

Biotypes of the seed beetle *Callosobruchus maculatus* have differing effects on the germination and growth of their legume hosts

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- Abstract**
- 1 Populations of the seed beetle *Callosobruchus maculatus* (F.) exhibit considerable differences in body size and larval behaviour. We examined whether such variation modifies the relationship between beetle infestation and host plant performance.
 - 2 Larvae from African and Asian biotypes were reared in seeds of four hosts that represented an almost four-fold variation in seed mass. We estimated mass lost to larval consumption, and compared germination rates and seedling growth between infested and control seeds.
 - 3 In seeds bearing a single larva, the larger-bodied, contest-competing larvae of the Asian biotype caused a 38–47% greater reduction in seed mass compared with the smaller-bodied, scramble-competing larvae of the African biotype. The amount of seed mass lost per larva remained similar in seeds with one or two scramble-competing larvae but decreased significantly in seeds bearing two contest-competing Asian larvae.
 - 4 Differences in larval consumption and behaviour produced striking differences in the frequency of germination. Germination of singly-infested mung bean (i.e. the smallest host) was 71% for African-infested seeds versus 11% for Asian-infested seeds. In cowpea (i.e. the largest host), 76% of Asian-infested seeds germinated, whereas the germination rate of African-infested cowpeas (92%) was similar to that of uninfested seeds.
 - 5 Effects of beetle origin persisted after germination. Seedlings derived from Asian-infested seeds had greater cotyledon damage 7 days after germination, and displayed lower height and less biomass 15 days after germination. Cotyledon damage was a good predictor of seedling performance (i.e. better than seed mass consumed) 15 days after germination.
 - 6 Previous studies have suggested that population differences in larval size and burrowing behaviour ('centripetal tendency') reflect adaptation to different-sized seeds. The present study demonstrates that these differences in turn influence the impact of larval feeding on host viability. Strong biotypic variation makes it difficult to generalize about pest impacts at the level of pest species.

Keywords *Callosobruchus*, germination, grain legume, larval competition, seedling growth, *Vigna* spp.

Introduction

Many insects have greatly expanded their geographic ranges as a result of human activity (Malacrida *et al.*, 2007; Smith *et al.*,

2007; Kenis *et al.*, 2009). If the distribution of such species is discontinuous and gene flow is sufficiently reduced (Garant *et al.*, 2007), introduced populations may diverge genetically from their ancestral population, and from each other, as a result of genetic drift or local selection (Phillips *et al.*, 2008; Ahern *et al.*, 2009; Diamantidis *et al.*, 2009). In pest species, genetic differentiation is sometimes recognized by the formation of

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biotypes (i.e. genetically distinct populations that differ in key traits, such as the ability to use a particular plant or resist an insecticide) (Lushai *et al.*, 2002; Kim *et al.*, 2008). Biotype formation may be especially prevalent among stored-product pests, which often occur as a series of disjunct populations after long-distance transport of infested grains (Credland, 1990; Tran & Credland, 1995).

Biotypes of herbivorous insects may differ in more than just host range or preference; by virtue of differences in size, demography, growth rates, or phenology, they may also have different impacts on their hosts (Via, 1990; Credland & Dendy, 1992; Harris *et al.*, 2003; Delatte *et al.*, 2009). In the present study, we compared the effects of African and Asian biotypes of a seed beetle on the performance of four legume hosts. *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) is most likely native to Africa (Smartt, 1985; Shade *et al.*, 1999) but has become a cosmopolitan pest of grain legumes (Tuda *et al.*, 2006). Geographic populations exhibit substantial genetic differentiation in behavioural, morphological, physiological and life-history traits (Credland *et al.*, 1986; Messina & Mitchell, 1989; Savalli *et al.*, 2000; Fox *et al.*, 2004). Some of this variation appears to reflect adaptation to hosts that differ in seed size or nutritional quality (Taper, 1990; Tuda & Iwasa, 1998; Messina & Karren, 2003; Messina, 2004a).

Two larval traits, body size and competitive behaviour, may have especially strong effects on tissue damage inside seeds, and thus on host viability and growth. Larvae from one Asian biotype (South India population) are relatively large-bodied, and engage in an extreme contest-type competition within seeds (Messina, 1991a, b; Toquenaga, 1993). By contrast, the smaller-bodied larvae of an African biotype (Burkina Faso population) actively avoid direct contact and produce a scramble-type outcome (Takano *et al.*, 2001) and (Mitchell, 1990; Mano & Toquenaga, 2008a, b). Larvae from the Asian biotype develop into adults that are 10–35% larger than those of the African biotype (depending on rearing host, temperature and sex; Stillwell *et al.*, 2007). Other variable traits that may affect the extent of seed damage include metabolic efficiency and burrowing behaviour (i.e. the tendency to burrow toward the centre of the seed or remain on the periphery) (Toquenaga *et al.*, 1994; Guedes *et al.*, 2003). Larvae from different biotypes are known to consume different amounts of seed mass (Credland & Dick, 1987) but the effects on subsequent seed germination and seedling growth are less well-studied.

