2.2	13.3
<i>Vicia tuberosus</i>	0	13.6		40-42	100	28.0	0	44.4
<i>Vicia isum sativum</i>	0	20.5	46-101	40-162	93.2	7.3	4.6	62.5

According to ILDIS (International Legume Database & Information Service) <https://ildis.org/index.shtml> [74]. Accessed Oct. 2020; <sup>2</sup>From L1's entering the seeds to adult emergence (min-max values). Empty cells mean missing data. \*Although the median was zero, a very low number of adults emerged from intact seeds (mean  $\pm$ SE: 1.12 $\pm$ 0.6, N=17)

## Figures



**Figure 1**

Distribution of the number of eggs laid/female bean weevil (*Acanthoscelides obtectus*) by leguminous tribes. Data are means ( $\pm$ SEs), points present outlier values. The order of tribes corresponds to the phylogenetic relations presented by [24]

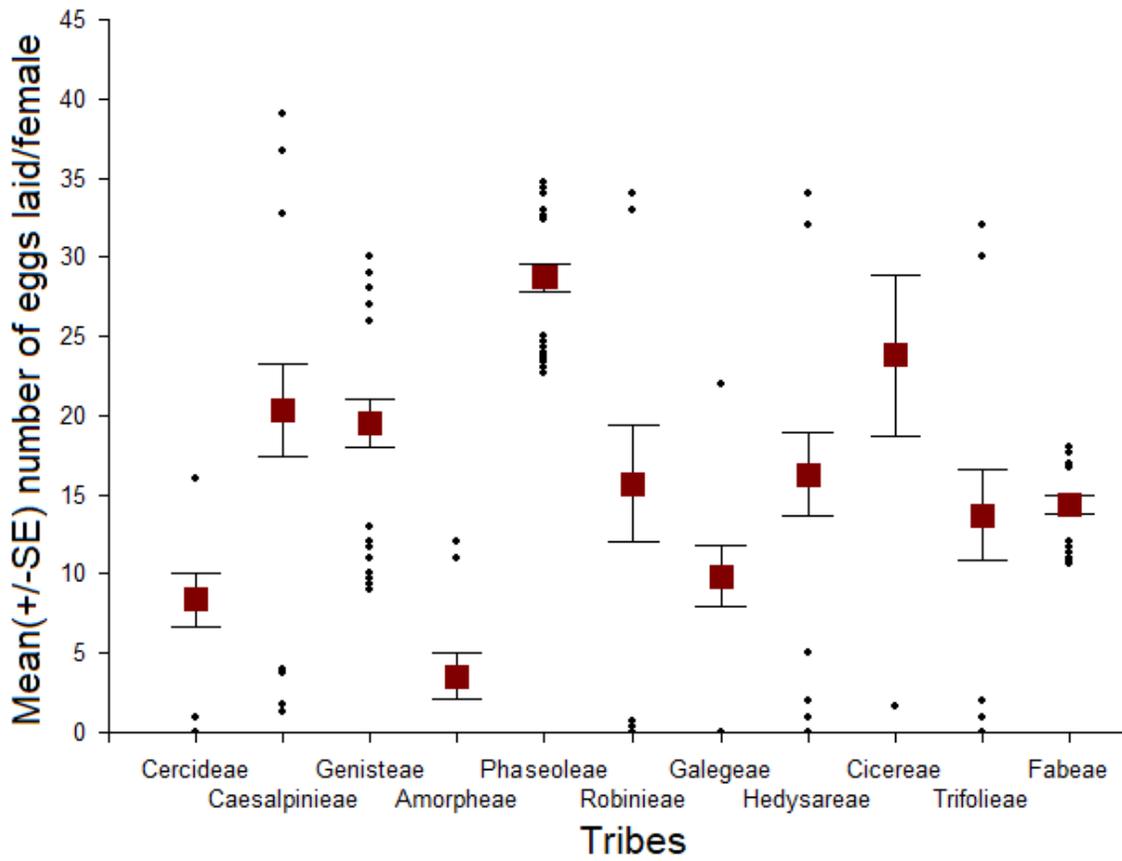
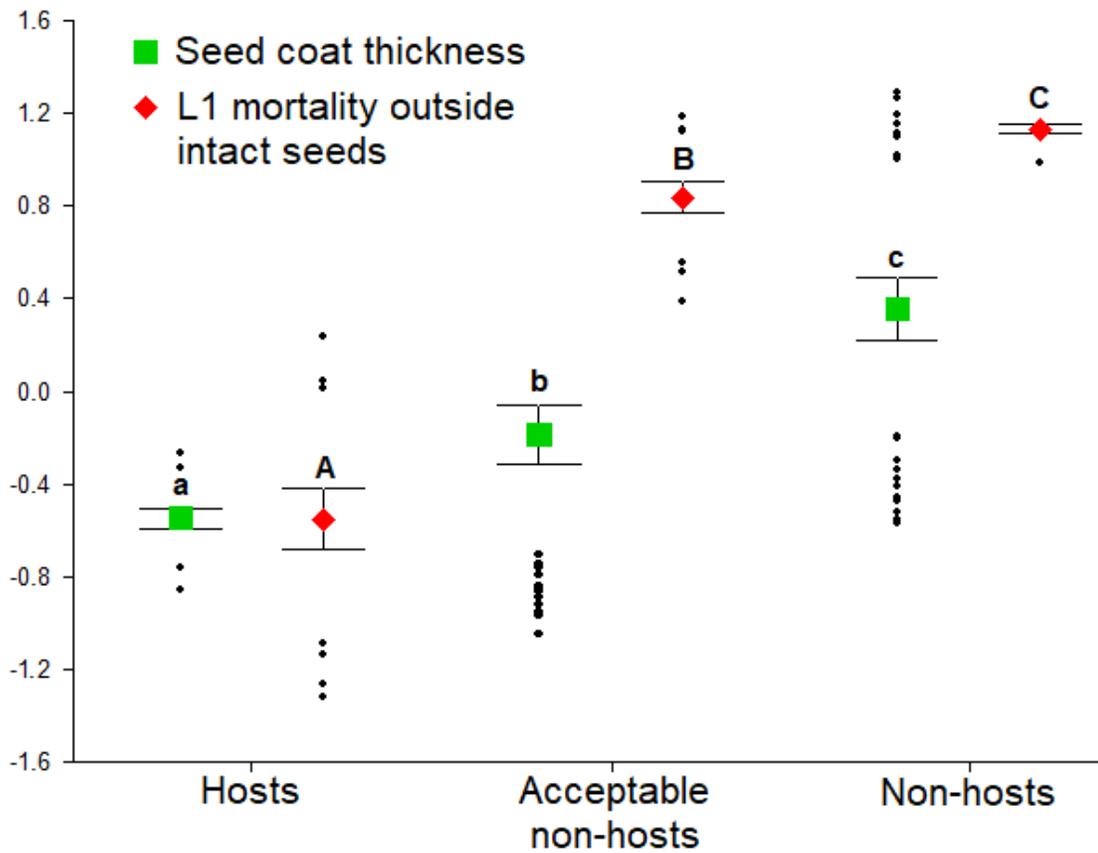


Figure 2

Distribution of the number of eggs laid/female bean weevil (*A. obtectus*) by leguminous tribes. Data are mean ( $\pm$ SEs), circles present outlier values. The order of tribes corresponds to the phylogenetic relations presented by Lewis et al. (2005)



**Figure 3**

Interaction of seed coat thickness and mortality of first instar larvae of the bean weevil (*A. obtectus*) outside intact leguminous seeds on H, ANH and NH plants. As original units were in mm and percentages, resp., data were standardized in order to make the figure metric free. Standardization did not affect statistical relations. Black points represent outliers. Means ( $\pm$ SEs) signed with different small (seed coat thickness) and large (L1 mortality outside seeds) letters are significantly different

## Supplementary Files

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- [SzentesiBMCEcolElectronicSupplementaryMaterialsrevised.pdf](#)