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TECHNICAL NOTE

Dummy caterpillars as a simple method to assess predation rates on invertebrates in a tropical agroecosystem

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Introduction

Attention to the role ecosystem services play in maintaining the world's habitats and their inhabitants is increasing as both our understanding and opportunities to estimate the economic value of these services increase. Generally, these services are of immense importance, not only from an anthropogenic viewpoint; they are also necessary to maintain the health and functioning of ecosystems (MA, 2005). The recently completed Millennium Ecosystem Assessment highlighted the need for methods to evaluate these services, but existing knowledge already indicates that many ecosystem services are not in good shape (MA, 2005).

Agricultural management is very important from this perspective (Tilman et al., 2002; Dale & Polasky, 2007). Ecosystem services tend to be more important in developing than in developed countries (Mertz et al., 2007), mainly (but not only) because in those agroecosystems, external resources are often not available to substitute for ecosystem services, especially in rural communities (Mertz et al., 2007). Biological control/natural pest control through predation and parasitism by an array of naturally occurring organisms (native natural enemies) is one of the recognised ecosystem services (de Groot et al., 2002). However, our knowledge about the level of biological control provided by ecosystems is not too detailed, and especially lacking from developing countries in the tropics.

Studies on natural enemies often assume density as a measure of natural enemy importance or intensity of function (Waage & Mills, 1992), arguing that a doubling in natural enemy densities equates to a two-fold increase in predation pressure. This is not necessarily so, because several factors, including predator satiation, intraguild predation, and intra- and interspecific competition can complicate the picture (Schmitz, 2007). Therefore, there is a need for functional biological control studies which measure levels of natural enemy pressure on pests under field conditions. Natural enemy activity in general is difficult to detect, especially by invertebrates. Assessing parasitism rates is less problematic because the hosts of parasitoids often become immobile and are easily recognised. However, predation often leaves no trace, or only fragments of the consumed prey can be found. A predation event usually happens quickly, predators often hide while consuming prey, and many of them are active at night (Crawley, 1992). Such factors make field assessments of predation intensity difficult. These inherent problems have curtailed our knowledge about the kind and extent of predation in different habitats by different natural enemies (Crawley, 1992). Consequently, we have several examples of quantitative parasitoid food webs (Müller et al., 1999), but fewer of predator food webs.

Predation can be studied by video recording (Varley et al., 1994), by indirect means, using dyes, markers, and analysis of predators for prey remains, or by using sentinel prey (Jervis & Kidd, 1996). The last method monitors the rate of disappearance of prey provided by the experimenter, and is the easiest one to obtain quantitative data on predation pressure. Immobile (eggs, pupae) or immobilised stages of arthropods are often used as sentinel prey. This approach has several inherent problems, such as generating sufficient numbers (which often requires mass rearing), putting them out into the field, and recording/identifying the natural enemies responsible for prey disappearance. Several of the above difficulties can be avoided or circumvented if artificial

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(not live) prey are used. In this note, we report on the use of such an artificial sentinel prey, lepidopteran 'caterpillars' made of plasticine.

We could find five published studies, viz., Loiselle & Farji-Brener (2002), Koh & Menge (2006), Posa et al. (2007), Richards & Coley (2007), and Faveri et al. (2008), that used the method as part of their research, but none of them provides a detailed description. We believe artificial caterpillars made of plasticine have wide applicability in experiments on predation. The technique is simple, inexpensive, does not require sophisticated equipment or procedures, and can be used under field conditions and a wide range of situations. Our intention with this short note is two-fold: (1) to provide a detailed description of the technique, and (2) to demonstrate the utility of this method by providing an illustration of its application to quantify predation in an Ugandan cotton field. By doing so, we hope to raise the attention of ecologists and entomologists, especially those engaged in research in biological control in tropical countries, to the potential usefulness of this method in field-based studies.