The effect of seed-beetle infestation on host performance can be complex (Southgate, 1979). Direct damage to embryos often prevents germination (Tomaz *et al.*, 2007), and larval feeding creates openings for pathogenic bacteria and fungi (Cipollini & Stiles, 1991). Even if infested seeds do germinate, emergent seedlings may exhibit appressed cotyledons, which can inhibit the formation of true leaves (Hegazy & Eesa, 1991), and depletion of cotyledon reserves may decrease growth and establishment (Baier & Webster, 1992). On the other hand, some legumes (including some crop species) are quite tolerant of moderate levels of infestation (Miller, 1994; Mack, 1998; Dalling & Harms, 1999). Larval feeding may actually improve the rate and timing of germination by enhancing the uptake of water (Epperlein, 1992; Mucunguzi, 1995). In a previous study, scarification of seeds by seed beetle penetration was a

prerequisite to successful germination (Takakura, 2002). For four legume cultivars, we estimated seed mass lost to larval consumption, and subsequently compared germination rates and seedling growth among African-infested, Asian-infested and uninfested seeds.

Materials and methods

Beetle populations and legume hosts

Females of *C. maculatus* lay eggs singly on the seed surface, and larvae burrow into the seed directly beneath the oviposition site. Larvae complete development within a single seed and adults emerge from circular exit holes. The Asian biotype used in the present study is from a population in South India (SI) that was collected from infested mung beans *Vigna radiata* (L.) Wilczek and the closely-related black gram *Vigna mungo* (L.) Hepper in Tirunelveli, India (Mitchell, 1991). The African biotype is from Burkina Faso (BF) and was collected from infested cowpeas, *V. unguiculata* (L.) Walpers, in Ouagadougou, Burkina Faso (Messina, 1993). Each population had been maintained on their respective hosts (SI on mung bean, BF on cowpea) for >100 generations in the laboratory before the start of the present study. For most of this period, each new generation was formed by adding 1500–2000 newly-emerged adults (estimated by volume) to a 2-L jar containing approximately 750 g of seeds.

We compared the effects of BF and SI larvae on two cultivars of cowpea, 'California Blackeye No. 5' (Blackeye cowpea) and 'Magnolia' (Magnolia cowpea), as well as one cultivar each of azuki bean, *Vigna angularis* (Willd.) Ohwi & Ohashi (cv. 'Erimo') and mung bean (cv. 'Berken'). These hosts are all highly suitable for *C. maculatus* (Messina, 2004a, b) but present an almost four-fold variation in average seed mass (Table 1). They may therefore be expected to show a wide range of tolerance to larval feeding.

Seed treatment

To establish a range of larval densities in the infested seeds, we isolated pairs of beetles in 30-mm Petri dishes (one pair per dish) with five pre-weighed and individually marked seeds. Providing only five seeds per female was meant to increase the frequency of seeds receiving multiple eggs. Females were allowed to lay eggs for 24–48 h and then discarded. For the BF population, eggs were scraped from seeds before hatching to create densities of one to four eggs per seed. SI females strongly avoid adding eggs to egg-laden seeds, especially if seed size is small

Table 1 Seed mass (mean \pm SD) of four grain-legume hosts of *Callosobruchus maculatus*

Host	Mass (mg)	<i>n</i>
Blackeye cowpea, <i>Vigna unguiculata</i>	243.1 \pm 36.6	1349
Magnolia cowpea, <i>Vigna unguiculata</i>	171.3 \pm 27.6	1346
Azuki bean, <i>Vigna angularis</i>	92.3 \pm 25.6	923
Mung bean, <i>Vigna radiata</i>	65.1 \pm 11.4	1335

(Messina & Karren 2003). Because of the limited period of female exposure to seeds, we could obtain seeds with only one or two SI larvae per seed for the two cowpea varieties, and with only one larva per seed for mung bean and azuki bean. Thus, we use only singly-infested seeds to compare the effects of BF and SI larvae on host performance, and examine the effects of density per se using only larvae from the BF population.

Infested seeds were placed in a growth chamber at 25 °C and under an LD 15 : 9 h photoperiod. Control (unmanipulated) seeds were systematically interspersed with infested seeds throughout the chamber to ensure that all seeds experienced the same conditions. Upon beetle emergence, we carefully removed beetle frass inside exit holes by tapping and with a small paint brush, and then reweighed each seed to estimate the mass removed by beetles. All analyses treat the number of hatched eggs on a seed as an estimate of larval density within seeds; almost all larvae (>95%) from each population survived to adult emergence in each host.

Germination and seedling growth

Control and previously infested seeds were sown in round pots (diameter 10 cm, volume ~640 mL) in a greenhouse furnished with supplemental lighting and maintained at 29 ± 1 °C (day) and 27 ± 1 °C (night) and under an LD 15 : 9 photoperiod. For the germination trials, we added a third seed treatment: artificial scarification. To scarify seeds, we removed approximately 5% of the seed coat with sandpaper, which was judged sufficient to promote imbibition of water without affecting the underlying cotyledons. Similar to control seeds, scarified seeds lost no cotyledon tissue; similar to infested seeds, they possessed an opening in the seed coat for greater ingress of water.

Planting occurred in weekly or biweekly blocks over one calendar year, with most hosts and seed treatments represented in each block. Each pot was filled with loosely-packed ProMix (Premier Horticulture, Canada) and received a single seed approximately 2.5 cm below the soil surface. Pots were watered and inspected daily. Plants were considered to have germinated when seedling tissue became visible above the soil surface. Seeds were classified as having failed to germinate if no tissue was visible after 1 month. We thus failed to detect the germination of seedlings that died without emerging from the soil. However, when the pots with no aboveground tissue were later discarded, casual inspection indicated that very few seedlings fell into this 'cryptic germination' category.

