ELEMENTAL MARKING OF ARTHROPOD PESTS IN AGRICULTURAL SYSTEMS: SINGLE AND MULTIGENERATIONAL MARKING

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ABSTRACT

Use of elemental markers to study movement of arthropod pests of field crops is reviewed. Trace elements, rubidium (Rb) and cesium (Cs), have provided a nondisruptive method of marking natural adult populations via developmental stage consumption of treated host plants. Multigenerational marking occurs with the transfer of elemental markers from marked adults to reproductive products, including eggs, egg masses, and spermatophores. For highly mobile insects such as Lepidoptera, recovery of marked eggs is superior to the more typical recapture of marked adults. Preliminary studies required to refine marking and detection techniques are described, and results of ongoing field studies of meso-scale movement in *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie) using elemental marking and recovery of eggs are reported. Additionally, the problem of mark variability is discussed.

INTRODUCTION

The feasibility of elemental marking has been most widely examined among arthropods in agricultural systems, and in particular in field crops (reviewed by Hayes and Hopper 1987). Insect pests in four orders and ten families have been studied (Table 1). While several trace elements have been tested in field trials published to date, only the elements rubidium (in chloride form, RbCl) and, to a lesser extent, cesium (CsCl) have been used in foliar treatments of field crops to mark natural populations of target pests. Field studies of adult dispersal using elemental marking have been conducted on seven field crop pest species including black-faced leafhopper, *Graminella nigrifrons* (Forbes) (Alverson *et al.* 1980), boll weevil, *Anthonomus grandis* Boheman (Wolfenbarger *et al.* 1982), tarnished plant bug, *Lygus lineolaris* (P. deB.) (Fleischer *et al.* 1988), and five lepidopteran species including imported cabbageworm, *Pieris rapae* (L.) (Stimmann 1974), pink bollworm, *Pectinophora gossypiella* (Saunders) (Van Steenwyk *et al.* 1978b), cotton bollworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Graham *et al.* 1978).

It is not an accident that elemental marking methods were developed (Berry *et al.* 1972) and most frequently implemented for the study of lepidopterous agricultural pests (Table 1). Beyond the general problems solved by a nondisruptive marking method (i.e., no physical contact with the organism is needed), use of trace

TABLE 1. Demonstrated Feasibility of Elemental Marking in Field Crop Pests.

Species Order: Family	Stage[a]	Element	Crop	Condition[b]	Sources[c]
Coleoptera: Coccinellidae					
Mexican bean beetle	a	Rb	snap bean	g	1, 2
Coleoptera: Curculionidae					
Boll weevil	1/a	Rb	cotton	g/f	3
Heteroptera: Miridae					
Tarnished plant bug	a	Rb	mustard, cotton	g/f	4, 5
Homoptera: Aphididae					
Aphids	a	Rb	- - - -	g	6
Homoptera: Cicadellidae					
Black-faced leafhopper	a	Rb	barley	c/f	7
Homoptera: Delphacidae					
Plant hoppers	a	Rb	rice	l/f	8
Lepidoptera: Gelechiidae					
Pink bollworm	a	Rb/Cs/Sr	cotton	l/g/c/f	9, 10, 11, 12
Lepidoptera: Noctuidae					
Tobacco budworm	e/sp/l/a	Rb/Cs/Sr	cotton pigeon pea	l/g/c/f	13, 14, 15, 16
Cotton bollworm	e/l/a	Rb/Cs/Sr	cotton, corn sorghum	l/g/c/f	17, 18, 14
Beet armyworm	ec/a	Rb	cotton, alfalfa	l/g/f	19
Fall armyworm	l/a	Rb	corn	f	18
Cabbage looper	e/a	Rb/Cs	cotton	l/g/f	20, 21
Lepidoptera: Pieridae					
Imported cabbageworm	e/a	Rb	collard	f	22
Alfalfa caterpillar	e/a	Rb	vetch	l/g	23
Lepidoptera: Pyralidae					
European corn borer	ec/a	Rb	corn	l/g	24

