

Oviposition substrate affects adult mortality, independent of reproduction, in the seed beetle *Callosobruchus maculatus*

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Abstract. 1. Seed beetles (Coleoptera: Bruchidae) are commonly used to study the influence of reproduction on life-span and senescence. To study the physiological trade-off between reproduction and mortality, many experiments rely on manipulating access to oviposition substrates to manipulate the reproductive rate of females.

2. The presence of host seeds, independent of reproduction, results in increased mortality of female *Callosobruchus maculatus*. This influence on mortality varies between two host seed species, suggesting a role of either allelochemicals or energetic costs associated with behaviour on hosts.

3. The influence of host seeds on survivorship, independent of reproduction, confounds the interpretation of cost-of-reproduction studies with seed beetles. This complication must thus be considered in the design and interpretation of life-history studies of seed beetles and other insects.

Key words. Cost of reproduction, life-span, survivorship.

Introduction

Seed beetles (Coleoptera: Bruchidae) are excellent subjects for many empirical studies of life-history and evolutionary theory (Wasserman & Futuyma, 1981; Mark, 1982; Møller *et al.*, 1989a, b, c; Credland, 1990; Messina, 1991; Siemens *et al.*, 1991; Smith, 1991; Tanaka, 1991; Fox, 1993a, b, c; Tatar *et al.*, 1993). They are easy to cultivate and handle, and laboratory conditions resemble their semi-natural storage environment. Recently, bruchids have been used to study the influence of reproduction on life-span and senescence, including both genetic analyses (Møller *et al.*, 1989a, c; Tucić *et al.*, 1990; Tanaka, 1991) and manipulative experiments (Møller *et al.*, 1989b; Smith, 1991; Tatar *et al.*, 1993). In particular, manipulating access to host seeds can produce cohorts with high and low reproduction, and thus treatment differences in survivorship can be interpreted as a mortality cost of reproduction (Møller *et al.*, 1989b; Smith, 1991). Similar host deprivation procedures have been used for studying the influence of reproduction on mortality in other insects (e.g. Carey *et*

al., 1986; Partridge *et al.*, 1987). Here we demonstrate that the presence of host seeds is a previously unconsidered extrinsic source of mortality, independent of reproduction, which may confound the interpretation of these experiments. We argue that this complication must be considered in the design and interpretation of life-history studies of seed beetles and other insects.

Methods

Callosobruchus maculatus (Fabricius) is a cosmopolitan pest of stored legumes (Fabaceae), particularly seeds of the genus *Vigna*. Females cement their eggs to the surface of host seeds. Approximately 4–5 days later (at 26–28°C), the eggs hatch and the first-instar larvae burrow through the seed coat and into the seed. Larval development and pupation are completed entirely within a single seed. Adults eclose and emerge from the bean with some mature eggs and are immediately able to mate and oviposit. At 25°C, the life cycle takes about 25–30 days. Adult *C. maculatus* are facultatively aphagous, and do not feed on the oviposition substrates used in these experiments.

Our beetle stock was collected from stored azuki seeds (*Vigna angularis*) near San Francisco, California, and

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maintained for approximately twenty-two laboratory generations prior to this experiment (see Fox, 1993a).

One hundred and eighty virgin females were collected from isolated azuki seeds within 12 h of adult emergence and transferred individually to 35 mm petri dishes containing either: (a) twenty-five azuki seeds (*Vigna angularis*), (b) sixteen cowpea seeds (*V. unguiculata*), or (c) no seeds. Food and water were withheld. In each treatment the number of seeds was sufficient to cover the entire bottom of the 35 mm petri dish which minimized the rolling of seeds and reduced the potential for mechanical damage of the beetles. Dishes were interspersed on two mesh trays and kept at $26 \pm 0.5^\circ\text{C}$, continuous light, <25% r.h. Time of death was recorded within 12 h.

Results

Exposure to host seeds reduced adult survivorship (Fig. 1). Mean life-span without seeds was 28.9 ± 0.8 days ($n = 60$) compared to 18.5 ± 0.5 days ($n = 58$) on azuki and 15.7 ± 0.4 days ($n = 60$) on cowpea (Mann-Whitney U-tests; cowpea seeds versus no seeds, $U = 27.0$, $P < 0.001$; azuki seeds versus no seeds, $U = 191.0$, $P < 0.001$). Mean longevity on cowpea was shorter than on azuki (Mann-Whitney U-test, $U = 2454.5$, $P < 0.001$).