Seven days after germination, we determined cotyledon damage on each seedling. Cotyledon damage was visually estimated, by individuals who were blinded to a plant's assigned treatment, as the proportion of missing tissue. At 15 days post-germination, we measured seedling height and then harvested each plant. Extracted seedlings were carefully washed to remove any attached soil, divided into above- and belowground portions, and dried to constant weight in an oven at 60 °C.

Statistical analysis

The proportion of seeds germinating was analyzed using logistic regression, with legume host or seed treatment as the independent variable. All other traits were analyzed using analyses

of variance (ANOVA) to test for heterogeneity among classes (PROC MIXED) using the SAS statistical package (SAS Institute Inc., Cary, North Carolina). Seedling size variables (height and mass) were log-transformed before analysis. Linear contrasts (planned comparisons) were performed using the ESTIMATE statement in SAS. In some analyses, seed mass prior to infestation and mass lost to larval consumption were included as covariates. We present *F*-statistics for the overall ANOVA and analyses of variance (ANCOVA) results, and a *t*-statistic for linear contrasts.

For all parametric analyses, 'block' was included as a random effect. This effect was almost always significant but was included only to control for among-block variation and is not discussed further below. Because of significant block effects, we present least-squares (LS) means to remove block effects on plant traits. LS means were calculated with SAS PROC MIXED (Littell *et al.*, 1996). Estimates of these means depend on the particular statistical model and thus differ slightly between analyses that incorporated different treatment combinations.

Results

Loss of seed mass

There was significant variation among hosts in the amount of seed mass removed by a single *C. maculatus* larva ($F_{3,1855} = 9.0$; $P < 0.001$). Individual larvae from both the BF and SI populations removed more seed mass from the two cowpea hosts than from the two smaller-seeded hosts (one-larva treatments in Table 2). The proportion of seed mass lost by singly-infested seeds also varied among hosts ($F_{3,1855} = 935.9$; $P < 0.001$); despite losing more absolute mass, the larger cowpea seeds lost a much lower proportion of their total mass compared with azuki beans and mung beans (Table 1). On average, infestation by a single larva caused mung beans to lose 22–33% of their mass, whereas the average proportional loss for Blackeye cowpea (the largest host) was ≤10% (Table 2).

In addition to a host effect, there was a highly significant effect of beetle population on the amount of mass consumed by a single larva. When developing alone in a seed, SI larvae removed 38–47% more seed mass than did BF larvae ($F > 23.4$; $P < 0.001$ for each of the four hosts; Table 2). The host × beetle population interaction was not significant ($F_{3,1855} = 0.25$; $P = 0.86$), which suggests that the BF and SI populations responded similarly to the four hosts with respect to the absolute amount of mass removed by a single larva.

As expected, seeds lost more mass as they received more larvae, although the amount of mass lost per larva generally declined with increasing larval density (Table 2). This result is expected if there is resource competition between co-occurring larvae. Because SI females rarely laid more than one egg on the two smallest hosts and rarely laid two eggs on the larger, cowpea hosts (see Materials and methods), we could compare the effects of larval density between beetle populations only for cowpeas (Blackeye and Magnolia) that bore either one or two larvae. The effect of larval density on mass lost per larva did differ between beetle populations (i.e. there was a significant interaction between the effects of density and beetle population) ($F_{1,1003} = 46.4$; $P < 0.001$). For BF larvae, the mass lost per

Table 2 Seed mass lost (mean \pm SE) to *Callosobruchus maculatus* larvae from the Burkina Faso (BF) and South India (SI) populations

Host	Larval density	No. of seeds		Total mass lost (mg)		Mass lost per larva (mg)		Percentage of total mass lost	
		BF	SI	BF	SI	BF	SI	BF	SI
Blackeye cowpea	1	197	238	16.0 \pm 1.0	22.0 \pm 0.7	16.0 \pm 1.0	22.0 \pm 0.7	6.7 \pm 0.4	9.3 \pm 0.3
	2	65	12	30.8 \pm 0.7	38.0 \pm 3.5	15.4 \pm 0.4	19.0 \pm 1.7	13.2 \pm 0.4	16.8 \pm 1.1
	3	48	ND	47.2 \pm 0.8	ND	15.7 \pm 0.3	ND	19.6 \pm 0.5	ND
	4	27	ND	61.0 \pm 2.0	ND	15.2 \pm 0.5	ND	25.6 \pm 1.1	ND
Magnolia cowpea	1	203	233	15.3 \pm 0.3	22.4 \pm 1.3	15.3 \pm 0.3	22.4 \pm 1.3	9.2 \pm 0.2	13.3 \pm 0.3
	2	62	7	29.9 \pm 0.7	35.9 \pm 3.8	15.0 \pm 0.3	18.0 \pm 1.9	18.1 \pm 0.5	20.3 \pm 2.6
	3	44	ND	43.3 \pm 1.3	ND	14.4 \pm 0.4	ND	25.2 \pm 0.8	ND
	4	14	ND	54.0 \pm 2.1	ND	13.5 \pm 0.5	ND	30.2 \pm 1.1	ND
Azuki bean	1	125	185	12.7 \pm 0.7	18.6 \pm 0.4	12.7 \pm 0.7	18.6 \pm 0.4	16.2 \pm 0.7	22.5 \pm 0.5
	2	41	ND	23.8 \pm 0.9	ND	11.9 \pm 0.4	ND	24.8 \pm 0.1	ND
Mung bean	1	207	475	14.0 \pm 0.4	20.3 \pm 0.2	14.0 \pm 0.4	20.3 \pm 0.2	22.1 \pm 0.5	32.5 \pm 0.3
	2	76	ND	27.1 \pm 0.5	ND	13.6 \pm 0.3	ND	43.4 \pm 1.1	ND