TABLE 1 (Continued)

[a] Stage: e=egg, ec=egg cluster, sp=spermatophore, l=larva, a=adult.
[b] Condition: l=laboratory, g=greenhouse, c=field cage, f=field.
[c] Sources: numbers refer to Literature Cited:

1) Shepard and Waddill (1976), 2) Wolfenbarger et al. (1982), 3) Mitchell et al. (1982), 4) Fleischer et al. (1986), 5) Fleischer et al. (1988), 6) Frazer and Raworth (1974) 7) Alverson et al. (1980), 8) Padgham and Cook (1984), 9) Akey (1988), 10) Moss and Van Steenwyk (1982), 11) Van Steenwyk et al. (1978a), 12) Van Steenwyk et al. (1978b), 13) Graham and Wolfenbarger (1977), 14) Hayes and Hopper (1987), 15) Hayes (1989), 16) Hayes and Reed (1989), 17) Culin and Alverson (1986), 18) Graham et al. (1978), 19) Pearson et al. (1989), 20) Moss and Van Steenwyk (1984), 21) Stimmann et al. (1973), 22) Stimmann (1974). 23) Hayes and Claussen (1988), 24) Legg and Chiang (1984).

elements has the potential to solve marking problems specifically associated with the surveillance of these highly mobile insects. In addition to their mobility, lepidopterous pests are often polyphagous and multivoltine. As a result, densities vary greatly in time and space, making species such as *Heliothis virescens* (F.) and *H. zea* extremely difficult to sample.

Low capture rate is a chronic problem of mark-release-recapture (MRR) studies and is exacerbated by various aspects of lepidopteran biology, such as the fact that many target species are nocturnally active. The distance a moth or butterfly can fly, patchy distribution, and relatively low population density of adults make the operational ability to conduct thorough studies prohibitive. Although elemental marking has been used in field situations for dispersal testing, the number of these studies is still surprisingly small. The degree of success experienced with the technique has and will be limited by recapture capability. Recovery of marked insects by hand capturing (*e.g.*, with nets) is inefficient, and while recovery by trapping is more efficient, it is often biased by trap design and positioning or is age or sex-limited.

The most cost-effective solution to this problem is to select the life stage with the least mobility and greatest availability. For Lepidoptera, which typically exhibit a type III survivorship curve (*i.e.*, produce large numbers of offspring and suffer high juvenile mortality [Pianka 1974]), the best life stage to target for an MRR study is the egg stage. The immediate advantage is that measurement of dispersal of fertilized eggs provides a direct measure of gene flow, which can only be inferred from adult dispersal studies.

A MRR study that targeted marked egg recovery was initially proposed and used by Jones *et al.* (1980) in an elegant study of long-distance movement of the imported cabbageworm. In their study, adult insects reared in the lab on an artificial medium containing a dietary dye were released, and the colored eggs these insects produced were recovered. The obvious limitations of this study are the possible problems associated with the use of laboratory-reared insects. This complaint can be eliminated given the findings of Legg and Chiang (1984) and others (see Table 1); elemental marking of lepidopteran adults would produce marked eggs and make possible egg-labeling with native populations.

Preliminary field studies of egg-labeling of lepidopterous species with elemental markers have been successful (*e.g.*, Hayes and Hopper 1987), and other studies indicate the method can be used widely among phytophagous insects for a variety of purposes (*e.g.*, Jackson *et al.* 1988). The method has been refined and tested most thoroughly by Pearson *et al.* (1989) to examine short-range movement of beet armyworm and Hayes and co-workers (Hayes 1989, Hayes and Claussen 1988, Hayes and Hopper 1987, Hayes and Reed 1989) to study meso-scale movement of *H. virescens*, *H. zea*, and *Colias* spp.

