

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 of the dry bean weevil, *Acanthoscelides obtectus* Say (Coleoptera: Bruchinae), a seed predator of beans, is not properly known. Occasional use of leguminous seeds other than beans is reported, however the sphere of possible wild and cultivated hosts is uncertain. Female oviposition preference and larval performance relationship is complicated by the respective importance of seed coat and cotyledon, because paradoxically, females must exercise oviposition preference on the basis of stimuli provided by the seed coat alone, without directly being able to assess the quality of cotyledon's suitability for larval development.

Results Host specificity and host range investigations carried out on seeds of 62 grown and naturally occurring legume species and 82 cultivars of *Phaseolus*, *Pisum*, *Glycine*, *Lens* and others in Hungary, using no-choice tests for egg-laying, and intact or pierced seed coat for larval development in seeds, showed that there were 18 plant species (35% of them *Lathyrus*) that supported larval development to adults, however, only nine species (4 of 17 *Glycine max* accessions, *Vigna unguiculata*, *V. angularis*, *Phaseolus vulgaris*, *Ph. coccineus*, *Cicer arietinum*, *Vicia faba*, *Lathyrus sativus* and 13 of 27 *Pisum sativum* accessions) allowed it if the seed coat was intact. Furthermore, there was no overall positive correlation between oviposition preference and larval performance, with the exception for the 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 Host range expansion is not probable with the bean weevil, primarily because it would require the recognition of basically different oviposition substrates (pods) among outdoor conditions.

Background

In a seminal paper entitled "How southern cowpea weevil larvae (Bruchidae: *Callosobruchus maculatus*) die on nonhost seeds" Janzen [1] for the first time 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 ovipositions 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 progenies' 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 the offspring's 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 the base of which the female makes a decision on egg-laying 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 of the enormous advance in chemical analysis and the wealth of information of seed chemical composition in Fabaceae [15], 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 bruchids *Acanthoscelides obtectus*, *Callosobruchus* spp., and *Zabrotes subfasciatus*, 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*. Tucić et al. [18], then Milanović and Gliksman [19] showed that after rearing *A. obtectus* on chickpea (*Cicer arietinum*) for ≥ 50 generations, it had lower survival rate on the main host, *Phaseolus vulgaris*. 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 tests the preference-performance relations of the bean weevil *Acanthoscelides obtectus* Say (Coleoptera, Chrysomelidae, Bruchinae). This bruchid is unusual in that the first instar larva possesses legs, therefore can exercise selection behaviour, even against its mother's preference, if a choice of seeds is available. Due to its indoor life cycle, dry seeds are accessible for this bruchid; therefore the females can impart higher fitness to progeny if her choice is optimal. Several questions are pending, including:

how wide is the host range, what kind of 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 [20], but are they able to judge the suitability for larval development, based on information provided solely by the seed coat? I also investigated the following hypotheses: (1) Seed coat is both *i*) a physical barrier, therefore thinner seed coats represent lower resistance to L1 larvae to enter seeds successfully and in greater proportions than seeds with thicker testa, and *ii*) a chemical barrier, causing mortality if consumed. (2) It is the suitability of cotyledon that defines first instars' development to adult stage if seed coat as a physical barrier is eliminated.

Results

Female oviposition preference

Bruchid females deposited significantly decreasing number of eggs on seeds in the order of H, ANH and NH species (Fig. 1, Kruskal-Wallis ANOVA: $H_{2,140} = 48.644$, $p < 0.001$). Females laid 36.9 ± 1.8 (mean \pm SE) eggs on H species (N = 22 SCAs), 18.9 ± 1.3 eggs on ANH species (N = 55 SCAs), and 13.4 ± 0.89 eggs on NHs (N = 63 SCAs). The females laid significantly more eggs on larger seeds (Fig. 1, Kruskal-Wallis test: $H_{2,132} = 73.057$, $p < 0.001$). Mean mass of H seeds was 340.8 ± 24.0 mg, that of ANHs 235.3 ± 22.9 mg, and that of NHs 69.5 ± 9.7 mg. 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. Figure 2 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*) was the least preferred. Comparable responses were noted to some species within Caesalpinieae, Genisteae, Robinieae, Cicereae and Fabaeae (ESM Table 1). Although none of them being a host for the bean weevil, within the tribe of Caesalpinieae *Gleditsia delavayi*, within Genisteae *Laburnum alpinum* and *L. anagyroides*, and within Robinieae *Robinia viscosa* received high numbers of eggs. *Cicer arietinum* and *Vicia faba* are known as occasional hosts. The number of eggs laid on them fell into the medium and high categories, respectively. Among the legumes the lowest numbers of eggs were laid on *Vicia tenuifolia*, *Robinia pseudoacacia* and *Amorpha fruticosa*, but on some members of the Fabaeae tribe, comprising *Vicia* and *Lathyrus* species, the mean number of eggs was less than 10. Females accepted the pilules incorporated with seed coat as an oviposition substrate and laid comparable number of eggs to control (Table 3).

Table 1

Mean seed mass and seed coat thickness of leguminous plants from which bean weevil (*A. obtectus*) adults emerged. *Glycine max* comprises 17, *Vigna unguiculata* 2, *Phaseolus vulgaris* 21, *Vicia faba* 6, *Lens culinaris* 2, *Pisum sativum* 27 cultivars, and *Lathyrus tuberosus* 2 samples

Plant tribe and species ¹	Seed mass ² (mg) ±SE (N)	Seed coat thickness ³ (mm) ± SE (N)
Genisteae		
<i>Lupinus albus</i> L.	197.2 ± 4.1 (46)	0.20 ± 0.007 (25)
Phaseoleae		
<i>Glycine max</i> (L.) Merr. ⁴	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.	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. ⁴	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)
Cicereae		
<i>Cicer arietinum</i> L.	189.6 ± 4.3 (35)	0.17 ± 0.004 (15)
Fabeae		
<i>Vicia faba</i> L. ⁴	613.9 ± 10.1 (210)	0.18 ± 0.004 (110)
<i>Lens culinaris</i> Medik. ⁴	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)

¹Species' and authors' names are according to ILDIS (International Legume Database & Information Service) <https://ildis.org/index.shtml> [70]. Accessed 20 May 2020; ²Measured at room temperature; ³Measured under the dissection microscope; ⁴Cultivars/accessions were included in the measurements.

