A NOVEL TECHNIQUE FOR LABELING PARASITOIDS OF COTTON PESTS

James Hagler and Glen Jackson
USDA-ARS, Western Cotton Research Laboratory
Phoenix, AZ
Matt Ciomperlik
USDA-APHIS-PPQ, Mission Biological Control Center
Mission, TX

Abstract

A laboratory study was conducted to examine the efficacy of a novel protein marking technique on insect parasitoids. Adult parasitoids were marked with the mammal protein, rabbit immunoglobulin G (IgG), by three different application methods. Adult parasitoids were marked internally by feeding them a honey solution "spiked" with rabbit IgG and externally by contact exposure or topical mist. Marked individuals were then assayed using a sandwich enzyme-linked immunosorbent assay (ELISA) for the presence of the protein marker using an antibody specific to rabbit IgG. Data indicate that the protein marker was retained throughout the entire adult lifespan in almost every (>95%) individual parasitoid assayed, regardless of the species examined and the application method used.

Introduction

To understand the population dynamics of insect parasitoids, dispersal behavior must be precisely monitored. Generally, parasitoid dispersal has been researched by mark-release-recapture techniques (M-R-R). M-R-R involves marking insects with an environmentally persistent material, releasing them into the field, and recapturing them at a given time interval. A wide variety of materials have been used to mark insects for dispersal studies (Southwood 1978, Akey et al. 1991). However, marking parasitoids has proven to be a special challenge because most parasitoids are very small and delicate. Conventional marking materials such as paints, dyes, dusts, and tags are ineffective on small parasitoids because they are heavy and can inhibit normal dispersal behavior.

Probably the best markers to date for studying parasitoid dispersal have been the trace elements (e.g., Rubidium) (Jackson et al. 1988, Jackson & Debolt 1990, Akey & Burns, 1991). Trace elements are environmentally safe and relatively persistant in insect tissues. However, there are several drawbacks to using trace elements as biological markers (see Akey et al., 1991).

Recently, a protein marking procedure was developed to mark insects that proved simple, rapid, safe, inexpensive, and stable (Hagler et al. 1992; Hagler 1997). Because the protein marking procedure worked so well on large insects, we planned to investigate the feasibility of protein marking on very small parasitoids.

Materials & Methods

Test Insects

The parasitoids examined in this study are given in Table 1.

Parasitoid Marking Procedures

Three different methods were used to label the parasitoids: 1) ingestion (internal label), 2) contact exposure (external label) and 3) topical mist (external label). For details on the marking procedures see Hagler & Jackson (In Press).

ELISA Procedure

A sandwich ELISA was performed on each parasitoid as described by Hagler (1997).

Negative Parasitoid Controls

Adult parasitoids known not to contain any of the rabbit protein were assayed by sandwich ELISA (Hagler 1997). Parasitoids known to have been exposed to rabbit protein were scored positive for the presence of the marker if the ELISA optical density value exceeded the mean negative control reading by three standard deviations (critical value) (Hagler et al. 1992).

Results

Negative Parasitoid Controls

The mean ELISA optical density values yielded by the negative control parasitoids were lower than the mean value for the PBS blanks (i.e., ca. 0). These data suggest that there are no proteins in these parasitoids that cross react with the rabbit protein marker.

Retention of IgG by Ingestion

The percentage of parasitoids scoring positive for rabbit IgG remains after ingesting a honey solution containing rabbit IgG are given in Table 2. Virtually all of *C. curvimaculatus* and *A. iole* and most of the *E. formosa* and *E. mundus* that fed on the honey solution containing rabbit protein yielded a positive ELISA reaction throughout their adult lifespan.

Retention of IgG by Contact Exposure

The percentage of individuals scoring positive for rabbit IgG after contact exposure to the wetted filter paper are given in Table 2. Almost all of *C. curvimaculatus*, *A. iole*, and *E. formosa* and most of the *E. mundus* that walked on the IgG soaked filter paper scored positive for protein remains.

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Retention of IgG by Topical Mist

The percentage of individuals scoring positive for rabbit IgG after receiving a topical mist spray of rabbit IgG are given in Table 2. Again, almost all of the parasitoids that were sprayed with the IgG solution yielded a positive ELISA reaction for the presence of rabbit IgG.

Discussion

In this study we report for the first time that vertebrate protein can be used to successfully mark minute parasitoids for M-R-R studies. This marking procedure should expedite future field studies aimed at determining parasitoid dispersal patterns. Protein markers offer many practical advantages over many of the more conventional marking techniques. For instance, thousands of parasitoids can be quickly labeled with small quantities of the inexpensive protein. The ELISA procedure is also simple, rapid, and economical. Moreover, laboratory equipment needed to run an ELISA can be found in most laboratories and ELISA reagents are inexpensive and safe.

The best method for applying protein marker will ultimately depend on the nature of the experiment, the characteristics of the parasitoid being marked, and the preference of the investigator. We expect to use this assay for further parasitoid dispersal studies. The immunoassay's specificity, sensitivity, and persistence suggests that we can label *in vitro*-reared parasitoids, release them into the field, and recapture them to examine their dispersal behavior. This novel immunolabeling procedure will expedite research aimed at evaluating insect pest and natural enemy dispersal patterns.

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Table 1. The parasitoids examined in this study for retention of the protein marker.

Parasitoid	Host	Host Stage Attacked
Encarsia formosa	Whitefly	Nymphal
Eretmocerus mundus	Whitefly	Nymphal
Chelonus curvimaculatus	Pink bollworm	Egg/larval
Anaphes iole	Lygus bug	Egg

Table 2. Frequency of positive ELISA responses for the presence of protein label on each parasitoid species over time (n= ca. 30 per treatment).

		Percent Positive by ELISA Marking Method		
Parasitoid Species	Retention (Days)	Ingestion	Contact	Topical
E. formosa	0	100.0	100.0	100.0
	1	97.4	100.0	100.0
	2	86.4	100.0	100.0
	3	90.0	100.0	---
	4	87.9	95.0	---
	5	75.0	97.5	---
	6	---	82.4	---
E. mundus	0	92.9	100.0	100.0
	1	96.2	83.0	100.0
	2	76.3	100.0	80.0
	3	54.8	92.3	---
	4	95.7	58.6	---
	5	52.2	66.0	---
C. curvimaculatus	0	100.0	100.0	100.0
	1	100.0	100.0	100.0
	2	100.0	100.0	100.0
	3	100.0	100.0	100.0
	4	100.0	100.0	100.0
	5	100.0	100.0	100.0
A. iole	0	100.0	100.0	100.0
	1	100.0	100.0	100.0
	2	100.0	96.7	100.0
	3	100.0	100.0	94.3
	4	100.0	100.0	100.0
	5	100.0	100.0	90.6
	6	100.0	100.0	95.0
	7	100.0	100.0	100.0
	8	95.0	100.0	100.0