In this paper, I will describe some of the results of the experiments that were required to address preliminary questions, and I will present results of our ongoing study of movement of *H. virescens* using elemental marking and recovery of eggs. Additionally, the problematic aspects of this method, primarily mark variability, will be discussed. In our study of *H. virescens* and *H. zea*, our goal has been to determine the scale needed for an area-wide suppression program. In particular, we wanted to determine how far the average female distributes her eggs in a generation. Our specific questions included, among other things, the following:

Mark: Can the insect and its offspring be reliably marked without deleterious effects? And, can this be accomplished in the field?

Release: Can the emergence timing and abundance of marked moths be accurately monitored under field conditions?

Recapture: How large must the recovery area be? And, what is an appropriate sampling strategy?

Problematic aspects: What impact does variability have on mark detection and interpretation?

MARKING AND DETECTION

Can The Insect And Its Offspring Be Reliably Marked Without Deleterious Effects? Our first step was to establish the ability to reliably label *H. virescens* or *H. zea* eggs via treated diet. *Heliothis virescens* were reared on artificial diet treated with four elements (Rb, Cs, Sr, and Dy) alone or in combinations of two or three (Hayes 1989). We were able to detect Rb with 100% reliability and Cs with *ca.* 70% in individual eggs regardless of the element combination. Indigenous levels of Sr overlapped with quantities found in treated adults and eggs, and Dy was not detectable using our methods. Rb- and Cs-treated adults were 100% reliably marked, and spermatophores produced by Rb-treated males were >80% reliably labeled. With the exception of the triply labeled group, no loss of viability was detected at the treatment quantities used (1000 ppm Rb and Sr; 2000 ppm Cs and Dy). Differences were found in element content of eggs produced over a 7-day period; detectability of Rb was not affected, but Cs detection declined by more than 10% on some days. There was high egg-to-egg variability among eggs from any one day.

Can Marking Be Accomplished In The Field? In the next step, laboratory-reared *H. virescens* and *H. zea* adults were released in cages placed over host plants treated with Rb, Sr, or Cs (Hayes and Hopper 1987). Host plants included sorghum and corn for *H. zea*, sesame and pigeon pea for *H. virescens*, and cotton for both species. Host plants were treated twice, once at flowering and again as fruit were set, at a rate of 1 kg/0.4 ha for Rb and Sr, and 2 kg/0.4 ha for Cs. Eggs from adults reared on the caged, treated host plants were analyzed by atomic absorption spectrophotometer (AAS). Incorporation of Rb into the eggs of both species was achieved, however neither Sr nor Cs was found in greater than 50% of the samples. The presence of Rb was detectable in nearly 100% of the eggs of *H. zea* reared on treated corn, and detectable quantities of Rb were found in 95% of the eggs of *H. virescens* reared on treated pigeon pea. Failure to detect marks in eggs from cotton and sorghum was attributed to inaccurate timing of element applications relative to the fruiting stage of those hosts. Egg-to-egg variability was high, and the critical nature of the timing of host crop treatments became evident.

In proceeding to open field work, host plants were chosen that would support *H. virescens* and *H. zea* development during each generation. In succession, these crops were then treated twice, as described above, with foliar applications of Rb by high clearance or backpack sprayers.

RELEASE

Can The Emergence Timing And Abundance Of Marked Moths Be Accurately Monitored Under Field Conditions? "Release" in the marking of native populations via host plants is a misnomer, since physically releasing marked insects is unnecessary. Instead, marked insects will emerge from pupation as part of the natural population; thus, operationally, MRR (mark-release-recapture) is reduced to

MR (mark-recapture). However, it is critical that emergence time and mark abundance (*i.e.*, density) be measured for analysis of recovery data. In our work with *H. virescens* and *H. zea*, "release" was monitored by pheromone trap captures and with collections from field cages or emergence cones placed over treated host crops. Actual timing and rate of emergence, densities, and sex ratios were determined.