Plant tribe and species ¹	Seed mass ² (mg) ±SE (N)	Seed coat thickness ³ (mm) ± SE (N)
<i>Lathyrus tuberosus</i> L.	37.0 ± 0.8 (70)	0.15 ± 0.003 (63)
<i>Pisum sativum</i> L. ⁴	243.2 ± 1.6 (975)	0.09 ± 0.001 (489)

¹Species' and authors' names are according to ILDIS (International Legume Database & Information Service) <https://ildis.org/index.shtml> [70]. Accessed 20 May 2020; ²Measured at room temperature; ³Measured under the dissection microscope; ⁴Cultivars/accessions were included in the measurements.

Table 3

Egg-laying and larval development of the bean weevil (*A. obtectus*) on artificial seeds incorporated with bean seed coat powder

Type of pilule ¹	Number of eggs laid/female (mean ± SE)	Dead L1 larvae 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

¹Pilules 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 pilules 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 ($F_{3,48}=0.8445$, $p = 0.4763$) and Scheffé post-hoc test were not significant at $p < 0.05$.

Results of larval performance

Development 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 pierced status of seeds. The seed coats of H and ANH species were significantly thinner (H: 0.088 ± 0.002 mm, mean ± SE, N = 22; ANH: 0.103 ± 0.005 mm, N = 55) than those of NHs (0.126 ± 0.006 mm, N = 63) (Fig. 3, Kruskal-Wallis ANOVA: $H_{2,140}=17.48$, $p = 0.0002$). L1 mortality outside intact seeds was significantly different among plant species groups (Fig. 3, Kruskal-Wallis ANOVA: $H_{2,140}=77.9$, $p < 0.001$). It was lower for Hs (24.75%,

9.1–53.3 median and quartiles, N = 22) and ANH (93.3%, 80.5–100, N = 55) SCAs, in comparison with NHs (100%, N = 63). For H and ANH species Table 1 provides data of seed coat thickness, whereas ESM Tables 2 and 3 give similar information for NHs and SCAs of Hs and ANHs. Remarkably, seed coat substantiates a barrier even on its primary host, the beans (*Ph. vulgaris*), for the bean weevil. Contrary to the very low larval mortality inside seeds, there were substantial mortality outside and especially in case of intact seeds of bean cultivars (27.3%, 13.6–53.3, median, lower and upper quartiles, N = 21), and 4.4% (0-11.4) for pierced 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* and *V. angularis*, where seed coats were extremely thin (Tables 1 and 2). A remarkable case is the runner beans (*Ph. coccineus*) with 0% (intact seed) and 2.2% (pierced seed) larval mortality outside seeds.

Table 2

Emergence and length of development of bean weevil (*A. obtectus*) adults and mortality of stages in host and acceptable non-host plant species. Where data were obtained in percentages, results were presented in medians and quartiles (numbers in smaller fonts). *Glycine max* comprises 17, *Vigna unguiculata* 2, *Phaseolus vulgaris* 21, *Vicia faba* 6, *Lens culinaris* 2, *Pisum sativum* 27 cultivars, and *Lathyrus tuberosus* 2 samples

Plant tribe and species ¹	Adult emergence (%) from seeds		Length of development (days) ²		L1 mortality (%)		Larval-pupal mortality (%)	
					Outside seeds		Inside seeds	
	Intact	Pierced	Intact	Pierced	Intact	Pierced	Intact	Pierced
Genisteae								
<i>Lupinus albus</i>	0	3.5			100	18.8	0	77.7
Phaseoleae								
<i>Glycine max</i>	0*	0	68–101	67–92	97.7	2.3	2.2	95.5
		0-2.2			93.2–100	2.2–4.4	0-6.7	90.9–97.8
<i>Lablab purpureus</i>	0	7.0			100	10.3	0	82.7
<i>Vigna unguiculata</i>	65.2	93.3	30–40	28–51	32.5	3.3	2.3	3.4
	39.5–90.9	88.9–97.7			6.8–58.1	0-6.7	2.3–2.3	2.3–4.4
<i>V. angularis</i>	11.4	63.6	51–154	41–68	34.1	4.6	54.6	31.8
<i>V. radiata</i>	0	86.7		30–40	100	2.2	0	11.1
<i>Phaseolus vulgaris</i>	72.7	93.2	31–59	31–86	27.3	4.4	0	2.2
	46.7–86.4	86.1–97.6			13.6–53.3	0-11.4	0-2.2	0-2.3
<i>P. coccineus</i>	100	97.8	37–44	37–44	0	2.22	0	0
Cicereae								
<i>Cicer arietinum</i>	2.2	97.8		33–58	97.8	2.2	0	0
Fabeae								

¹According to ILDIS (International Legume Database & Information Service)

<https://ildis.org/index.shtml> [70]. Accessed 20 May 2020; ²From 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 ± SE: 1.12 ± 0.6, N = 17).