Materials and methods

The materials needed for this method are few: plasticine, to produce the 'prey caterpillars,' either by hand or by an instrument, quick-setting glue to fix them onto the desired surface, and a hand-held magnifying glass to control them for signs of predation after a set exposure period. The technique relies on the malleability of hobby plasticine which can be rolled by hand into the cylindrical shape of a caterpillar whose diameter and length are at the researcher's discretion. Prey caterpillars can also be produced using a strong garlic press. Using an adhesive agent, these artificial caterpillars can be attached to any substrate (plant leaf, stem, or soil). Care should be taken to minimise the amount of glue used as this may give unwanted chemical signals to predators. The distribution of this artificial prey source is at the investigator's discretion. After a designated duration (we suggest 24 h in order to check for potential problems, ease of finding the prey items again, and for ease of calculation to obtain the level of predation per day) the artificial caterpillars are inspected in situ, or removed for inspection. This can be done using either a magnifying glass, for on-site inspection, or the artificial caterpillars can be taken to a laboratory and examined under a microscope. In this last case, care should be taken to protect the caterpillars from accidental marking and subsequent spoiling of samples. It is often best not to remove the prey from the surface, but collect the whole leaf or stem on which the prey item was fixed. The malleability of the plasticine allows one to observe marks caused by predator's mandibles, teeth, beak, or ovipositor. Using the counts of predation/parasitism on artificial caterpillars, levels of predation can be calculated (expressed as percentage attacked caterpillars per unit of time).

In an experimental cotton field at the Makerere University Agricultural Research Institute, Kabanyolo, Wakiso district, Uganda (0°27′N, 32°37′E), artificial caterpillars (3.5 × 25 mm; Figure 1A) made of light green plasticine (Eberhard Faber, Neumarkt, Germany) were attached with contact glue (Super Attak gel control; Loctite, Henkel, Düsseldorf, Germany) to leaves of cotton plants [*Gossypium hirsutum* L. (Malvaceae)] (Figure 1A). Plant heights varied from 11–64 cm at the start of the experiment to 11–80 cm 4 weeks later when the experiments were finished. The first squares on fruiting branches appeared during the middle of the sampling period. The field (46.5 × 20.3 m) was divided into three blocks in each of which eight 5 × 5 m plots were allotted. Each plot contained up to 96 cotton plants, but due to drought-related stress after sowing, several plots contained fewer. Distances between plots were 1.7–2.2 m. Cotton plants were spaced 30 cm from each other, and row distance was 0.9 m. Cotton plants were either treated once with a systematic insecticide (an organophosphate, TAFGOR 40 EC, active ingredient:

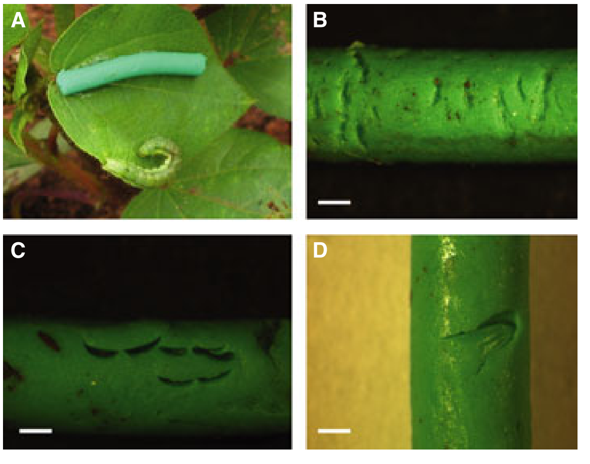

Figure 1 Artificial plasticine caterpillars showing signs of 'predation.' (A) An artificial caterpillar on the same cotton leaf together with a *Helicoverpa armigera* (Hübner) caterpillar. (B–D) bite marks on artificial caterpillars, made by (B) a chewing arthropod, (C) a small rodent, and (D) a bird. Bars on panels B-D indicate 1 mm. Digital photos (B–D) were modified for better contrast, using Leica Application Suite software.

dimethoate 40% EC) or were insecticide-free. Plasticine caterpillars were randomly assigned to leaves on plants and the minimum distance between dummy caterpillars was approximately 15–30 cm, due to overlapping plant stems. Artificial caterpillars (in total 1 802) were exposed over 10 non-consecutive days; with approximately 180 larvae exposed per 24 h. This gave rise to a total prey availability of 18 020 prey-days.

