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The rearing of the gregarious koinobiont endoparasitoid *Microplitis tristis* (Hymenoptera: Braconidae) on its natural host *Hadena bicruris* (Lepidoptera: Noctuidae).

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Abstract

We describe a method for the rearing of the oligophagous herbivore *Hadena bicruris*, and its gregarious koinobiont endoparasitoid *Microplitis tristis* under laboratory conditions. With flowers from the host food plant *Silene latifolia* available, female moths lay on average 414 eggs during their lifetime, starting at day 3 after eggression and with a peak number per night on day 8. Caterpillars can be reared with 56 % survival on an artificial diet. Individually mated parasitoids succesfully parsitised individually presented L4 caterpillars. Clutch size of the reared parasitoids (13.4) is lower than clutch sizes found in natural populations (17.7). Age of the females might be an important factor influencing clutch size. Diapause is differently induced in the host and in the parasitoid. Under long day conditions low night temperature induces diapause in hosts but not in prasitoids. Under short day conditions both species enter diapause at low night temperature.

Introduction

Parasitoids constitute an important and well-studied group of insects. Their larvae develop on or inside the bodies of other arthropods, usually other insects, eventually killing the host. Most of these parasitoids are in the family Hymenoptera. Around 50.000 species have been described (Godfray, 1994). Most parasitoids of which the biology and ecology are well known are studied because of their significance for biocontrol of agricultural pests. However, to better understand their importance and dynamics in natural systems, more knowledge about naturally occuring (non-agricultural) species is necessary. To successfully perform experiments with large numbers of individuals, it is necessary to have a solid working knowledge of the systems under investigation.

The Lepidopteran herbivore *Hadena bicruris*, a seed predator of white campion, *Silene latifolia*, and its parasitoid *Microplitis tristis* are currently used as a model system to study effects of habitat fragmentation on a multitrophic system. Along the river Waal, populations exhibiting different degrees of fragmentation are studied. However, to investigate top-down control in the system it is neccessary to establish artificial populations and to carry out cage experiments. For these studies it is essential to be able to rear both the host and the parasitoid in large quantities.

Here, we present the first description of the rearing of *Microplitis tristis* on its host *Hadena bicruris* in large quantities. We will also discuss some unsolved issues that need further attention.

Species natural history

The host

Hadena (Schrank, 1802) species are specialist Lepidoptera (Noctuidae) on plants in the pink family Caryophyllaceae. For an extensive overview of the *Hadena* family see Hacker (1996). *Hadena bicruris* (Hufn., 1766) (see a habitus description in Bretherton et al. (1979)) has a strong preference for *Silene latifolia (Pioret,)(= S. alba = Melandrium album)* as foodplant, but eggs and caterpillars can also be found on *S. dioica, S. vulgaris, Saponaria officinalis* and presumably on other Caryophyllaceae (Wirooks & Plassmann, 1999). The adult moth is a major pollinator of *Silene latifolia* (Jürgens et al., 1996). After drinking nectar from the flower the female can decide to lay an egg on the flower (Brantjes, 1976b). Usually one egg per female flower is deposited on the developing seed capsule (Brantjes, 1976a). After hatching, the first instar caterpillar will mine into the developing seed capsule leaving a small hole. It feeds on the developing seeds inside and excretes frass through the hole that increases in size over time. This red frass is usually visible and a clear sign that a seed capsule contains a developing larva. When the caterpillar reaches instar 4 or 5 (hereafter L4, L5) the “primary” seed capsule becomes empty and the caterpillar abandons it to seek other “secondary” seed capsules. It will start consuming these from the top, exposing its abdominal segments. Fully grown L5 caterpillars leave the capsule and drop to the soil where they spin a loose cocoon just below the surface.

The parasitoid

Microplitis tristis (Nees) (Braconidae) is a specialist Hymenopteran endoparasitoid that attacks caterpillars of *Hadena bicruris* (Shenefelt, 1973). It is a gregarious species that can lay approximately 1 - 50 eggs in one host. Larvae develop and feed exclusively on the haemolymph of the host and make a firm cocoon after emerging, usually in the soil but occassionaly in an empty seed capsule.
In 53 populations along the Waal, 30% of 600 collected caterpillars appeared to be parasitised by *M. tristis* with an average clutch size of 17.7 ± 0.66 in September 2001. In 71% of the populations the parasitoid was present. Thus far in the field only L4 (12%) and L5 (40%) instars of *H. bicruris* have been found to be parasitised by *M. tristis*. No early instars L1- L3 have been recovered parasitised. We assume at the moment that the parasitoid, which has a short ovipositor, can only attack caterpillars that have left the primary seed capsule i.e. instar 4 or instar 5 caterpillars.