ND, No data available because SI females failed to produce enough cowpea seeds bearing >2 eggs or enough azuki beans and mung beans bearing >1 egg.

larva did not differ between one-larva and two-larvae seeds ($F_{1,521} = 0.29$; $P = 0.59$). By contrast, SI larvae consumed significantly less mass per larva when there were two larvae per seed compared with when there was one larva per seed ($F_{1,484} = 3.93$; $P = 0.048$; compare the one- versus two-larva treatments in Table 2). Consequently, the percentage of seed mass lost was more similar between the BF and SI biotypes for two-larva seeds than for one-larva seeds (Table 2). The interaction between cowpea variety (Blackeye versus Magnolia) and beetle density was not significant ($F_{1,1003} = 0.03$; $P = 0.86$), which suggests that the amount of seed mass lost per larva decreased similarly on the two hosts as larval density increased from 1 to 2.

Germination

Control seeds of all hosts showed a high frequency of germination ($\chi^2 = 3.8$; d.f. = 3; $P = 0.29$ from logistic regression; Fig. 1A). Scarification did not improve the germination of any host (for simplicity, this treatment is not shown in Fig. 1). Indeed, it inexplicably reduced germination of Magnolia cowpea from 92 to 81% ($\chi^2 = 4.9$; d.f. = 1; $P = 0.03$, although this is not significant after a sequential Bonferroni correction for multiple comparisons). For the other three hosts, germination frequencies of control and scarified seeds were statistically indistinguishable ($P > 0.36$ for these hosts).

Infestation by a single *C. maculatus* larva significantly reduced the frequency of germination of each host ($\chi^2 = 230.0$; d.f. = 3; $P < 0.001$, for the treatment effect in the full-factor logistic regression; Fig. 1A). Planned comparisons indicated that germination frequencies of singly-infested seeds were consistently below those of control seeds ($\chi^2 > 11.8$; d.f. = 1; $P < 0.001$ for all hosts). As expected, the magnitude of the infestation effect scaled according to seed size. Germination of mung bean was most affected; Blackeye cowpea was least affected (Fig. 1A). These hosts also incurred the highest and lowest proportions of seed mass lost, respectively (Table 2).

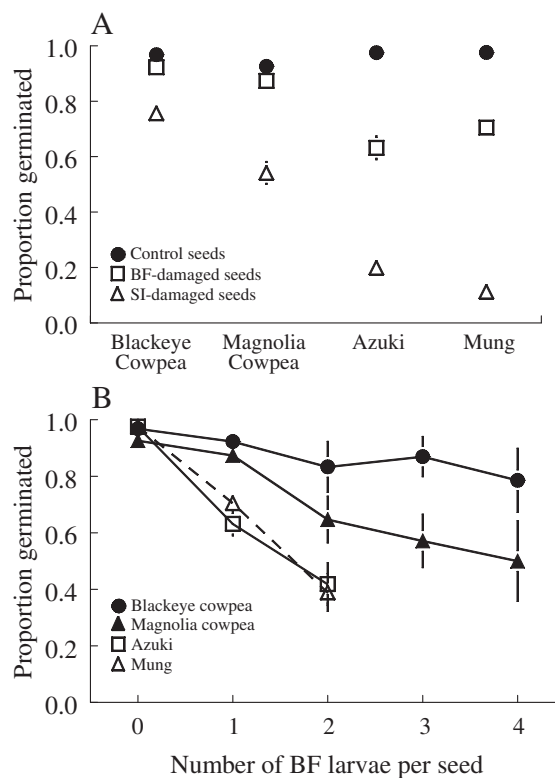


Figure 1 Frequency of germination (mean \pm SE) (A) for control seeds and seeds infested by a single *Callosobruchus maculatus* larva from the Burkina Faso (BF) and South India (SI) populations, and (B) for seeds infested with 0–4 BF larvae. Some error bars are not visible because they are smaller than the overlying points.

Because larvae from the SI and BF populations consumed different amounts of seed mass per larva, they should have different effects on germination success. Indeed, the difference in germination frequencies between BF- and SI-infested seeds

was especially striking for two smallest hosts: azuki bean and mung bean (Fig. 1A). Approximately 11% of SI-infested mung beans germinated but almost three-quarters of BF-infested seeds did so. Infestation by a single SI larva significantly reduced germination frequencies of all hosts relative to control seeds ($\chi^2 > 13.7$; d.f. = 1; $P < 0.001$ for all hosts; Fig. 1A), whereas infestation by a single BF larva reduced germination frequencies of azuki bean and mung bean ($\chi^2 > 9.3$; d.f. = 1; $P < 0.003$) but had no detectable effect on germination of the two cowpea cultivars ($\chi^2 < 1.7$; d.f. = 1; $P > 0.19$).