Plant tribe and species ¹	Adult emergence (%) from seeds		Length of development (days) ²		L1 mortality (%)		Larval-pupal mortality (%)	
	min	max	min	max	min	max	min	max
<i>Vicia faba</i>	6.7	38.4	32–68	35–79	83.9	35.6	4.7	19.2
	2.3–20.5	35.9–41.9			73.3–95.4	30.2–51.3	2.3–15.6	9.3–31.8
<i>Lens culinaris</i>	0	2.3			100	51.2	0	48.8
		0-2.3			95.6–100	34.9–56.8	0-4.4	40.9–62.8
<i>Lathyrus hirsutus</i>	0	20.0		84	100	32.0	0	48.0
<i>L. latifolius</i>	0	4.2		85	100	87.5	0	8.3
<i>L. odoratus</i>	0	12.0		85	100	32.0	0	56.0
<i>L. pratensis</i>	0	46.7		84	100	6.7	0	46.7
<i>L. sativus</i>	57.8	75.6	33–90	33–47	40.0	11.1	2.2	13.3
<i>L. tuberosus</i>	0	13.6		40–42	100	28.0	0	44.4
		0-28.9				26.7–59.1		27.3–72.0
<i>Pisum sativum</i>	0	20.5	46–101	40–162	93.2	7.3	4.6	62.5
	0-4.4	8.9–34.9			83.7–95.6	4.5–18.9	2.2–7.7	46.3–85.0

¹According to ILDIS (International Legume Database & Information Service)

<https://ildis.org/index.shtml> [70]. Accessed 20 May 2020; ²From 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 ± SE: 1.12 ± 0.6, N = 17).

Mortalities of various developmental stages inside intact seeds were substantially different from those of pierced ones. Furthermore, there were differences among host-types too (Kruskal-Wallis ANOVA: $H_{2,140}=28.39$, $p < 0.001$). Table 2 provides results for H and ANH species, and further data are available in ESM Tables 2 and 3 for bean, pea and soybean varieties, as well as for NH species. For NHs mortality inside intact seeds was practically nil due to the inability of L1s to penetrate the seed coat, whereas for pierced 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* seeds, both intact and pierced, had such traces). It is worth remarking that ca. 10% of L1 entered the intact *Gleditsia delavayi* seeds. Inside the seeds many L1s died by various manners. Some perished on the surface of the

cotyledon, others following preparation of tunnels of various lengths. Although it is well documented that additional *A. obtectus* larvae may enter through the hole made by a pioneer larva [21], the ratio of L1-made and artificial holes was 1.5:1 on the most preferred bean seeds (*Ph. coccineus*), i.e., most L1 larvae did not use the pre-prepared holes on this host. In some instances (*Caragana*, *Onobrychis*) 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 making 1–2 mm long tunnels. In cases where the cotyledons were soft (several *Glycine* cultivars/accessions and *Caragana*) larvae made longer paths before dying.

Development in artificial seeds. The experiment proved that besides being a physical barrier, seed coat also inhibited larval development at the lowest concentration incorporated into artificial seeds (Table 3).

Adult emergence. Significantly more adults emerged from H species/cultivars than either from ANH species/cultivars or NH species, and whether intact or pierced (Table 2). From intact seeds of Hs 75.3% (46.7–90.5), from ANHs 0% (0–5, medians and quartiles), and from NHs 0% adults emerged (Kruskal-Wallis ANOVA: $H_{2,140}=120.88$, $p < 0.001$). From pierced seeds of Hs 93.3% (86.1–97.7 medians and quartiles), from ANHs 26.7% (8.9–48.8), and from NHs 0% adults emerged (Kruskal-Wallis ANOVA: $H_{2,140}=93.61$, $p < 0.001$) (Fig. 1). Of the 62 legume species *A. obtectus* larvae developed into adults in 18 (29%) in four tribes (Table 2), however, the picture varied considerably concerning SCAs, and whether the seed coat was intact or not (ESM Table 3). Although adult emergence in *Ph. vulgaris* was generally high, at 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, soybean (*Glycine*) varieties and hyacinth bean (*Lablab*) did so only sporadically. This is also supported by the length of developmental time needed until adult emergence (Table 1). In some cases (*G. max*, *V. angularis* and *P. sativum*) 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 for 17 *G. max* cultivars/accessions: adult emerged from six (35.3%), and only from four of these if having 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*, from intact, the rest of them from pierced seeds), there was only one such case among vetches, the faba beans (*Vicia faba*). Both intact and pierced seeds yielded adults from faba beans, although it had a relatively thick testa. Although larval development proceeded in some NHs 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 0.06% malformed adults in beans, 33.0% in peas, 31.6% in *L. tuberosus*, 6.67% in *L. sativus*, and 0.72% in *V. faba*.

Female preference and larval performance relation

Correlations. Table 4 provides the most important 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., the number of eggs laid and the adult emergence. The seed mass and the number of eggs laid positively, but not significantly, correlated only in Hs. 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.

Table 4
Nonparametric (Kendall τ) correlation coefficients between seed traits and/or insect responses of the bean weevil (*A. obtectus*)

Correlations between seed traits and/or insect responses	H	ANH	NH
Seed mass vs. Number of eggs laid per female	0.2739	0.0189	0.0866
Seed coat thickness vs. L1 mortality outside seeds	-0.3414	0.2139	0.2264
Number of eggs laid per female vs. Adult emergence	0.1400	0.3088	-

H = hosts, ANH = acceptable non-hosts, NH = non-hosts. Bold numbers are significant correlations ($p < 0.05$).