Rearing methods

Source populations

In June, 2000, several caterpillars were collected from different *S. latifolia* populations in the province of Gelderland, the Netherlands. These caterpillars were reared on seed capsules collected from plants in the experimental garden of NIOO (Heteren) and allowed to pupate in vermiculite in a large container. Wasp larvae emerged from several of these caterpillars.

Rearing of the host, *Hadena bicruris*

Eggs

To collect eggs, several female and male moths were placed together in a plexiglass cage (30 x 40 x 30 cm) with small holes closed with mesh gauze. Each day ± 5 female *S. latifolia* flowers for oviposition in a small vase were refreshed. One male flower was placed in a 2 ml Eppendorf cup filled with 50% honeywater attached to the side of the cage as an adult moth food source.

Female moths started laying eggs after 3 days at 25/15 º C 14/10 hrs L/D. The number of eggs laid per night quickly rised and after peaking at around 8 days, it decreased again (Figure 1), a pattern similar to that observed in the noctuid *Mamestra brassicae* (Rojas, 2001). The four moths used produced on average 414 eggs during their lifetime, which is considerably higher than the 119 eggs produced by the moths in the study of Brantjes (1976b). Similarly, the longevity of moths in the study of Brantjes (1976b), which averaged 8.7 days, was shorter than in this study (avg. 18.3, max. 30 days). While females in natural populations will ususally lay a single egg, carefully placed on the central part of the ovary, the captive females layed up to 40 eggs on a single flower at various places, including petals.

Eggs were collected daily from flowers; only few eggs were found on other places in the cage. Using a wet toothpick eggs were placed in a Petri dish with moist filterpaper. Eggs that stuck firmly together were carefully separated. After 24 hours at 25º C 14/10 hrs L/D, fertilised eggs turn brownish while unfertilised eggs stay white. Eggs will hatch after three days.

Larvae

As also described by Peschken and Derby (1990) for *H. perplexa*, *H. bicruris* larvae are cannibalistic, not only in their first instars as described by Brantjes (1976b) but also in larger instars, and have to be reared in separate vials. Newly hatched larvae will also eat conspecific eggs, which has been described in other lepidopteran species as well (Watanabe & Oh'ura, 1997). For *H. bicruris* (egg) cannibalism might be an adaptation to prevent competition for food and/or shelter on a single seed capsule (Brantjes 1976b). To rear *H. bicruris,* we used plastic Coulter-counter cups (30 ml). In every cup we placed a small block (± 0.7 mg) of artificial diet. The diet is modified from Poitout & Bues (1974) and is often used for the rearing of noctuid species like S*podoptera exigua*. It contains per l H_2O: 28g agar, 160g cornflour, 50g yeast powder, 50g wheat germs, 2g sorbic acid, 1.6g nipagin, 8g ascorbic acid, 0.1g streptomycin. Peschken & Derby (1990) reared *H. perplexa* on artificial diet with dried flowers added. When reared from the egg, *H. bicruris* did not need this. When growing caterpillars were transferred from natural food to artificial diet, more caterpillars accepted the food when mashed seed capsules were mixed into the diet (unpub. data).

Forty-eight hours after being collected, brown eggs were placed with a tootpick on the side of the cup which is closed with the lid. Typically, eggs hatch within 24-36h.
After seven days holes were made in the lids with a needle. From this point onwards food was refreshed every fourth day. Growth curves at 25/25 and 25/15º C are shown in figure 2. When caterpillars reached L5 the cup was filled half with vermiculite with the food on top. Approximately two days before pupation, the caterpillars started constructing a loose cocoon in the vermiculite. At 25/25º C it took about 16 days before the moths emerged (see unsolved issues about hibernation). Total survival from egg to pupa was 56% (figure 3). Pupal survival was around 95%.