RECAPTURE

How Large Must The Recovery Area Be? Without prior knowledge of the movement capabilities of the target insect, sampling in series of concentric circles at regular intervals away from a release site, with equivalent sample density per circle, is ideal. However, under most circumstances this approach presents a number of practical problems. Principally, the number of samples necessary at the extreme edge of the recovery area becomes financially and physically prohibitive. One means of improving this situation is the use of multiple labels and simultaneous "releases" from the corners of a uniformly sampled recovery area, thereby quadrupling the information obtained from each recapture. A potential flaw in this design, however, is the necessary assumption that dispersal is nondirectional or random. (For more information about experimental design and analysis, see Hopper, this supplement).

We began our studies of *H. virescens* and *H. zea* movement, with a single reliable field marker (Rb), limited resources, and information that suggests these moths move directionally and on the order of kilometers (*e.g.* Sparks 1979). The initial experimental design involved a 16 x 16 km grid, with uniform sampling and a central "release" point and included plans to realign or expand the area if necessary. Thus, the recovery area would literally be defined by the insects themselves and by operational feasibility.

What Is An Appropriate Sampling Strategy? In our work, the area was divided into 1.6 km^2 block sampling units, and in each block a single cotton field was selected to be routinely sampled (Fig. 1). Over 70 of the 100 possible sites were sampled, reflecting the fact that *ca.* 65% of the arable land in the recovery area is planted in cotton each year; other crops include soybean, sorghum, rice and corn. A sample consisted of 30 minutes spent by two samplers collecting all *H. virescens* and *H. zea* eggs laid on cotton (or velvet leaf) that could be found. The recovery area was searched twice per week. Eggs were returned to the laboratory, a portion was reared for species identification, and the remainder was prepared for AAS analysis.

In 1987, we treated host plants during each of three field generations (as determined by area-wide pheromone trap captures). However, it was only with the first generation that we were able to synchronize our treatment with both insect and crop phenology. Additionally, our sampling effort was not consistent, given precipitation, irrigation, and spray schedule complications. In three field generations, 6932 eggs were analyzed and a total of 378 marked eggs were recovered 1 or more km from the treatment area (Fig. 1). The inordinately high number of marked eggs recovered during the third generation may have resulted from adult moths feeding on the extra-floral nectaries of the Rb-treated cotton in the treatment plot; the problem of nontarget marking is discussed below. At least during the first generation, movement was coincident with wind direction. Female flight distances of 8 km were common.

In 1988, the recovery area was expanded to include a 16 x 19 km area, and sampling was carried out more consistently; however, each site was sampled by one sampler rather than two as in 1987. After planting and treating the early season *H. virescens* host, we were deterred from marking subsequent generations by prevailing

drought conditions. Instead we tested and used Rb-treated artificial nectar feeding stations to mark adults and their offspring (Hayes and Reed 1989; see below). From a 41-day collection period, 7326 eggs were analyzed and a total of 154 eggs were considered marked, with 18 of the marks deemed produced by spiked nectar treatment because of the extremely high Rb content (Fig. 2). Once again, female