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 (0.081 ± 0.001 mm, mean \pm SE, $N = 62$), in comparison with thick seed coat (0.147 ± 0.005 mm, $N = 78$; Wald test: 8.165, $df = 1$, $p = 0.0043$; log-likelihood: -48.4233; goodness of fit χ^2 : 17.986, $df = 1$, $p < 0.001$). However, 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: 27.2694, $df = 1$, $p < 0.001$; log-likelihood: -81.4425; $\chi^2 = 31.082$, $df = 1$, $p < 0.001$). 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 ± 0.004 mm ($N = 64$), whereas 'impenetrable' showed 0.119 ± 0.005 mm ($N = 76$), amounting only to 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' in comparison with 'unsuitable' cotyledon (Wald test: 27.5997, $df = 1$, $p < 0.001$; log-likelihood= -131.456; $\chi^2 = 29.7737$, $df = 1$, $p < 0.001$). The number of eggs on 'suitable' seeds was 26.25 ± 1.11 eggs/female (mean \pm SE, $N = 124$) vs. on 'unsuitable': 13.84 ± 0.72 , $N = 88$. Logistic regression for adult emergence could not be performed, because one cell of the χ^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 having < 50% mortality, if they happened to bore in a seed with seed coat thickness of < 0.1 mm, and risk difference (RD) and risk ratio (RR) also strengthened the finding (Table 5). However, the regression coefficient (φ^2) explained only 10% of variance of seed coat effect. On the other hand, when facing penetrable/impenetrable seed coat (results are not shown in table), L1 larvae got into the 'penetrable' seeds with ca. 3 times higher probability (RR = 3.01 ± 1.3, mean ± SE, CI₉₅ = 1.9 & 4.8), but effect size for larvae to enter a seed was large (OR = 7.5 ± 1.5, CI₉₅ = 3.5 & 16.1) in case of a penetrable testa, even if they died after the first bites from the cotyledon. Here, the regression coefficient explained a relatively high level (21%) of the variance.

Table 5

Chi²-table and risk effect sizes with 95% confidence intervals of bean weevil (*A. obtectus*) L1 mortality due to seed coat thickness on hosts and acceptable non-hosts (lumped together) vs. non-hosts

Dependent variable	Independent variable: Seed coat thickness			Marg. sums	Effect size
	< 0.1 mm	> 0.1 mm			
L1 mortality					$p_{<50\%}^3 = 0.95$ $p_{>50\%}^4 = 0.4917$
First instar mortality	< 50% ¹	19	1	20	RD ⁵ = 0.4583; CI ₉₅ ⁶ = 0.30 & 0.62
outside seeds	L1 mortality				RR ⁷ = 1.9322; CI ₉₅ = 1.57 & 2.38
seeds	> 50% ²	59	61	120	OR ⁸ = 19.7; CI ₉₅ = 2.55 & 152.4
	Marginal				logit d^9 = 1.6441
	sums	78	62	140	Pearson correlation (φ) = 0.3229
					Regression (φ^2) = 0.1043

¹Hosts and acceptable non-hosts; ²Non-hosts; ³Proportion of L1 died in case of hosts and acceptable non-hosts; ⁴Proportion of L1 died in the presence of non-hosts; ⁵Risk difference; ⁶95% Confidence Interval; ⁷Risk ratio; ⁸Odds ratio; ⁹Standardized mean difference; χ^2 for the table = 14.6, df = 1, p < 0.001.

The second phase of the preference-performance relationship is manifested in the adult progeny production as related to the number of eggs laid per species. The chance of < 30% mortality inside seeds for larvae increased ca. 2 times (RR = 1.95 ± 1.13, CI₉₅ = 1.52 & 2.49) in cotyledon suitable for reaching later developmental stages. The odds of outcome was high (OR = 4.85 ± 1.28, CI₉₅ = 2.96 & 7.94, results are not shown in table). The chance for reaching adulthood in seeds where > 10% of adults emerged was ca. 4 times higher (RR = 3.82 ± 1.16, CI₉₅ = 2.83 & 5.16), than in cotyledons where only 1–2 weevils could

successfully terminated development. The odds for adulthood in suitable seeds were extremely high (OR = 523.42 ± 1.23 , $CI_{95} = 348.74 \text{ \& } 785.60$) due to the asymmetry caused by the NH seeds (Table 6). The regression coefficient (φ^2) explained a high portion (54%) of the variance.

Table 6

Chi²-table and risk effect sizes of bean weevil (*A. obtectus*) adult emergence from hosts and acceptable non-hosts (lumped together) vs. non-hosts

Dependent variable	Independent variable: Suitability (suit.) of cotyledon				Effect size
	More suit.	Less suit.	Marg. sums		
					$p_{>10\%}^3 = 0.9946$ $p_{<10\%}^4 = 0.2603$
Adult emergence	> 10% ¹	92	0	92	RD ⁵ = 0.7343; $CI_{95}^6 = 0.69 \text{ \& } 0.77$ RR ⁷ = 3.8206; $CI_{95} = 2.83 \text{ \& } 5.16$ OR ⁸ = 523.4; $CI_{95} = 348.7 \text{ \& } 785.6$
	< 10% ²	31	89	120	logit $d^9 = 3.4534$
	Marginal sums	123	89	212	Pearson correlation (φ) = 0.7373 Regression (φ^2) = 0.5437
¹ Hosts and acceptable non-hosts; ² Non-hosts; ³ Proportion of adults emerged from hosts and acceptable non-hosts; ⁴ Proportion of adults emerged from non-hosts; ⁵ Risk difference; ⁶ 95% Confidence Interval; ⁷ Risk ratio; ⁸ Odds ratio; ⁹ Standardized mean difference; χ^2 for the table = 117.61, df = 1, p < 0.001.					

As expected, there was an interaction between penetrable/impenetrable and intact/pierced states of seed coats: the joint effect ($OR_{11} = 0.6718$) was larger than the multiplied value (0.4820) of their respective effects ($OR_{10} = 0.7179$ and $OR_{01} = 0.4820$).

A model selection procedure among seed traits by GLZ logit regression to test whether seed coat thickness or suitability of cotyledon for development was more influential in affecting bean weevil responses showed that seed coat was overwhelmingly deterministic in the subsequent fate of larvae and, therefore in adult emergence too (all Wald statistics were significant).