Rearing of the parasitoid, *Microplitis tristis*

Mating

To ensure that wasps produced both sexes in their offspring, male and female parasitoids were placed together until mating was visibly observed. In large Petri dishes with small drops of honey, ± 15 males and three females were released. Males will immediately start chasing the females and vibrating their wings when approaching a female. Although males ambush females very often, successful mating can only be ascertained when females remain motionless and female and male are attached for about 20 seconds at the abdomen. These females were then immediately removed and stored separately. When two or three females had mated the next three females were added.
In several Hymenoptera single-locus complementary sex determination has been demonstrated, and the phenomenon is apparently common in the Braconidae (Godfray 1994). There is no information available whether this phenomenon occurs in *M. tristis*. To minimise the chance of loss of allelic diversity (leading to the production of diploid males), we mated wasps from different clutches and sources (Cook 1993).
Wasps were kept at 10 ºC between different treatments.

Parasitising

Individually mated females (max. age 1 week) were placed under Coulter-counter cups with a droplet of honey. Although wasps can parasitise all instars, instar 4 is optimal in terms of fitness correlates (body size, development time and survival) of *M. tristis* (Elzinga, in prep.).

Caterpillars reared as described above were kept at 4 º C as L4. They can survive at least a month under these conditions (J. Elzinga, unpub. obs.). These caterpillars were presented individually with a forceps to female wasps. Larvae first dipped in a watery suspension of mashed *S. latifolia* seed capsules were more attractive than dry caterpillars, probably because of the emitting odours of the host plant.

Eight days after parasitism at 25/25º C, or 12 days at 25/15º C, vials with parasitised larvae were half-filled with vermiculite to allow caterpillars to spin a cocoon. If wasp larvae emerge from the caterpillar and are in contact with the artificial diet cocooning will fail, probably due to excessive moisture.

After 11 days at 25/25º C, or 17 days at 25/15º C, parasitoid larvae emerged and spun cocoons.

After cocooning it took on average 8 and 12 days, repectively, before the adult wasps emerged (but see unsolved issues about diapause).

Unsolved issues

Clutch size

Caterpillars parasitised in natural populations produced an average of 17.7 wasp larvae (figure 4). By contrast, lab-reared hosts produced an average of only 13.4 ± 0.89 wasps (n = 272). We do not know what causes this difference, but the age of the parasitising wasp might be an important factor. We performed an experiment with two groups of wasps with no previous parasitising experience. The first group was kept for 2 months at 10º C and the second for only one week. The results indicate that clutch size is strongly inversely correlated with the age of the female wasp (figure 4). Other experiments with *M. tristis* suggest that clutch size also decreases with the number of oviposition experiences (Elzinga, in prep.).

The relationship between age and clutch size in gregarious parasitoids has not been studied very intensively. Most studies focus on age-related effects on clutch size in parasitoids with hosts continuously available for parasitation (Moralesramos & Cate 1992, Tagawa 2000). Low numbers of available hosts lead to higher clutch sizes, but with high numbers of hosts available, clutch sizes show a decrease with parasitoid age due to oviposition experience and/or egg-depletion (Rosenheim & Rosen 1991).

Diapause

Continuous rearing was undertaken at 25/25ºC and 14/10 hours L/D. At theses temperature and light conditions both host and parasitoid did not enter diapause. Parasitoids pupated directly after completing their cocoon. However, when reared at 25/15ºC, under the same light conditions, host pupae enter diapause but parasitoids do not. When reared under 25/15º C and 10/14 L/D both species entered into diapause. Diapausing wasp larvae pupate and develop in the cocoons only after being kept at 4º C 10/14 L/D for a period of time. At present we do not yet know how long this period has to be, nor which factor is important for terminating diapause.

Photoperiod and temperature are the most important factors initiating and regulating diapause in polyvoltine insects, including noctuids and parasitoids (Beck, 1968, Tauber et al., 1983, Ishii et al., 2000). Temperature, photoperiod and moisture have been indicated as important factors for termination of the diapause (Alvi & Momoi, 1994, Ishii et al., 2000, Seymour & Jones, 2000) but this appears to be very species-specific.

The parasitoids that do not diapause can be kept at 4 º C for upto a month but after 2 months most wasps have died. Diapausing wasps can be kept in the cold at least for almost a year. Diapausing pupae of the host can be kept for more than a year.

More studeis of factors inducing, terminating and preventing diapause of the host and the parasitoid need to be performed to facilitate succesful storage of host and parasitoid pupae.