Increasing densities of BF larvae progressively reduced germination frequency of all hosts (logistic regression in which beetle density was a covariate; $\chi^2 = 100.7$; d.f. = 1; $P < 0.001$; Fig. 1B). There was a significant host \times beetle density interaction ($\chi^2 = 27.3$; d.f. = 3; $P < 0.001$), which means that the magnitude of the effect of increasing larval density varied among hosts. As expected, the effect of increasing larval density in reducing germination was most pronounced for azuki bean and mung bean, intermediate for Magnolia cowpea, and smallest for Blackeye cowpea (Fig. 1B). Even Blackeye cowpeas that bore four BF larvae were able to germinate at a frequency of almost 80%, which was comparable with the frequency observed for Blackeye cowpeas containing only a single SI larva (Fig. 1A, B).

The four host cultivars displayed innate differences in germination time. Control seeds of azuki beans germinated in approximately 6 days, whereas the other three hosts generally germinated 4–5 days after sowing ($F_{3,1187} = 37.7$; $P < 0.001$ for the host effect in ANOVA; Fig. 2A). Seed treatment (scarification or infestation) did not modify the timing of germination ($F_{3,1187} = 1.45$; $P = 0.23$; Fig. 2A) and there was no treatment \times host interaction ($F_{9,1187} = 0.90$; $P = 0.53$) for time to germination. Among seeds infested with multiple BF larvae, larval density surprisingly had no effect on time to germination (ANCOVA with density as a covariate; $F_{1,807} = 0.00$; $P = 0.94$; Fig. 2B). Seeds that had been infested by multiple larvae took approximately the same amount of time to germinate as control or singly-infested seeds (Fig. 2B).

Cotyledon damage

Many seeds infested by a single larva germinated successfully but the ensuing 7-day-old seedlings often exhibited substantial cotyledon damage (missing tissue). Cotyledon damage varied significantly among the hosts ($F_{3,587} = 16.2$; $P < 0.001$), with mung bean experiencing the most damage (Fig. 3A). Surprisingly, Magnolia cowpea suffered as much cotyledon damage as the smaller-seeded azuki bean (Fig. 3A). The amount of cotyledon damage was positively correlated with seed mass lost in singly-infested seeds ($F_{1,573} = 10.1$; $P = 0.002$ in an ANCOVA with mass lost, nested within legume host, as a covariate). Within each host, larger seeds suffered less damage; there was a significant negative relationship between initial seed mass and cotyledon damage ($F_{4,573} = 4.38$; $P = 0.002$ in an ANOVA with initial seed mass, nested within legume host, as a covariate). Control seedlings occasionally exhibited very small amounts of cotyledon damage (Fig. 3A).

Because SI larvae are larger and consume more seed mass (Table 2), they caused much more cotyledon damage than

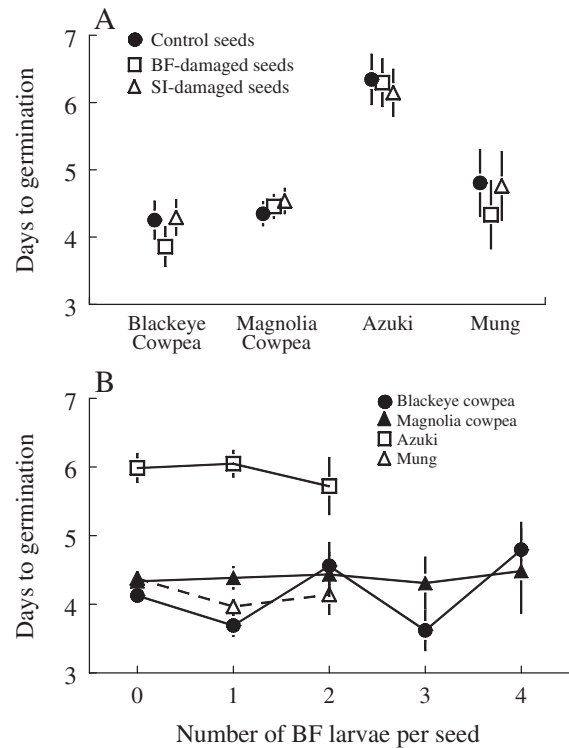


Figure 2 Time until germination (mean \pm SE) (A) for control seeds and seeds infested by a single *Callosobruchus maculatus* larva from the Bukina Faso (BF) and South India (SI) populations, and (B) for seeds infested with 0–4 BF larvae. Some error bars are not visible because they are smaller than the overlying points.

did BF larvae to seedlings grown from singly-infested seeds ($t = 7.8$; $P < 0.001$; Fig. 3A). On Blackeye cowpea, damage was greater than ten-fold higher if a seed was infested by an SI larva instead of a BF larva (Fig. 3A). On mung bean, all singly-infested seeds incurred considerable damage, and damage was only 1.5-fold higher among SI-infested seeds. The relationship between the amount of cotyledon damage and the amount of seed mass consumed differed between beetle populations [$F_{1,573} = 2.17$; $P = 0.004$ for the interaction between the covariate (mass consumed) and beetle population]. The amount of seed mass consumed adequately explained the effect of beetle population on cotyledon damage for Blackeye cowpea, azuki bean and mung bean (i.e. the population effect became nonsignificant when mass consumed by beetles was included in the model). For Magnolia cowpea, however, infestation by an SI larva caused significantly greater cotyledon damage than infestation by a BF larva, even after seed mass consumed was included as a covariate ($F_{1,137} = 17.0$; $P < 0.001$).