Dummy caterpillars were exposed for 24 h, after which they were collected into medical-type resealable plastic bags, labelled according to treatment, placed in a plastic box, and transported to the laboratory where they were examined for signs of predation under a stereomicroscope (Leica MS5, 10×0.63–4 magnification; for digital photos, Leica MZ12.5, 10×1.25 magnification attached to a Leica DFC 420 digital camera; Meyer Instruments, Houston, TX, USA). During one sampling period, 3 h of torrential rain dislodged all but a few caterpillars. This sample was omitted from the analysis.

Results

The artificial caterpillars were readily attacked by predators. Signs left by predatory and parasitoid arthropods and small mammals were identifiable (Figure 1). For example, of the 1 155 caterpillars attached to unsprayed cotton leaves, signs of predation were found on 43 of them. Arthropods accounted for 88.4% of predation events (81.4% mandibular marks and 7% parasitoids), whereas birds caused 9.3% of the recognisable predation events. Just over 2% of the marks could not be identified.

Results revealed higher counts of attacked artificial caterpillars in treatments without insecticide than in plots sprayed with insecticide. In cotton plots without insecticide sprays, the recorded mean (± SD) predation rate was $4.1 \pm 2.5\%$ ($n = 10$), whereas on sprayed cotton plots, the recorded mean predation was $3.7 \pm 2.2\%$ ($n = 7$). This difference was not significant (log-likelihood = -275.39, d.f. = 16, $P = 0.15$; using a binary logistic regression model (Proc GENMOD) from SAS 9.1, which analysed predation events in treatments by date). Daily predation rates varied considerably; in sprayed plots, there were 1.1–7.6% predation events per day and in plots without insecticide, we recorded 1.1–8.9% predation events per day.

Discussion

The dummy caterpillar method proved useful: the artificial sentinel prey were readily attacked by different predators in the cotton field, and they left identifiable marks. These revealed that arthropods, followed by birds, were responsible for the largest proportion of predation events on artificial lepidopteran larvae in both treatments.

The technique described above is not new, although to our knowledge its application in an agroecosystem is novel. Predation on such artificial caterpillars in forest ecosystems in tropical America and Asia shows varying, but usually high levels of predation. In treefall gaps and forest understories on Barro Colorado Island, Panama, predation can be between 6 and 18% per day (Richards & Coley, 2007). On the same island, Koh & Menge (2006) found that 60% of artificial caterpillars exposed over 48 h showed signs of predation. In an Amazonian forest, the recorded levels of predation ranged from 4.2 to 10.6% per day (Faveri et al., 2008).

Our results are, to our knowledge, the first published records from Africa, and indicate that predation rates in an agroecosystem may be comparable to predation levels obtained using the same technique from tropical forest habitats on other continents.

The choice of artificial caterpillar 'phenotypes'

Plasticine is available in different colours and artificial caterpillars can thereby be made in various colours as well as sizes, providing an opportunity to study the reaction of predators to them. Furthermore, as both the size and colour of plasticine caterpillars can be determined by the investigator, such dummy caterpillars can be modelled to resemble specific species (Loiselle & Farji-Brener, 2002; Koh & Menge, 2006; Posa et al., 2007; Faveri et al., 2008). Typically, well defended caterpillars have striking colours and/or hairs to signal their unpalatability to would-be predators (Edmunds, 1974). Green colours might be useful in comparative studies, because green caterpillars seem to be perceived by predators as palatable and undefended prey (P. Coley, pers. comm.). Therefore, we suggest to use plain, green plasticine to make the artificial caterpillars. However, such caterpillars are less conspicuous in the field and one has to be aware of the logistical difficulty in finding them again. Proper mapping and/or marking of plants with dummy caterpillars is important (it is prudent to mark a neighbouring plant).

