University of Kentucky

TITLE: Damage Thresholds, Risk Assessment, and Environmentally Compatible Management Tactics for White Grub Pests of Turfgrass

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PROGRESS REPORT
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DAMAGE THRESHOLDS, RISK ASSESSMENT, AND ENVIRONMENTALLY COMPATIBLE MANAGEMENT TACTICS FOR WHITE GRUB PESTS OF TURFGRASS

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Executive Summary:

The objectives of this project are to: 1) establish damage thresholds for root-feeding white grubs on cool-season turfgrasses, 2) evaluate the compatibility of turfgrass pesticides with beneficial invertebrates, 3) field test a pheromone-based system for predicting white grub densities, and 4) evaluate the potential for reducing white grub populations through non-chemical, cultural manipulations.

The impact of varying densities of Japanese beetle (JB) or masked chafer (MC) grubs on root and foliar growth and aesthetic quality of six different turfgrasses was measured in field tests using sunken enclosures and rooting boxes. Grub feeding preferences and tolerance of turf under differing management regimes were also evaluated in field and greenhouse tests. MC grubs are more damaging than JB grubs at equal densities, however, our results show that healthy turf can tolerate at least 20 MC grubs or 30 JB grubs/ft^2 before showing visible damage. Kentucky bluegrass is relatively susceptible, and tall fescue is relatively tolerant of grub damage. The tall fescue endophyte does not confer resistance to grubs of either species. Fall irrigation increased rooting strength and hastened recovery of turf from grub damage. Spring fertilization did not affect expression of grub damage the following fall. JB grubs showed significant preference for perennial ryegrass, whereas MC grubs showed no preference among grasses. Presence of one grub species did not affect the distribution of the other. These studies indicate that damage thresholds for white grubs are higher than previously thought, and that remedial irrigation should mask the injury from all but very severe infestations.

Impact of pesticides and growth regulators on earthworm populations was evaluated in two field tests conducted in spring and fall 1992. Of more than 40 products tested so far, only two fungicides (benomyl, thiophanate-methyl) and five insecticides (bendiocarb, carbaryl, ethoprop, diazinon, and fonofos) had significant impact on earthworms. This shows that most of the pesticides and related products used on golf courses are compatible with these beneficial elements of the soil fauna. Studies were initiated in 1992 to compare the abundance and diversity of predatory insects and spiders in meadows, lawns, and golf course roughs. Preliminary sorting of samples suggests that golf courses support populations of predators at levels similar to those found in lawns and meadows. Feeding studies confirmed that many of the more abundant predators readily consume eggs and larvae of turfgrass pests.

Efforts to identify the sex pheromone of masked chafers were bolstered by initiation of collaboration with Dr. J. Meinwald, one of the preeminent natural products chemists in the world. While collecting virgin females for analysis, we observed and then confirmed experimentally that the adult male beetles are attracted to both sexes of grubs. Presence of a chemical attractant in grubs was confirmed experimentally. This is the first report of attraction of adults to the larval stage for any insect species. This finding has considerable basic significance because it sheds insight on how sex pheromone communication systems may evolve. In practical terms, it extends the period during which we can collect and extract crude pheromone for chemical analysis. Identification of the attractant will increase the practicality of using traps to assess the risk of grub damage to particular sites.

Soil pH, fertilization, watering, soil compaction, and mowing height were manipulated in large field plots to determine how they would affect choice of egg-laying sites and subsequent densities of grubs. Even in this wet year, female JB and MC beetles were attracted to irrigated turf, resulting in much higher grub densities. We found about an 80% reduction in MC grubs in plots treated with aluminum sulfate, and about a 50% reduction in high-mown turf. Fertilization neither increased nor decreased grubs. Soil compaction did not affect subsequent grub densities, and use of a heavy (5000 lb) roller to crush the active grubs was not effective.

PROGRESS REPORT: 1992

OVERALL GOALS:

The goal of this project is to provide a better understanding of the ecology of root-feeding white grubs and to contribute to development of ecologically sound methods for their management on golf courses and other turfgrass sites. Although white grubs are the most important insect pests of cool-season turfgrasses, many aspects of their biology remain poorly known. We know relatively little about the relative resistance or tolerance of different grass species, the relationship between grub density and turf damage, or how management tactics affect the expression of injury. This information is important in developing damage thresholds or other guidelines for use in pest management decisions. Better methods of predicting densities of grubs are needed so as to reduce the need for preventative, and often wasteful pesticide applications. We seek understanding of the factors that affect the distribution and abundance of grubs on gold courses and other sites because this may reveal ways that the turf system can be manipulated to minimize insect outbreaks. Finally, we seek to understand the role of beneficial organisms within the turf habitat and to identify pesticides and other pest control tactics that provide the best compromise between efficacy and minimal negative impact on the environment.