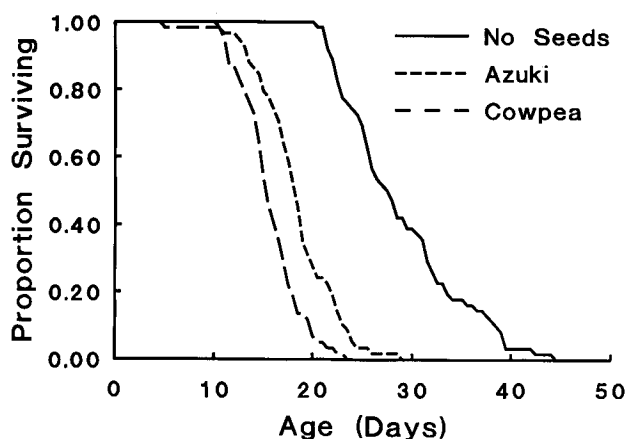


Fig. 1. The influence of oviposition substrate on survivorship of virgin adult female *Callosobruchus maculatus*, independent of reproduction. Sample sizes are sixty, fifty-eight and sixty females in the No Seeds, Azuki and Cowpea treatments, respectively.

These differences in mortality are not explainable by differences in reproductive rate: few females produced eggs (proportion of females laying: cowpea, 0.15; azuki, 0.19; without seeds, 0.12). Of females which laid eggs, there was no difference in the number of eggs produced among treatments (2.0 ± 0.5 eggs on cowpea; 1.8 ± 0.4 eggs on azuki, and 2.1 ± 0.6 eggs without seeds; Mann-Whitney U-test, $P > 0.05$ for all comparisons).

Discussion

The presence of host seeds, independent of reproduction, results in increased mortality of female *Callosobruchus maculatus*. This influence on mortality varies between two host seed species. We suggest four non-exhaustive explanations for these observed differences in mortality: (1) seeds contain allelochemicals which kill beetles; (2) seeds cause mechanical damage by rolling onto beetles when dishes are moved; (3) dried seeds contribute to desiccation of beetles; or (4) variation in beetle behaviour across treatments, resulting in variation in associated energy expenditures. Pesticide residues are not an explanation for the observed patterns because all seeds used in these experiments were grown and stored without chemical treatments for pest control (L. Perkins, Pacific Grain and Foods, pers. comm.). In addition, identical patterns of mortality were observed with seeds obtained from the Department of Vegetable Crops, University of California at Davis (M. Tatar, unpublished data). These latter seeds were likewise grown and stored without pesticides.

Mechanical damage and desiccation are unlikely explanations for the observed differences in mortality. Our handling methods and seed packing eliminated the rolling of seeds, and thus most sources of mechanical damage. Rates of desiccation could be increased through either high rates of transpiration due to hygroscopic properties of dry beans, or through abrasive dusts that decrease the water regulatory properties of the cuticle. However, this effect is unlikely since supplementing adults with water (Leroi, 1981) and varying relative humidity (Giga & Smith, 1983) do not influence adult survivorship. However, because the relative humidity of our incubators (<25%) was substantially lower than the experimental humidities of Giga & Smith (1983; 60–80% r.h.), desiccation cannot be entirely excluded as a potential explanation.

The differences in survivorship between adult beetles exposed to the two seed species suggests that allelochemicals or behaviour-associated energy costs contribute to mortality. Allelochemicals are common in legumes and represent some of the best examples of trophic coevolution. Insecticidal effects are described for larvae which involve interference with digestion or assimilation (Rosenthal, 1981). In contrast, a toxic effect on aphagous adults (this experiment) must be transcutaneous or respiratory. Similarly, behaviour-associated energy costs, such as the energy expended to search hosts, can vary with host presence or absence. This effect was considered by Roitberg (1989) in his examination of the cost of reproduction in *Rhagoletis basiola*, but has not been examined in bruchid beetles.

Our main concern, however, is not the mechanism producing the observed survivorship differences, but the implication of these survivorship differences for the appropriate choice of controls in life-history studies. For *C. maculatus*, the reduction of life-span in the presence of host seeds, relative to the absence of seeds, has been interpreted as a physiological cost of reproduction (Møller *et al.*, 1989b; Smith, 1991). However, the influence of

seeds on survivorship, independent of reproduction, confounds this interpretation. Mortality costs of reproduction must be assessed when the treatments do not produce bias in the absence of egg laying (Tatar *et al.*, 1993).

Confounding mortality effects may occur in other experimental insect systems as well. For example, reproductive effort of *Drosophila* can be manipulated by controlling the presence or absence of egg-laying media (e.g. Partridge *et al.*, 1987). Mortality factors such as pathogen infections may be associated with media containing yeast. These infections may not occur in control vials using non-nutritional media that reduce egg laying. Similarly, behavioural costs, as discussed above, can vary with host presence or absence. These costs must be standardized, as has been done in studies on the cost of reproduction in *Rhagoletis basiola* (Roitberg, 1989). In each case the oviposition substrate may cause mortality that is independent of the physiological costs of egg laying.

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