Discussion

This study could not prove an *overall* positive correspondence between host preference and host suitability. However, 16 leguminous plant species (Table 2) did show positive significant relationship between oviposition preference and larval performance (Table 4), and these I call *acceptable non-hosts* [22]. In spite of the fact that a preference for larger H seeds has been proven in bruchids in choice-tests [20, 23], the seed mass of ANH plants had negligible effects on the number of eggs laid, therefore the positive preference-performance relationship in these species must be attributable to traits of cotyledons. 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, 24], and occasional infestation is present in stores on garden peas or faba beans (Szentesi unpubl. results). How females assess host suitability of ANHs 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 (Table 2, Figs. 1 and 3, ESM Tables 2 and 3) among plant groups and results of logistic regressions (Tables 5 and 6) overwhelmingly emphasize the importance of seed coat in allowing or blocking the use of a seed. For instance, regression coefficients (φ^2) explained the variance of the effect of seed coat thickness and 'penetrability' in 10% and 21%, respectively, supporting the hypothesis that several yet unknown physical and chemical factors are also important. In the present study only a limited number of variables (seed mass, number of eggs laid and seed coat thickness) among those possible was taken into consideration, that may affect preference-performance relationships. Other important factors (chemicals, further physical, environmental, life-history traits, mobility, predators, host genotype, etc.) that influence host selection [8, 10, 25, 26, and others) were not investigated. In addition, experience may promote acceptance of ANHs [27, Szentesi unpubl. results], and may even lead to host-race formation [28], 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 ANHs and NHs was "erroneous" or "defective", as there are possible alternative explanations of the behaviour [29].

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 [30] 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, this was very different from those obtainable in choice tests, and even more different concerning larval development (Szentesi unpubl. results, ESM Table 4). 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, e.g., sphericity or size of substrate. In this study females laid ca. 1/3 eggs on NHs of those deposited on H plants. This means that elements of acceptability of

different NHs could induce the motivational state to accept a lowered egg-laying threshold as time passed.

The host-range of the bean weevil

The bean weevil is narrowly specialized even among wild and cultivated *Phaseolus* species [31]. 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 [32]. Some species and cultivars of the following genera belong to ANHs: *Lupinus*, *Glycine*, *Lablab*, *Vigna*, *Cicer*, *Vicia*, *Lens*, *Lathyrus*, and *Pisum* (Table 2), largely agreeing with findings of [5, 16, 33, 34]. Differences depend on whether the authors applied a wide enough spectrum of cultivars, because differences in acceptance among cultivars can be as large as or larger than those between species (ESM Tables).

Pierced seeds contributed to the understanding of host-range by how important chemical constituents of the cotyledons were, if seed coats did not function as barriers. The surprisingly high ratio of *Lathyrus* species supporting development to adulthood (Table 2) was possible only by pierced seed coats. It is noteworthy that most of those plant species that allowed some larval development were members of the tribe Fabeae, and specifically of the *Lathyrus* genus 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 pierced.

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 direction, Thiery [35] 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. There was a high L1 mortality with intact seed testa in case of naturally grown plants in this study (ESM Table 2). These complementary findings indicate that both physical (thickness, smoothness and hardness) and chemical traits of the seed coat jointly contribute, and to my knowledge no study has attempted to separate the respective effects.

Chemical factors in the seed coat

One obvious factor that should direct our attention to phytochemicals in seed coats is the observation with several bruchid species [1, 36, 37, 38], 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 [39] and in this study (Table 3) 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 Genisteeae tribe, due probably to quinolizidine alkaloids that were present in the testa [40].

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 [41]. Not only their remarkable amounts inside seeds

(up to 8% of dry weight [42]), but the taxonomic distribution in Fabae that is important. 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 [43, 44]. In this study, within the Fabae tribe, *V. faba* was the only species that allowed development of *A. obtectus* to adulthood (Table 2). 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 adults 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 a chemical resistance profile of a seed [45]. Indicators of the effects of this complex milieu are malformations and slower development, and presumably higher costs of handling substances in the cotyledon.

Evolutionary considerations

The seed coat not only excludes potential exploiters, but it effectively divides the “perception space” of egg-laying insects too. 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 [46], this would not necessarily happen in nature. These findings also direct attention to the need of more tests with pods of differently 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 pierced. 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 suitability of *Lathyrus* seeds for larvae when only pods are available in nature. (During 30 years of collecting legume seeds from *Lathyrus* species, *A. obtectus* has never been reared, Szentesi and T. Jermy unpubl. obs.)

As in several other instances [47], the egg-laying by bean weevil females onto ANH seeds also raises the question whether host range expansion occurred. The first steps in host-shifts are behavioural events [48, 49, 50], *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, 51, 52], there must be genetic covariance between preference and performance to adapt to a new plant species [53, 54]. Egg-laying should be accompanied by physiological adaptation of larvae to handle compounds metabolically once they are inside seeds [55, 56]. Unless preadaptation played a role [57], host-shift with the bean weevil is not probable.

Conclusions

The following traits maintain bean weevil in the current host specialization: (a) first instar larvae are not constrained to enter an ANH seed in a choice environment, because they have legs and until their energy reserves allow they can actively seek suitable hosts; (b) as data of this paper demonstrate, L1s rarely enter ANH seeds having intact seed testa; and (c) the life cycle of the *A. obtectus* is dual, i.e. taking place both in stores and in the field, and this spatial segregation regularly interrupts potential breeding and selection on potential additional hosts. Even if generations of beetles were produced on ANH seeds, the recognition of these plants as suitable oviposition sites in the field would, as a first step, require substantial genetic changes.