OBJECTIVE 1: Objective 1 is to quantify relationships between grub density, root damage, foliar growth, and aesthetic quality of different cool-season turfgrasses to establish damage thresholds for making management decisions. We are also studying how management practices such as watering and fertilization affect expression of grub injury on different grasses.

Methods: Galvanized steel enclosures (1 ft^2) were implanted into replicated field plots of Kentucky bluegrass, perennial ryegrass, creeping bentgrass, hard fescue, and endophyte-infected and endophyte-free tall fescue. Japanese beetle (JB) or masked chafer (MC) grubs were introduced at densities ranging from 0 to 60/ft^2. Aesthetic quality ratings, clipping yields, root weights, and grub survival were measured. Wooden rooting boxes established in Kentucky bluegrass or tall fescue were managed under different fertilizer and irrigation regimes and then infested with grubs to study interactions between management, feeding damage, management, and root strength. Grub feeding preferences and tolerance of different grasses were also evaluated in greenhouse tests.

Results: MC grubs are more damaging than JB grubs at equal densities. However, our data show that healthy turf can tolerate at least 20 MC grubs or 30 JB grubs/ft^2 before showing significant damage. Kentucky bluegrass and hard fescue are relatively susceptible, and tall fescue is relatively tolerant of grub injury. The tall fescue endophyte does not confer resistance to grubs of either species. Fall irrigation increased rooting strength and hastened recovery of turf from grub damage. Spring fertilization did not affect expression of grub damage the following fall. JB grubs showed significant preference for perennial ryegrass, whereas MC grubs showed no preference among grasses. Presence of one grub species did not affect the distribution of the other.

Significance: These studies indicate that damage thresholds for white grubs are higher than previously thought. Treatment of infestations $<$ 10-15 grubs/ft^2 would generally not be justified unless the turf is additionally stressed. Remedial irrigation in August and September should mask the injury from all but very severe infestations.

OBJECTIVE 2: Objective 2 is to evaluate the relative compatibility of turfgrass pesticides with beneficial invertebrates, including earthworms, predators, and parasites, and to investigate the importance of these beneficials in maintaining the stability of the turfgrass system. Earthworms are abundant and beneficial components of turfgrass soils, where their activity alleviates soil compaction, improves aeration and water infiltration, and breaks down thatch. Predatory invertebrates are also abundant, and preserving their populations where possible may be important in buffering the turf against pest outbreaks.

Methods: Impact of pesticides and growth regulators on earthworm populations was evaluated in two field tests conducted in spring and fall 1992. Replicated plots (7 x 10 ft) were treated at label rates in April or September, with post treatment irrigation. Formaldehyde drenches were used to sample the earthworms after 1 and 3 weeks. The samples were returned to the laboratory, where they were identified, counted, and weighed.

Studies were initiated in 1992 to compare the abundance and diversity of predatory insects and spiders in meadows, lawns, and golf course roughs. Pitfall traps were operated from April to October at four sites of each type. Samples were emptied weekly, and sorting and identification are underway. The more abundant species of predators were tested in the lab for their capacity to attack and eat fall armyworms, white grubs, and other turf pests. Final sorting and analyses of data were completed for our 1991 field test involving impact of insecticides on predators and predation. A manuscript was submitted to the Journal of Economic Entomology.

Results: Of more than 40 products tested so far, only two fungicides (benomyl, thiophanate-methyl) and five insecticides (bendiocarb, carbaryl, ethoprop, diazinon and fonofos) have shown severe impact on earthworms (Table 1). Recovery of worm populations to normal levels required at least 20 weeks in moderate-sized plots (30 x 30 ft) treated the most severe products, and might take much longer in larger treated areas such as fairways.

Preliminary sorting of the weekly samples suggests that golf roughs support high populations of predators such as ants, rove beetles, and ground beetles, and that their levels are similar to those found in lawns and meadows. Feeding studies confirmed that many of the more abundant predators will readily consume eggs and larvae of turfgrass pests.