Furthermore, marks left by predators in plasticine are identifiable, at least to a higher taxonomic level. Allocating predation to different kinds of predators is not unequivocal, and depends on the knowledge of characteristic marks left by the predation attempt. Detailed investigations of mandible marks would further aid differentiation of arthropod predators, and may enable the researcher to distinguish between attacks by, e.g., spiders (Araneae), predatory Heteroptera, beetles (Coleoptera), and ants (Formicoidea) – at least for species observed at the local scale. Such local 'calibration' would considerably improve the reliability and sophistication of the field data.

Clay caterpillars provide a conservative measure of predation

The use of sentinel or artificial prey is not without problems. For example, artificial caterpillars do not emit chemical cues which may be important for prey recognition by some predators (Vet & Dicke, 1992) and host location by parasitoids (Vinson, 1984). Unpublished data by Coley (cited by Richards & Coley, 2007) show no differences between attack rates on artificial lepidopteran larvae and real undefended caterpillars, but whether this is the general rule remains unclear. Another cue predators and parasitoids may use are synomones from an attacked plant (Turlings et al., 1990; Drukker et al., 1995). Obviously, artificial caterpillars do not impact plants as real herbivores would, and the lack of plant-derived cues or chemical signals may also prevent predators from finding the offered prey. There is also a lack of frass from artificial herbivores, another possible signal that natural enemies may employ in their search for prey (Weiss, 2006). The immobility of artificial caterpillars might also influence prey recognition. On the other hand, plasticine caterpillars do not move, hide, or retaliate, which inflates the recorded predation rate with respect to real predation. Furthermore, it is unlikely predators are satiated by plasticine. This, too, could result in an increased number of predation events which would further contribute to inflating the level of predation pressure.

Overall, this method of assessing predation pressure provides results that are probably conservative. The use of artificial prey may not reflect actual predation levels, but the number of predation incidents will be relative (Brodie, 1993) and thereby comparable among habitats, provided experimental set-ups are similar. Despite the difficulty of recording predation events on invertebrates in the field, the use of artificial caterpillars enables the assessment of in situ predation pressure.

Recommendations

It is important to produce caterpillars whose surfaces are completely smooth. During caterpillar production, tiny artefactural marks are sometimes made, that can only be seen under high magnification. There could be tiny 'air holes' made during production which could resemble parasitoid puncture marks. If such marks are unavoidable, they ought to be identified prior to field exposure so as not to be mistaken for marks made by natural enemies. This is important because some predator marks are too small to detect with a magnifying glass alone, and thus require higher magnification to be seen.

When using artificial caterpillars, we suggest the following should be considered: (1) the caterpillars have a smooth surface to ensure predator marks can be clearly detected, (2) the plasticine used should be unscented, to avoid attraction or deterrence by predators, (3) the glue used binds instantly and keeps caterpillars adhered to the substrate throughout the sampling period, (4) the amount of glue used is kept to a minimum, (5) spatial arrangements and densities of artificial caterpillars are representative of naturally occurring prey, (6) marking of caterpillars' positions on plants does not disturb predator movement, and (7) the caterpillars are of uniform size and colour in the areas to be compared.

Extreme weather, for example torrential rain can cause problems because of dislodging the caterpillars. During pilot studies in Uganda, we found that attaching artificial caterpillars to cotton plants during the warmest period of the day resulted in spoilt caterpillars, due to the plasticine almost melting. However, artificial caterpillars attached at cooler times, e.g., early mornings, were not negatively affected by heat during the day. These occasional technical problems neither destroy the experiment, nor cause significant damage to the experimenter's budget, and are easy to amend.