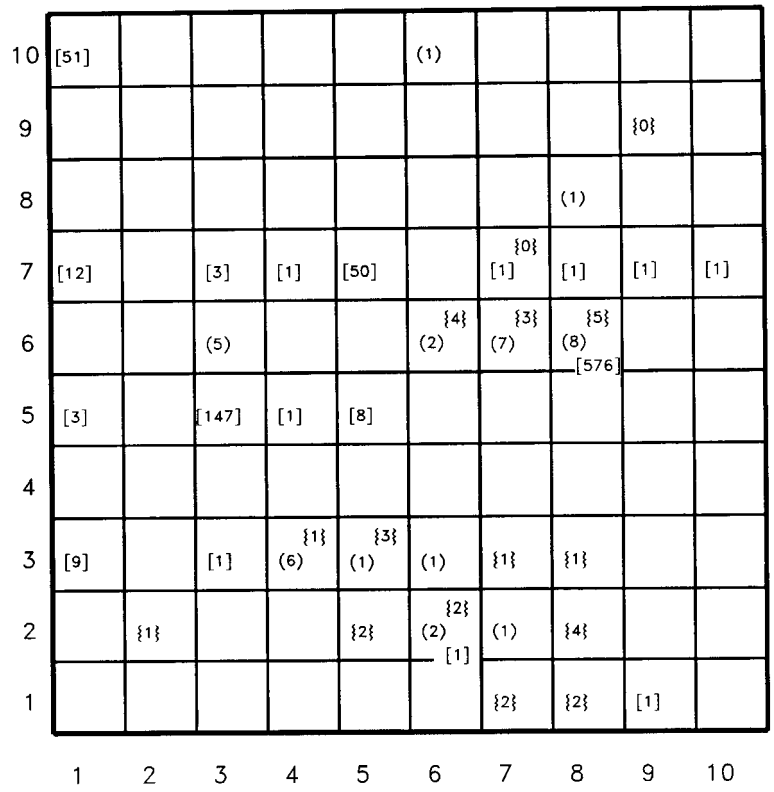

FIG. 1. Schematic grid map of 1987 MRR recovery area (sample site = 1.61 km^2). Recovery, per sample site, of *H. virescens* and *H. zea* eggs containing detectable levels of the trace element marker rubidium (Rb) is indicated by generation: {#} indicates number of individual marked eggs recovered from DOY (day of year) 152 to 170 (gen, 1), (#) represents number of individual marked eggs recovered from DOY 174 to 212 (gen. 2), [#] represents number of individual marked eggs recovered from DOY 215 to 266 (gen, 3). All marks originate from sample site 8,6 (x,y).

movement of 8 km was common, and distances of 19 km have been recorded. For this reason, in 1989 we expanded our recovery area to 24 km in at least one direction (north-south).

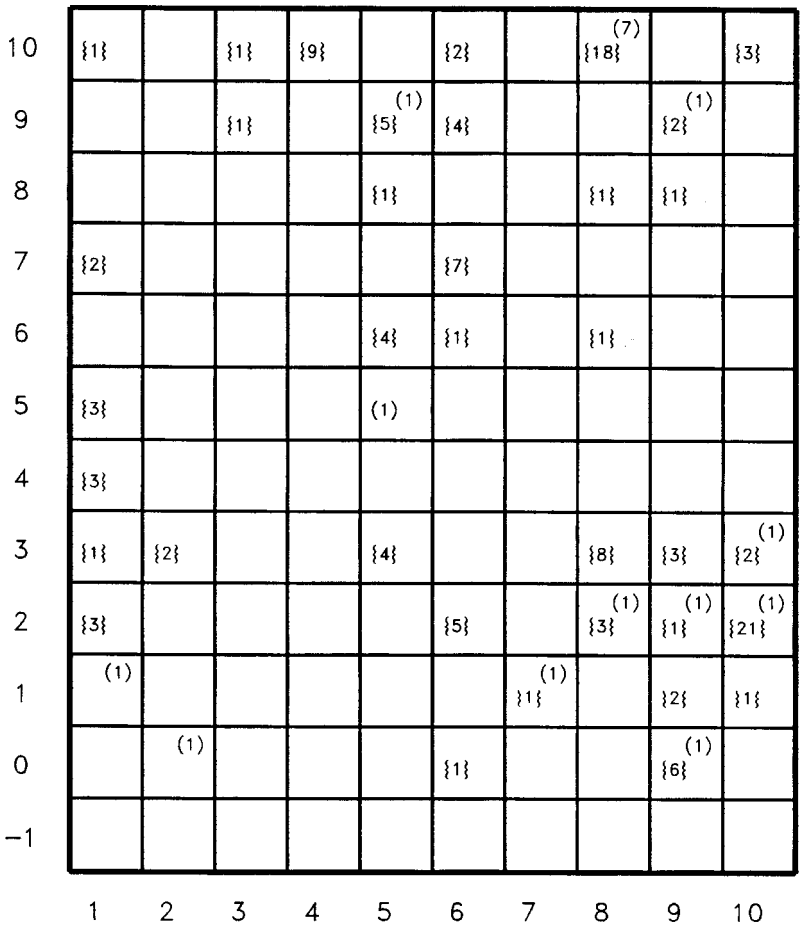

FIG. 2. Schematic grid map of 1988 MRR recovery area (sample site = 1.61 km^2). Recovery, per sample site from DOY 148-189, of *H. virescens* and *H. zea* eggs containing detectable levels of the trace element marker rubidium (Rb) are indicated: {#} represents number of individual marked eggs originating from sample site 8,6 (x,y) and (#) represents number of individual marked eggs originating from sample site 8,10.