Results of the 1991 field test showed that isazofos (Triumph) caused the greatest short-term reduction in predators, with effects of carbaryl (Sevinmol) and cyfluthrin (Tempo) somewhat less severe. Fall armyworm pupae and JB eggs implanted into plots sustained losses to predation as high as 60% and 74%, respectively, within 48 h. Predation on pupae was not affected by insecticides, but predation on JB eggs was reduced by as much as 70% in plots treated with either isazofos or carbaryl. Plots treated with isazofos in June had significantly higher grub populations in August, evidently because of interference with egg predation. The manuscript covering this work was accepted for publication with highly favorable reviews (see Appendix).

Significance: Most of the pesticides and related products used on golf courses are compatible with preservation of earthworms and other beneficial soil invertebrates. Effective alternatives for those products having severe impact have been identified for use in situations where preservation of earthworms is a concern. Golf courses support abundant and diverse populations of predatory invertebrates which help to regulate pest populations. Predators and parasites are impacted to varying degrees by insecticide treatments. Awareness of these potential interactions can serve as an incentive for professional turf managers to use insecticides more selectively. However, other projects being conducted in our laboratory show that biological alternatives such as milky disease and entomopathogenic nematodes are generally not sufficiently effective or reliable to substitute for conventional insecticides at the present time. At present, careful and selective use of insecticides and other pesticides is often the only way to avoid significant damage from heavy or unexpected pest outbreaks in fine turf.

Objective 3: Objective 3 is to develop and field test a pheromone-based system for predicting densities of root-feeding white grubs on golf courses and home lawns. Such a system would allow turfgrass managers to target for treatment only those sites that are at high risk for grub injury, while reducing costs and overall insecticide use.

Current Status: Our basic hypothesis for this objective is that the numbers of male masked chafer beetles captured in pheromone-baited sticky traps during adult mating flights can provide an estimate of population density, and will be correlated with subsequent numbers of grubs at particular sites. Results of our initial field test of this concept on a golf course were discussed in last year's report. While the traps themselves were quite effective at catching males, we were unable to predict local grub densities based on pheromone trap captures. The experiment was repeated using 30 home lawn sites. In this test we did obtain a significant, albeit relatively weak correlation. A manuscript describing these trials has been submitted for publication in Journal of Entomological Science.

Both the golf course and home lawn tests were limited by availability of pheromone bait, which presently must be extracted from field-collected, virgin female beetles. Thus, in the aforementioned tests we were only able to run the traps for two nights at each site. Since the flight period lasts for about 4 weeks, this provides only a "snapshot" of the overall activity at a site. Identification of the pheromone would allow large quantities of synthetic bait to be produced at relatively low cost. The pheromone could be formulated into dispensers which discharge it slowly over a period of several weeks, making extended trapping more practical. Efforts to identify the sex pheromone of masked chafers were bolstered by initiation of collaboration with Dr. J. Meinwald, one of the preeminent natural products chemists in the world (see Appendix).

While collecting virgin female beetles on a golf course, we observed adult male beetles to be attracted to some grubs that had evidently been dug up by a skunk or other animal. These grubs would not normally have been present during the adult flight period, except that their development had been delayed by milky disease. Because of the unusual nature of this phenomenon, we conducted experiments to investigate it more fully:

Methods: Healthy masked chafer grubs were collected in May, separated by sex, and held in soil in a cooler to delay their development until adult flights had begun. Traps were baited with freshly caught virgin females, male or female grubs, or extracts prepared by soaking grubs in solvent, and set out on golf fairways during beetle flights.

Results: As expected, virgin females were highly attractive to male beetles. However, males were equally attracted to both sexes of living grubs and to their extracts. Adult males were not attractive to other males. This confirms that the chemical sex attractant is present in both male and female larvae, and then lost during final development of adult males.

Significance: Remarkably, this is the first report of attraction of adults of any insect species to its own larval, or immature stage. This finding has considerable basic significance because it sheds insight on how sex pheromone communication systems may evolve. It has always been assumed that sex pheromones are "gained" in the ontogeny of the female, rather than "lost" by males. A manuscript on this work (reprint enclosed) was recently published in Journal of Chemical Ecology and is generating considerable interest among scientists. It is notable that after hundreds of other studies on pheromone communication, such a revelation should come from experiments conducted with a turfgrass pest on a golf course.