Although some problems, e.g. hibernation, have not yet been solved, *Microplitis tristis* can be reared effectively on *Hadena bicruris*. The fact that the host caterpillar does not need its natural food is very convenient. Experiments for which large quantities of *H. bicruris* and its parasitoid *M. tristis* are needed, are eminently possible when combined with laboratory methodology.

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Figures

Figure 1.

Daily egg production of *H. bicruris*. Data based on four female moths with lifetimes after eggression of 13, 13, 17 and 30 days (Numbers in bars indicate the number of moths still living). From day 18-23, in total 48 eggs (dashed line) were produced.

Figure 2.

Growth curves of *H. bicruris* caterpillars (± SE). Rearing took place at 25/25º C 14/10 L/D (closed symbols, n = 9) and 25/15 º C 14/10 L/D (open symbols, n = 26). For each treatment, the last datapoint represents pupal weight. Triangles indicate average hatching (h), moulting (2-5) and pupation (p) dates.

Figure 3.

Cumulative survival percentages per instar for lab-reared *H. bicruris* caterpillars from egg to pupation.

Figure 4.

Mean clutch sizes of old and young *M. tristis* in L4 parasitised caterpillars, and clutch sizes in field collected caterpillars (± SE). One week old: n = 46, clutch size 13.8 ± 0.69; 2 months old: n = 26, clutch size = 4.07 ± 0.47; filed caterpillars: n = 171, clutch size = 17.7± 0.66. Bars with different letters are significantly different from eachother ($p < 0.01$).

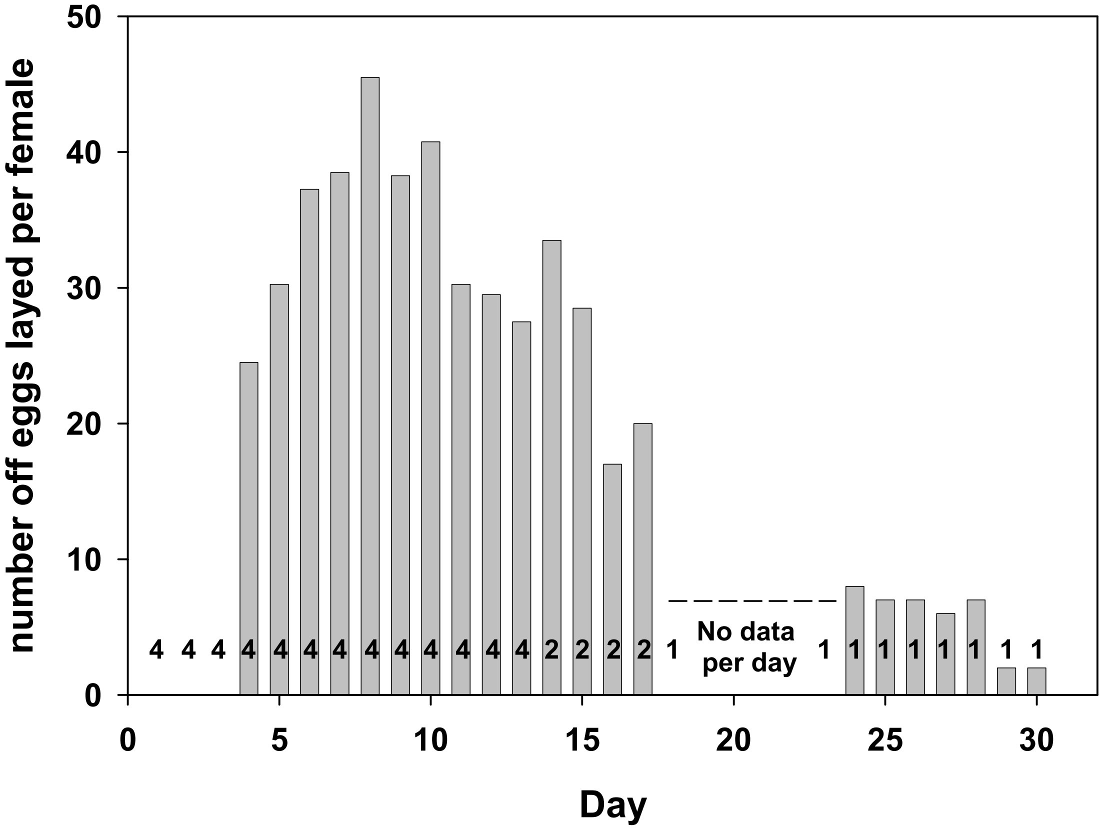

50
40
30
20
10
0
number off eggs layed per female
4 4 4 4 4 4 4 4 4 4 4 4 4 2 2 2 2 1
No data
per day
1 1 1 1 1 1 1 1
0 5 10 15 20 25 30
Day