Materials And Methods

The experimental insect

Bean weevil propagates on two species of cultivated beans (*Phaseolus vulgaris* and *Ph. coccineus*) in the temperate zone. In Europe, it can continuously reproduce in stored dry beans. It also yearly infests beans outdoors, laying some eggs on nearly mature seeds within pods [58]. At harvest, infested seeds are collected and in 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 and select sites on seeds to enter. While drilling through the seed coat, they spit out most of it [59], a behaviour observed with other bruchid species too [1, 37, 38]. Fine powder indicates the site of boring. Larvae tunnel in the cotyledon, develop through four instars and pupate within the seed. Adults live for one-two weeks at 25–28 °C in the laboratory.

In stores, bean weevil is an oligophagous species within the tribe Phaseoleae (Fabaceae) comprising *Glycine*, *Lablab*, *Vigna* and *Phaseolus* genera [60]. 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, 32, 33, 34, 46].

Plant species used in the tests

In this study, 62 species from the Fabaceae tribes Cercideae, Caesalpinieae, Genisteae, Amorpheae, 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 and 3 for species and cultivars/accessions [SCA] names). These species occur naturally in Hungary, are naturalized, or are grown as food or fodder. Those selected were only those which had seed masses large enough to support development of a bean weevil larva [61].

I follow de Boer and Hanson's [22] classification of the plant species as to developmental suitability. *Hosts* (Hs) 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* (ANHs) 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 stores. Although some adults may emerge, developmental time is typically long and larval mortality high. The third group, *non-hosts* (NHs), comprises plants that are nutritionally inadequate or toxic, and never supports development, although occasional egg-laying cannot be excluded. 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 pierced.

The seed samples originated from field collections or grown, 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 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 2 × 5 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. Test insects originated from the same generation of a continuous rearing of *A. obtectus* maintained on commercial beans for years at 26–28 °C and 18/6-light/dark regime as described by Szentesi [62] and were sexually mature. 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 SCAs were handled as described in the previous section. Seed mass was determined at room temperature with the help of an electronic balance (Sartorius A210P, Germany) operating with 0.1 mg accuracy.

I set up two parallel treatments with 45 replicates each. 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. I made one or two holes per seed 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 into corresponding sized vials individually and after positioning a single bean weevil egg onto the vial’s inner wall ca. 1–2 cm above the seed with the help of a fine wet brush, the vial was capped with a cotton stopper. The eggs were obtained

from the laboratory rearing by a specific egg-laying device [62]. Daily collection of eggs allowed precise timing of large numbers of the suitable developmental (“black-headed”) stage of the embryo. 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, I recorded the time of egg hatching, L1s’ boring into the seeds, and the emergence of adults.

At least four months were allowed for development, then the seeds were opened under a dissecting microscope and the dead stadia recorded. After establishing egg-mortality, the remaining number of instars outside and inside seeds was regarded as 100%, then was divided up among instars recorded. Bean weevil develops through four larval stadia. The age of a larva was checked by head capsule measurement. All developmental malformations were also recorded. During dissection of the seeds, seed coat thickness was measured on > 10 randomly selected seeds, using a micrometer 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 were prepared. The ca. 150 mg size pilules were prepared from finely pulverized cotyledons (seed coat removed) of 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 ± 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 block chemical deterioration from heat. The pilules consisted of a standard amount of bean powder (80%) mixed with 20% water-soluble potato starch whose adequate portions were substituted with seed coat to create the above concentrations. Addition of distilled water created a pastry from which 6 mm diam. pilules were made by hand. Pilules were dried at 40 °C for a day. The pilules containing only 20% potato starch served as controls. The acceptability of the artificial beans for egg-laying, and their suitability for larval development were tested with three males and females and three pilules, and with one “black-headed” egg placed on individual pilules in vials with 13 respective replicates for each treatment. The number of eggs laid, the percentage of larval stages dead without entering and inside pilules, as well as that of emerging adults were recorded.

Statistical procedures

Variance homogeneity of variables (larval mortality outside and inside seeds, and adult emergence), notwithstanding various transformations, did not meet conditions of parametric tests, therefore non-parametric ones were used. Between-groups comparisons were performed with Kruskal-Wallis ANOVA and Wald-Wolfowitz runs tests for variables by intact/pierced seed coat, by suitable/unsuitable seed cotyledon for larval development, and adult emergence. Wherever possible, the results are presented as medians and quartiles, because data were obtained in percentages.

As a non-parametric version of discriminant function analysis, logistic regressions of the generalized linear models [63, 64] were applied to reject or accept two major hypotheses: (a) L1 mortality outside seeds did not change as seed coat thickness increased, and (b) larval development to successful adult

emergence did not depend on the suitability of seed cotyledon. The continuous dependent variables indicated dichotomous distributions, therefore they were transformed to categorized variables at biologically reasonable cutpoints, and coding of χ^2 -table cells was accordingly done: 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 (Tables 4 and 5). Such zero-one “reference cell” coding [65] produced a χ^2 -table, where the left upper cell (A) was 11, and the right lower one (D) was 00.

For logistic regression STATISTICA ver. 6's [66] 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 χ^2 -statistics of log-likelihood were calculated.

With the categorical data, effect sizes (risk difference, risk ratio, odds ratio and confidence intervals, CIs) for L1 mortality and adult emergence were estimated by the methods described in [67, 68]. The calculations were based on 2×2 χ^2 -tables (Tables 4 and 5). 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 of the frequency table was zero. Interactions between categorical variables (e.g., penetrable/impenetrable and intact/pierced 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 [69]. 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 [66].

Abbreviations

H = host

ANH = acceptable non-host

NH = non-host

SCA = species/cultivar/accession

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.