This technique would further benefit from calibration studies in order to determine the integrity of predation measurements attainable. Comparisons with absolute measurements of predation on live caterpillars, using video recording of immobilised or enclosed live caterpillars or similar experiments, could indicate the level of bias resulting from lack of chemical cues, lack of plant cues, immobile prey, and natural enemy reaction to plasticine caterpillars or the adhesive agent.

However, a lack of such studies does not hinder the use of artificial caterpillars now and in the future. Much-needed field data can be collected in different habitats to assist building a more realistic picture of predation intensity, relative to different types of predators, in tropical habitats.

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References

Brodie ED (1993) Differential avoidance of coral snake banded patterns by free-ranging avian predators in Costa-Rica. Evolution 47: 227–235.

Crawley MJ (ed.) (1992) Natural Enemies. Blackwell, Oxford, UK.

Dale VH & Polasky S (2007) Measures of the effects of agricultural practices on ecosystem services. Ecological Economics 64: 286–296.

Drukker B, Scutareanu P & Sabelis MW (1995) Do anthocorid predators respond to synomones from *Psylla*-infested pear trees under field conditions? Entomologia Experimentalis et Applicata 77: 193–203.

Edmunds E (1974) Defence in Animals. Longman, Essex, UK.

Faveri SB, Vasconcelos HL & Dirzo R (2008) Effects of Amazonian forest fragmentation on the interaction between plants, insect herbivores, and their natural enemies. Journal of Tropical Ecology 24: 57–64.

de Groot RS, Wilson MA & Boumans RMJ (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecological Economics 41: 393–408.

Jervis M & Kidd N (1996) Insect Natural Enemies. Chapman & Hall, London, UK.

Koh LP & Menge DNL (2006) Rapid assessment of Lepidoptera predation rates in neotropical forest fragments. Biotropica 38: 132–134.

Loiselle BA & Farji-Brener AG (2002) What's up? An experimental comparison of predation levels between canopy and understory in a tropical wet forest. Biotropica 34: 327–330.

MA (2005) Millennium Ecosystem Assessment. Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC, USA.

Mertz O, Ravnborg HM, Lovei GL, Nielsen I & Konijnendijk CC (2007) Ecosystem services and biodiversity in developing countries. Biodiversity and Conservation 16: 2729–2737.

Müller CB, Adriaanse ICT, Belshaw R & Godfray HCJ (1999) The structure of an aphid-parasitoid community. Journal of Animal Ecology 68: 346–370.

Posa MRC, Sodhi NS & Koh LP (2007) Predation on artificial nests and caterpillar models across a disturbance gradient in Subic Bay, Philippines. Journal of Tropical Ecology 23: 27–33.

Richards LA & Coley PD (2007) Seasonal and habitat differences affect the impact of food and predation on herbivores: a comparison between gaps and understory of a tropical forest. Oikos 116: 31–40.

Schmitz OJ (2007) Predator diversity and trophic interactions. Ecology 88: 2415–2426.

Tilman D, Cassman KG, Matson PA, Naylor R & Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418: 671–677.

Turlings TCJ, Tumlinson JH & Lewis WJ (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. Science 250: 1251–1253.

Varley MJ, Copland MJW, Wratten SD & Bowie MH (1994) Parasites and predators. Video Techniques in Animal Ecology and Behaviour (ed. by SD Wratten), pp. 33–63. Chapman & Hall, London, UK.

Vet LEM & Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annual Review of Entomology 37: 141–172.

Vinson SB (1984) How parasitoids locate their hosts: a case of insect espionage. Insect Communication (ed. by T Lewis), pp. 325–348. Academic Press, London, UK.

Waage JK & Mills NJ (1992) Biological control. Natural Enemies (ed. by MJ Crawley), pp. 412–430. Blackwell, Oxford, UK.

Weiss MR (2006) Defecation behavior and ecology of insects. Annual Review of Entomology 51: 635–661.