Overall, cotyledon damage increased significantly with increasing densities of BF larvae ($F_{1,804} = 172.6$; $P < 0.001$, with density as a covariate; Fig. 3B), although the effect strongly depended on legume host ($F_{3,786} = 149.6$; $P < 0.001$ for the density \times host interaction). On mung bean, the presence of only two BF larvae was sufficient to remove >60% of cotyledon tissue, and even higher losses were observed when Magnolia cowpea was infested by three or four larvae. On

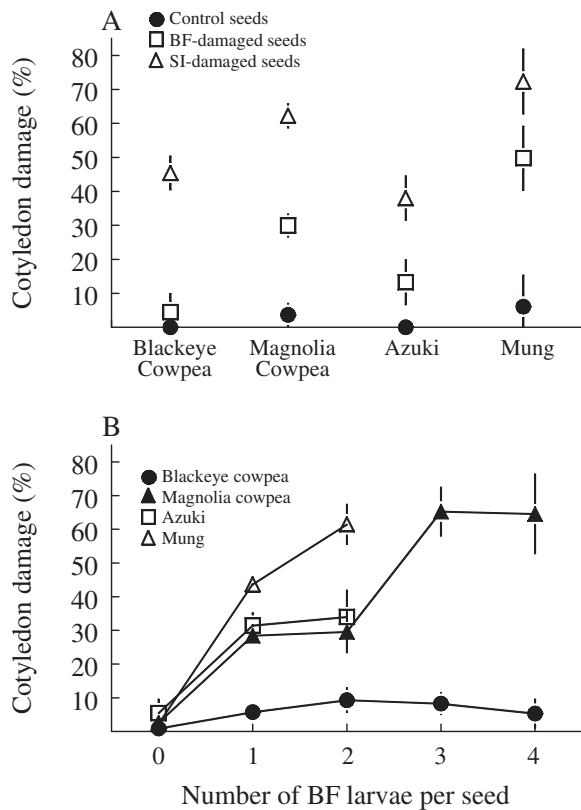


Figure 3 Percentage of cotyledon tissue missing (mean \pm SE) on seedlings that were 7 days post-germination and derived (A) from control seeds and seeds infested by a single *Callosobruchus maculatus* larva from the Bukina Faso (BF) and South India (SI) populations, and (B) from seeds infested with 0–4 BF larvae. Some error bars are not visible because they are smaller than the overlying points.

Blackeye cowpea, cotyledon damage remained surprisingly minimal at all densities of BF larvae (Fig. 3B).

Seedling growth

Fifteen days after germination, seedlings derived from singly-infested seeds were significantly smaller than those derived from control seeds [$F > 75.2$; $P < 0.001$ for the effect of treatment (uninfested versus SI-infested versus BF-infested) on height and both estimates of biomass; Fig. 4]. Linear contrasts between control seeds and all infested seeds (BF + SI) revealed treatment effects on height ($t_{907} = 11.2$; $P < 0.001$), aboveground mass ($t_{907} = 14.2$; $P < 0.001$) and belowground mass ($t_{838} = 12.8$, $P < 0.001$). However, the effect of infestation on seedling traits varied among hosts ($F > 4.2$; $P < 0.004$ for the seed treatment \times host interaction for all three traits). For most traits, seedlings of mung bean were most affected by infestation, although azuki bean experienced only a small (albeit significant) reduction in final biomass, despite a fairly large reduction in height (Fig. 4).

As expected from differences in both seed mass consumed and cotyledon damage, SI and BF larvae had different impacts on seedling traits. For all three measured traits, seedlings