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Figures

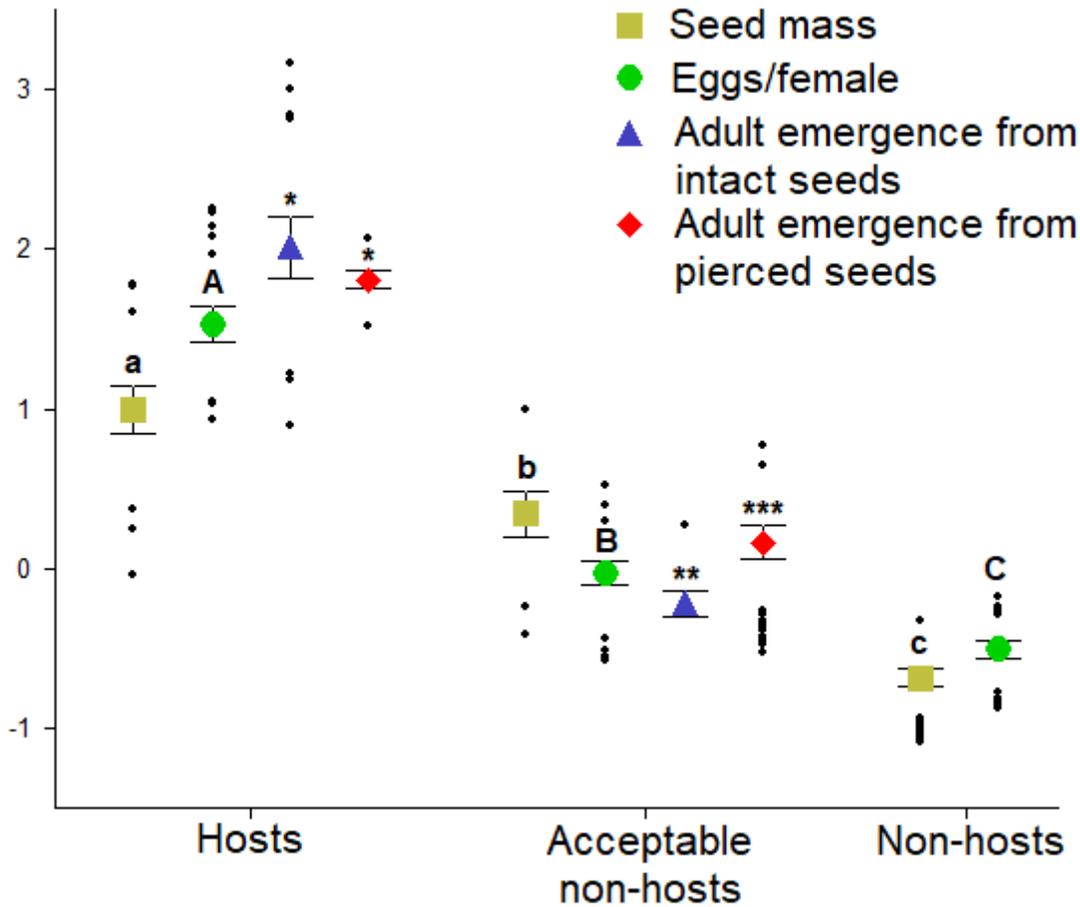


Figure 1

Seed masses, the number of eggs laid by bean weevil (*A. obtectus*) females, and adult emergence from intact and pierced seeds of H, ANH and NH leguminous plant species. Females laid eggs on NH plants too, however no adults emerged from these species, therefore no data are shown. As original units were in mg, number 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 (±SEs) signed with different small letters (seed mass), capitals (number of eggs laid) or asterisks (percentage of adults emerged), respectively, are significantly different by Kruskal-Wallis ANOVAs (see text) or by Wald-Wolfowitz Runs tests. Percentages of adults emerged from either intact or pierced seeds of H plants were not significant ($Z_{adj}=0.7627$, $p=0.4456$), whereas between H_{intact} and ANH_{intact} ($Z_{adj}=6.1818$, $p<0.001$), between $H_{pierced}$ and $ANH_{pierced}$ ($Z_{adj}=5.6180$, $p<0.001$), and between ANH_{intact} and $ANH_{pierced}$ ($Z_{adj}=3.3525$, $p<0.001$) they were