PROBLEMATIC ASPECTS

One of the most troublesome aspects of MRR using elemental marking is dealing with mark variability. Trace elements can be used to mark lepidopteran eggs

via labeled adults, but sources and significance of sample variability are relatively unexplored and are a major concern.

What Impact Does Variability Have On Mark Detection And Interpretation? We explored possible sources and effects of egg-to-egg variability in the alfalfa caterpillar, *Colias eurytheme* Boisduval (Hayes and Claussen 1988). *Colias eurytheme* was used because it is easily managed in the laboratory and because it shares essential features with *H. virescens* and *H. zea*; in particular, it lays its eggs singly. Female *C. eurytheme*, reared on Rb-treated food plants, and their eggs were analyzed for Rb content by AAS. Parent (three untreated and four treated females) and mean egg (25/female) element content were significantly correlated. Compared with untreated adults and eggs, treated samples were reliably marked, although significant egg-to-egg variability in Rb concentration was found within and between sib-groups. Differences among days of analysis were not significant. While adult sizes and element exposures may have contributed to between-group variance, maternal and genetic influences are potential sources of within-sib-group variance.

In another set of experiments, *H. virescens* were provided untreated or Rb-treated artificial nectar (2000 ppm RbCl in 10% sugar-water) for a 24-h period upon eclosion or 48-h post-eclosion (Hayes and Reed 1989). Eggs of treated females were collected every 48 h and analyzed by AAS for element content. Over 90% of the eggs from both treatment conditions contained reliably detectable levels of the maternal label, and Rb-treated female adults provided 100% reliable detection. The Rb level in the eggs from both nectar treatments was significantly higher and the egg-to-egg variability was lower than that found in eggs from adults reared on Rb-treated diet or host plants. Differences were found in element content of eggs produced over a 10-day period; however, detectability was not negatively affected.

We did, in effect, take advantage of mark variation the 1988 season by making use of the ability to discern a quantitative difference in eggs marked from two sources (host plant versus feeding station). But, it is obvious that this can be a confounding problem when the quantitative differences are not clear-cut.

In their study of movement in beet armyworm, *Spodoptera exigua* (Hübner), Pearson *et al.* (1989) also addressed problems of mark variation. The trace element Rb was used to mark adults and eggs. Marked eggs were produced in four ways: by moths reared as larvae on Rb-treated alfalfa and cotton, by moths reared as larvae on an artificial diet containing Rb, by females mated with Rb-treated males, and by adult females exposed to plants sprayed with RbCl. Increasing Rb levels were found in eggs produced by previously unmarked female moths after mating with Rb-marked males or after exposure to Rb-sprayed cotton. When Rb-marked females were fed a sucrose diet containing potassium in concentrations similar to that found in cotton nectar, decreasing levels of Rb were found in eggs produced. Potassium is the normal metabolite replaced by rubidium when insects consume treated host plants.

CONCLUSION

The use of elemental marking in studies of arthropod pests of agricultural systems is gaining acceptance. Because elemental markers are transferred to reproductive products (*i.e.*, eggs and spermatophores), a wide array of uses can be envisioned for multigenerational marking. The premise and basic methodology have been successfully demonstrated, but some problems remain as indicated in the last section. Refinement of marking and detection techniques and field recovery protocols will be necessary on a case-by-case basis. Advances in analytical

instrumentation and field sampling methods will enhance the use of single and multigenerational marking. In combination with multiple trophic level marking, multigenerational marking establishes the potential usefulness of elemental marking in much needed integrated studies at the systems level.

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