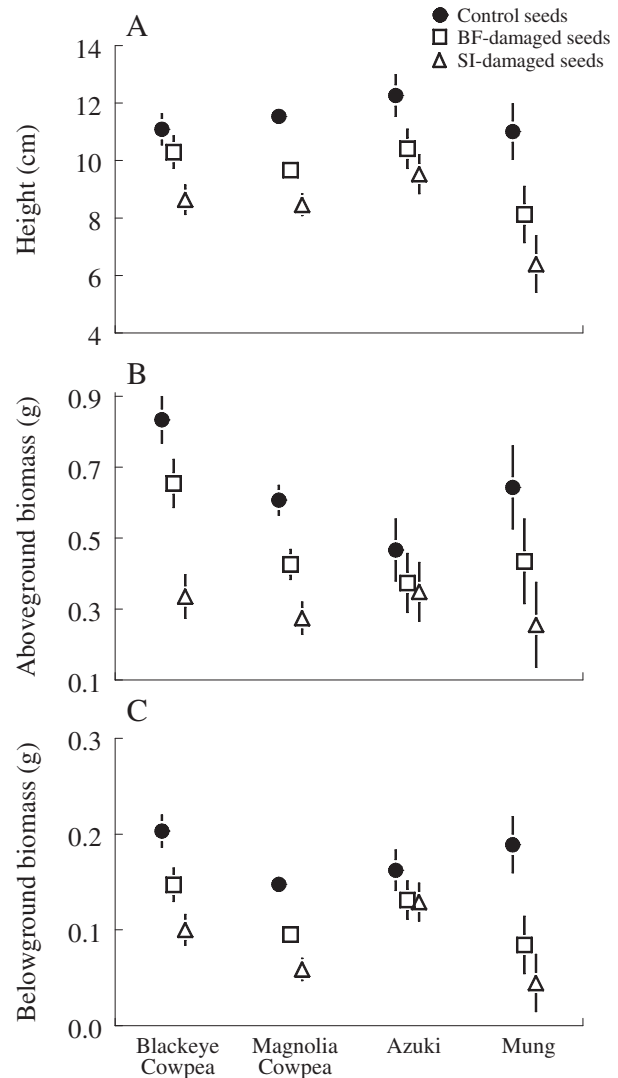


Figure 4 (A) Height and (B, C) dry mass (mean \pm SE) of seedlings 15 days after germination from control seeds and seeds infested by a single *Callosobruchus maculatus* larva from the Bukina Faso (BF) and South India (SI) populations.

derived from seeds infested by one SI larva were significantly smaller than those from seeds infested by one BF larva ($F > 20.9$; $P < 0.001$; Fig. 4). This effect of beetle population on seedling size did not vary significantly among the four hosts ($F < 2.3$; $P > 0.07$ for the beetle population \times host interaction for all three traits; Fig. 4).

When the amount of seed mass consumed by larvae was included as a covariate (nested within host), it had a significant effect on all seedling traits ($F > 4.7$; $P < 0.001$) but was not adequate to explain the effect of beetle population, which remained significant for all three seedling traits ($F > 7.0$; $P < 0.008$). In a second ANCOVA that included cotyledon damage (nested with host) as the covariate instead of mass consumed, cotyledon damage also had a highly significant effect on all traits ($F > 53.8$; $P < 0.001$ for each), and the effect of beetle population on seedling traits became nonsignificant for

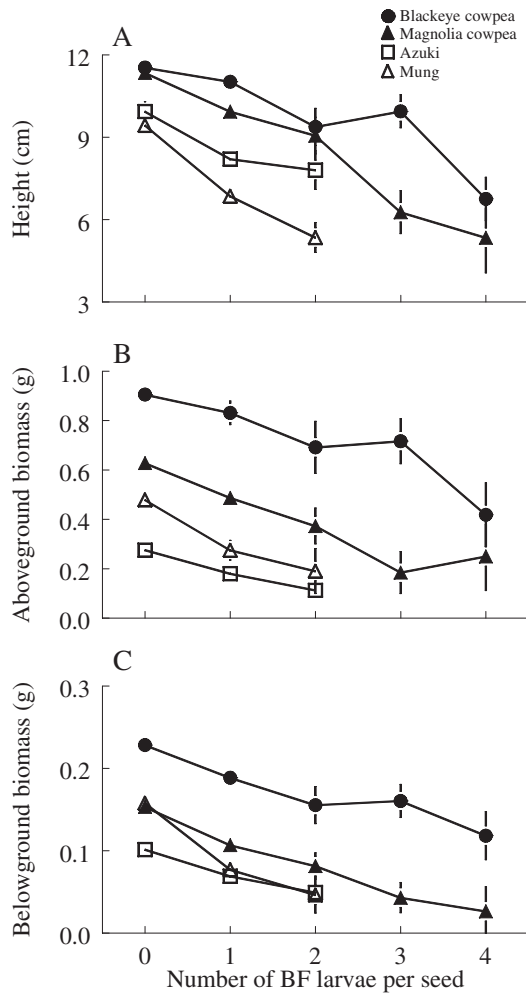


Figure 5 Effect of BF larval density on (A) height and (B, C) dry mass (mean \pm SE) of seedlings 15 days after germination. Some error bars are not visible because they are smaller than the overlying points.

each trait ($F < 1.30$, $P > 0.25$). This suggests that either the causal effect of larval damage on seedling growth is mediated by cotyledon damage or that cotyledon damage is simply a better predictor of embryo damage than is loss of seed mass.

Increasing the density of BF larvae reduced all three measures of seedling size ($F > 102.2$; $P < 0.001$ for each trait; Fig. 5). Including seed mass consumed by larvae as a covariate in the analysis made the density effect nonsignificant for both measures of seedling mass, although it was not adequate to explain the entire effect of larval density on seedling height. As we observed for the effect of beetle population, the effect of BF larval density was partially predicted by cotyledon damage. The effect of cotyledon damage (nested within host) on seedling growth was highly significant when added to the ANCOVA ($F > 50.9$; $P < 0.001$), and its addition to the model (along with mass lost to larvae) was adequate to explain the effect of larval density (which became nonsignificant) for above- and belowground mass ($F < 1.2$; $P > 0.28$) but not for plant height ($F_{1,424} = 7.25$; $P = 0.007$ for the effect of density on height).

Discussion

The BF and SI biotypes of *C. maculatus* had very different effects on host performance; SI larvae caused a relatively greater loss of seed mass, a greater reduction in germination frequency, more extensive cotyledon damage, and poorer seedling growth. SI adults are significantly larger-bodied than BF adults, and thus different resource requirements may account for some of the difference in mass lost by seeds to these two biotypes. However, other larval traits probably also contributed to the biotype effects observed here. For example, although SI adults are less than 35% larger than BF adults (Stillwell *et al.*, 2007), SI larvae consumed almost 50% more seed mass than BF larvae on some hosts (Table 1). SI larvae are known to have unusually high respiration rates (Guedes *et al.*, 2003), and differences in metabolic efficiency probably accounted for some of the variation in mass removed by a single larva (Credland & Dick, 1987).