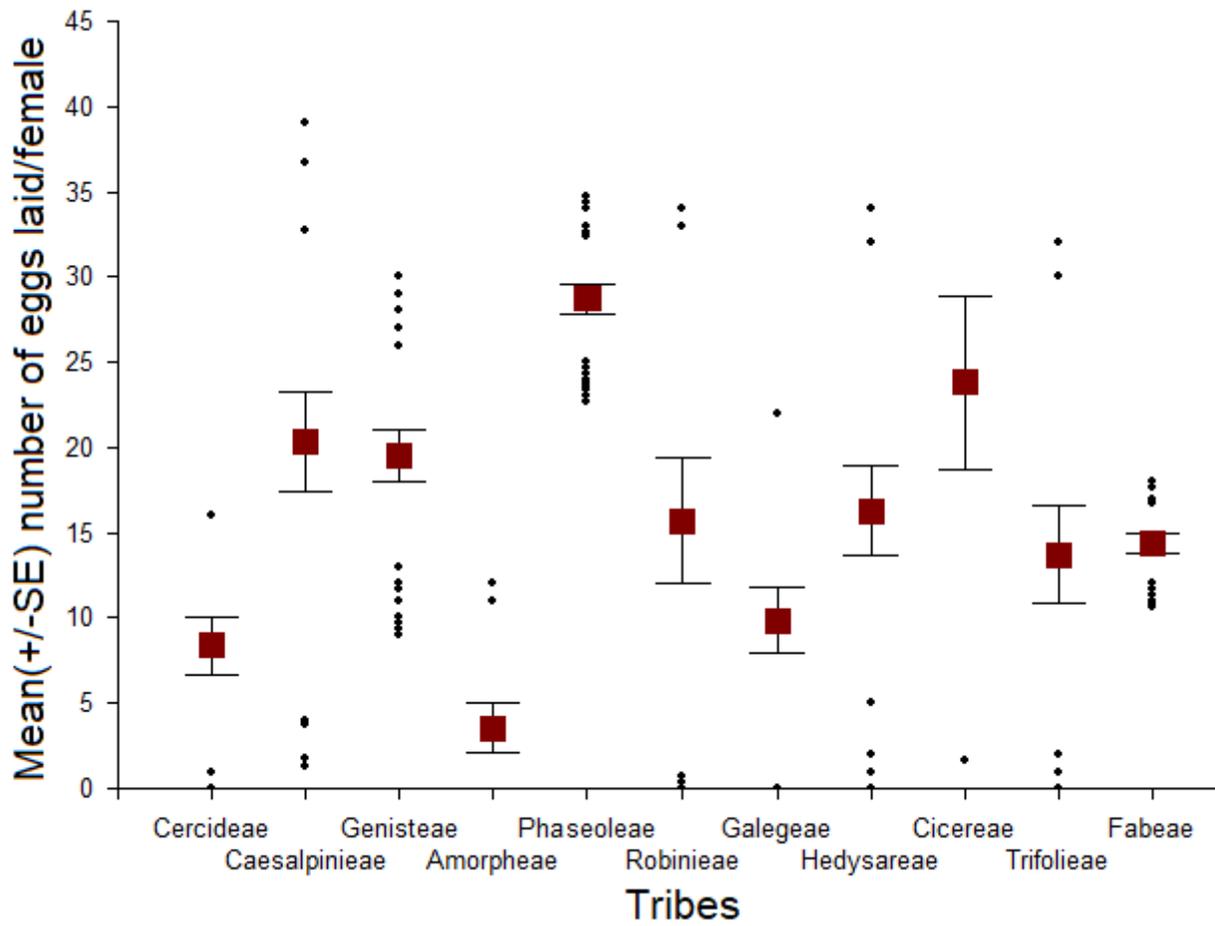


Figure 2

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)

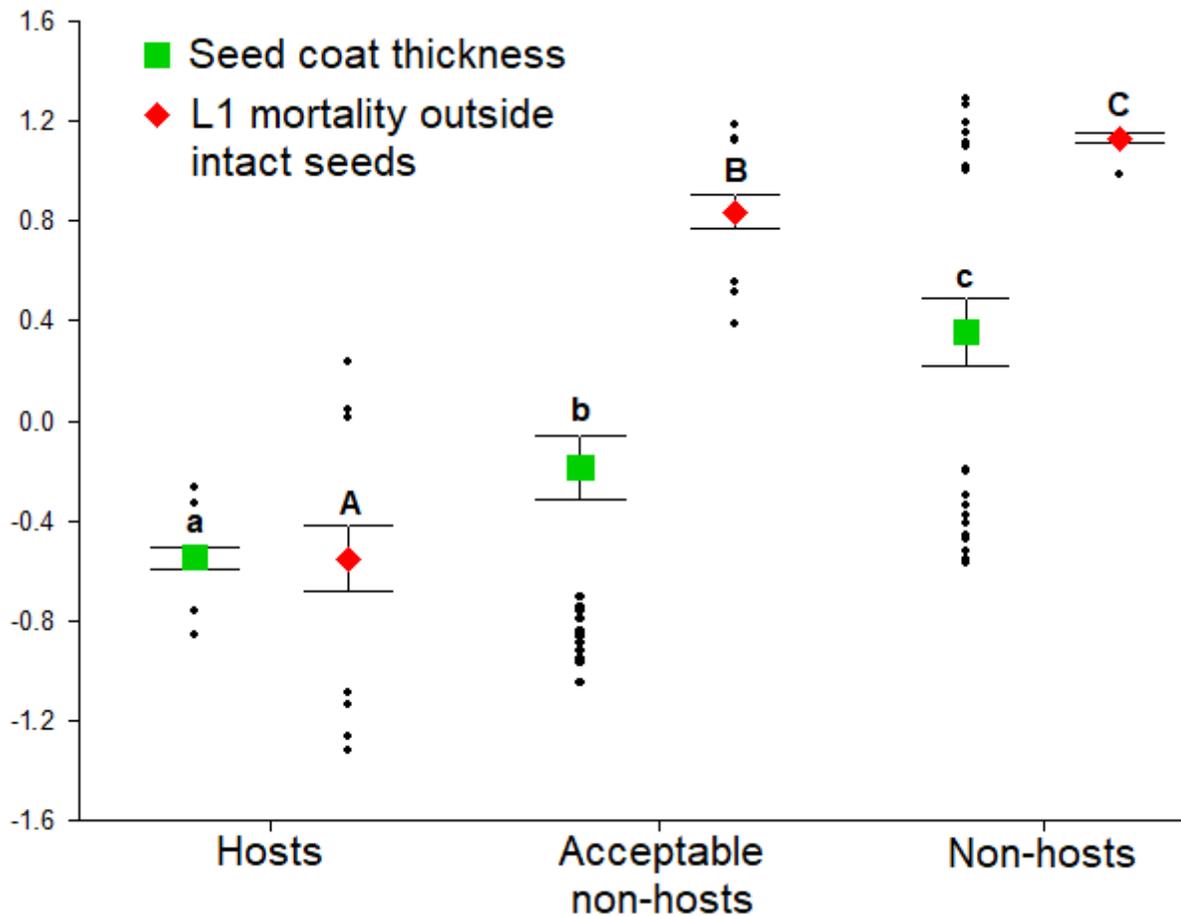


Figure 3

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

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