Figure 1

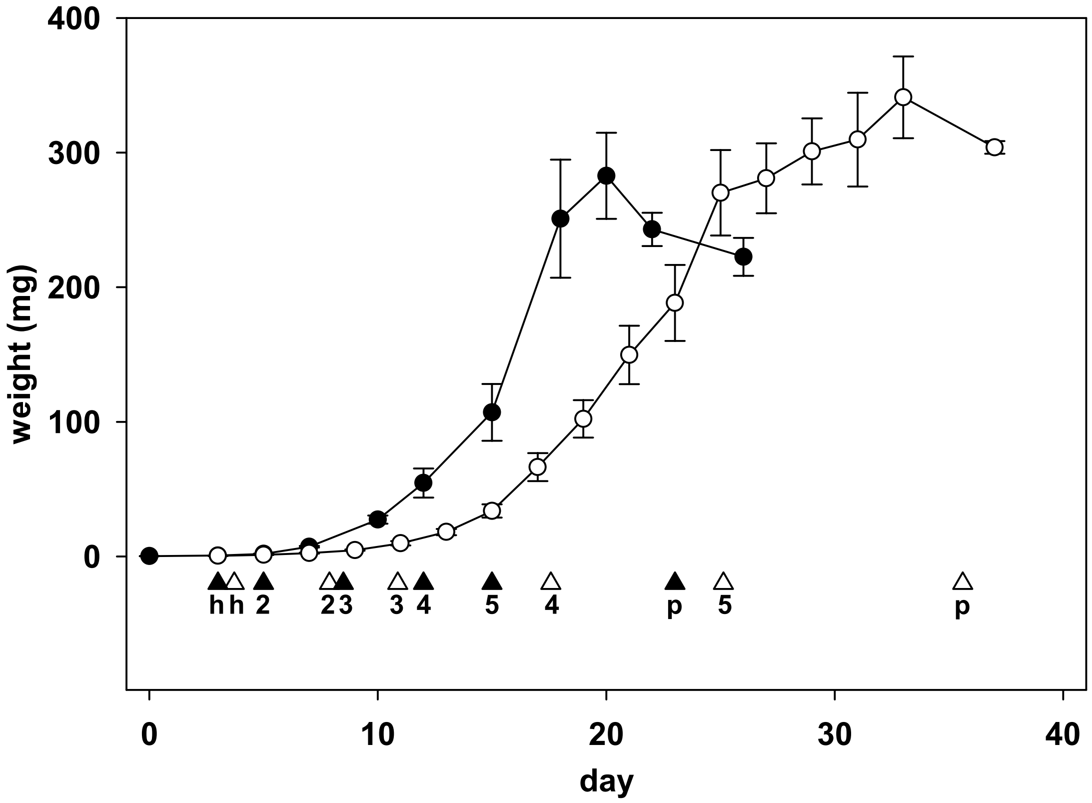
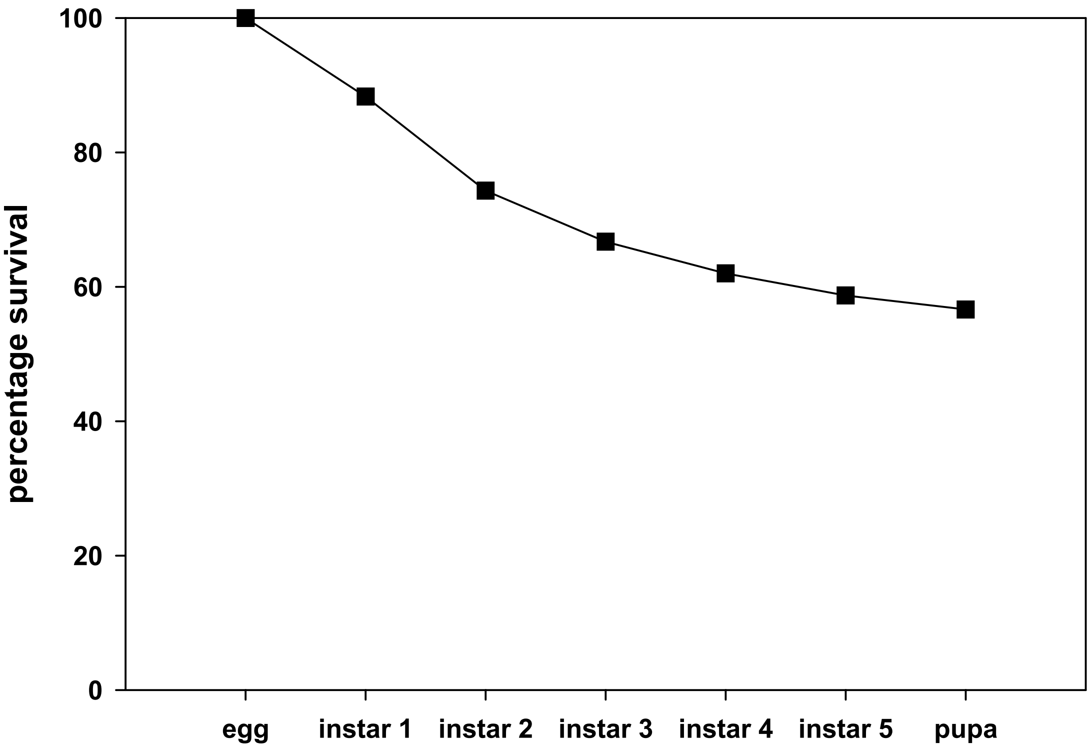