However, differences in dietary requirements, whether a result of differences in size or metabolic efficiency, do not appear to be adequate to explain the large differences in the effects of SI and BF larvae on germination and seedling growth. Despite a less than two-fold difference in seed mass consumption, there was a greater than six-fold difference in the germination frequencies of mung beans infested by BF versus SI larvae and a more than three-fold difference in the germination of azuki beans (Fig. 1A). Even in Blackeye cowpea, which contains ample resources to support multiple larvae, infestation by just a single SI larva significantly reduced germination success, whereas the germination frequency of BF-infested seeds was statistically indistinguishable from that of uninfested seeds. These differences in effects of seed germination and seedling growth were probably a result of biotypic differences in larval behaviour within seeds.

Larval competitive behaviour differs substantially between these two biotypes (Messina, 1991b). Larvae that engage in a scramble-type competition (such as the BF population) avoid each other within seeds, apparently by a stereotypical 'wall-making' behaviour (Mitchell, 1990; Mano & Toquenaga, 2008a, b), and tend to remain on the periphery of a seed (Toquenaga, 1993; Toquenaga *et al.*, 1994). By contrast, contest-competing larvae (including the SI population) typically burrow toward the centre of seeds, which increases the likelihood of direct contact and aggressive interactions. These differences in 'centripetal tendency' (i.e. whether a larva tends to move to the core of the seed or remain on the periphery) (Toquenaga *et al.*, 1994) may have a large effect on the likelihood of fatally damaging the embryonic axis (Cipollini & Stiles, 1991), including the epicotyl, hypocotyl and radicle.

It is clear that larval behaviour and/or interactions within seeds affected the amount of damage to seeds. For example, although seeds of the two cowpea cultivars used in the present study easily provide enough resources for two beetle larvae from either population (Table 1), the amount of seed mass consumed per larva varied with density only for SI larvae. If BF larvae do not interfere with each other within seeds, the amount of seed mass consumed per larva should be similar in one-larva and two-larva seeds (Table 2). The significant drop in seed mass lost per larva when seeds bore two contest-competing SI larvae

probably results from strong larval interference or inhibition (Thanthianga & Mitchell, 1987).

The substantial difference in cotyledon damage on seedlings derived from BF- and SI-infested seeds also may be a result of behavioural differences of larvae within seeds. For example, feeding in different regions and on different tissues may explain why Blackeye cowpeas infested by multiple BF larvae sustained less cotyledon damage than those infested by a single SI larva (Fig. 3A, B). Identifying the mechanisms underlying biotype effects on cotyledon damage may be particularly useful because this variable was a good predictor of seedling performance (better than seed mass consumed) 15 days after germination. Distinguishing the relative effects of larval size, metabolic efficiency, and behaviour on plant traits will require periodic dissections or X-ray analyses of seeds bearing larvae of known origin, densities and ages (Thanthianga & Mitchell, 1987; Mano & Toquenaga, 2008a, b). It would also be helpful to obtain information on larval growth, location and behaviour in both small and large hosts because the magnitude of the biotype effect on plant performance varied among hosts and the pattern of differences often varied with seed size.

Biotypic or geographic variation makes it difficult to form generalizations about the impact of particular pest at the level of the pest species. Southgate (1979), for example, noted that, although cultivated grain legumes (as in the genus *Vigna*) were fairly tolerant of low levels of *C. maculatus* infestation, even a single larva would almost always preclude successful germination of small-seeded mung beans. On the basis of the results obtained in the present study, this statement is largely true for SI-infested mung beans but is not at all accurate for BF-infested mung beans (which germinated at a frequency of 70%; Fig. 1). Similar complications could arise in any attempt to obtain general, robust estimates of tolerance in partially resistant cultivars (Credland & Dendy, 1992). Unchecked population growth of any *C. maculatus* biotype in grain-legume stores will eventually render virtually all seeds unfit for human consumption or planting. However, at low to moderate infestation levels, the origin of the pest population could have a major effect on the degree to which the infestation reduces either the edible mass of stored seeds or the frequency of successful germination among seeds used for the next growing season.

Because *C. maculatus* is a stored-product pest with a cosmopolitan but fragmented distribution, it is perhaps not surprising that interfertile populations have diverged in many behavioural, physiological, morphological and life-history traits (Messina & Mitchell, 1989; Mitchell, 1990; Fox *et al.*, 2004). Some of this variation may have arisen as a result of random genetic drift during bottlenecked generations (Credland, 1990; Tran & Credland, 1995). Nevertheless, at least some population differences appear to reflect adaptation to different legume hosts (Smith, 1990) and can evolve rather quickly. For example, switching replicate lines of the SI population from its ancestral mung bean host to Blackeye cowpea led to the evolution of reduced body size and a change in larval behaviour, so that larval competition shifted from the contest type to the scramble type in less than approximately 40 generations (Messina, 2004a). Conversely, Tuda & Iwasa (1998) found that switching a *C. maculatus* population to a small-seeded host led to

the evolution of larger body size and a more contest type of competition in less than 20 generations (Tuda, 1998). The findings of the present study suggest that these instances of local adaptation to different legume hosts can in turn influence the impact of beetle feeding on the host itself.

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