Figure 2



100
80
60
40
20
0
percentage survival
egg
instar 1
instar 2
instar 3
instar 4
instar 5
pupa

Figure 3

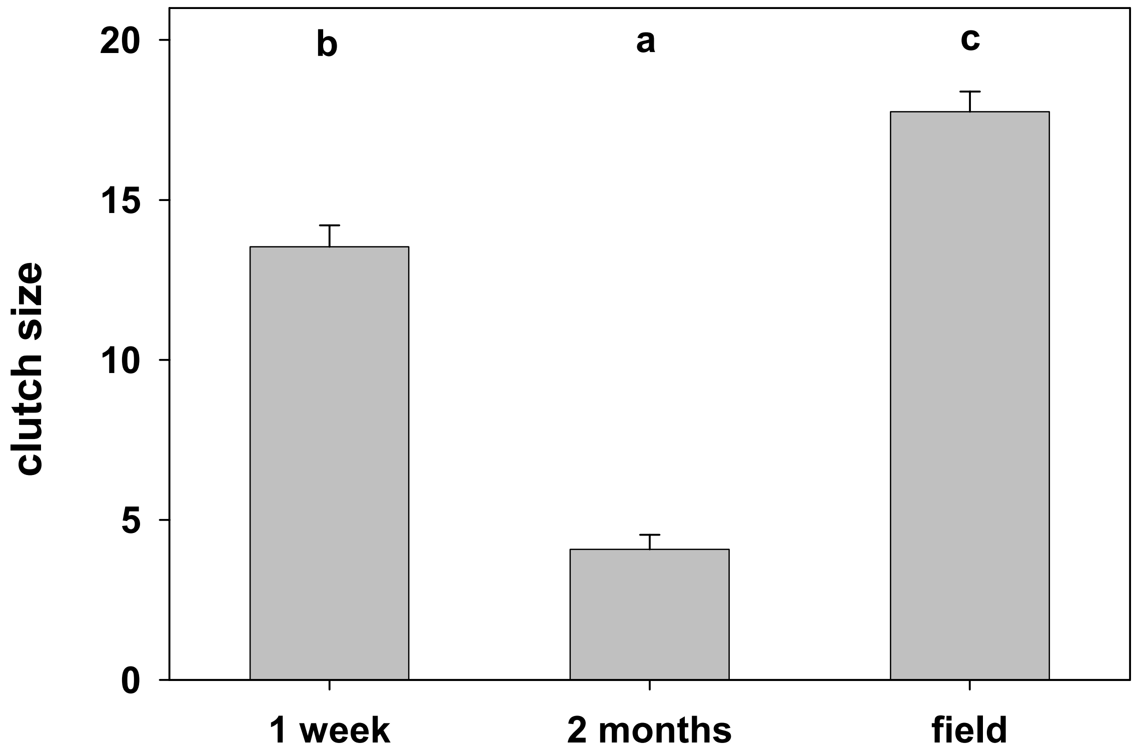


Figure 4