In practical terms, this finding extends the period during which we can collect and extract crude pheromone for chemical analysis. Identification of the attractant will increase the practicality of using traps to assess the risk of grub damage to particular sites.

Objective 4: The objective of this part of the project is to evaluate the potential for reducing white grub populations through non-chemical, cultural manipulations. This two-year field study, which was initiated in 1992, may also provide insight on the reasons why outbreaks of white grubs on golf courses occur when and where they do.

Methods: Soil pH, fertilization, watering, soil compaction, and mowing height were manipulated in large (1000 ft^2) field plots to determine how they would affect choice of egg-laying sites and subsequent densities of grubs. Soil pH was elevated or reduced with lime or aluminum sulfate, respectively. Irrigated plots were watered daily throughout the beetles' 6 week flight and egg-laying period. High-mown plots were left unmown during this time. Soil compaction was induced in selected plots by rolling the turf four times with a heavy (5000 lb) roller. Other plots were rolled in September, after the grubs had hatched and had reached nearly full size, to determine if remedial rolling would provide control by crushing the larvae.

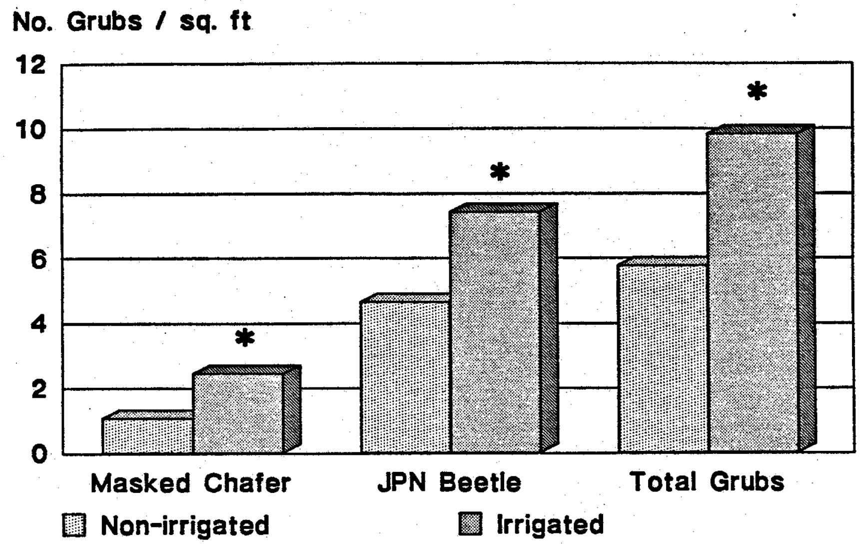
Results: Even in this wet year, female JB and MC beetles were attracted to irrigated turf, resulting in grub densities nearly twice as high in irrigated plots (Fig. 1). This response appears to be related to soil moisture thresholds required for egg survival and development. We found about an 80% reduction in MC grubs in plots treated with aluminum sulfate, and about a 50% reduction in high-mown turf (Fig. 2). JB grubs were also somewhat less abundant in sulfur-treated plots and high-mown turf, although this species seems less sensitive to these manipulations than is the MC. Development of both species of grubs was also delayed in the sulfur-treated and high-mown turf. Fertilization neither increased nor decreased grub density. Soil compaction before the flight period did not affect subsequent grub densities, and use of the heavy roller to crush the active grubs was not effective (Fig. 1).

Significance: This is apparently the first study to manipulate cultural factors to determine how this affects the abundance and distribution of white grubs. Both species of beetles preferred irrigated turf for egg-laying. This may explain the typical preponderance of grubs in tee banks, green banks, and fairways of golf courses that receive regular watering. We expect that this response would be even greater in drought years. Masked chafer grubs appear to be intolerant of acid soils, and the beetles also avoid laying eggs in high-mown turf. Japanese beetles show a less pronounced, but similar trend. The finding that fertilization did not affect grub densities is significant because it has been shown that certain other insect pests prefer fertilized host plants. Heavy rolling has been shown to be effective for controlling (crushing) grubs in pastures in New Zealand. This is apparently the first test of this concept in the U.S.

Table 1. Relative toxicity of turfgrass pesticides and related products to earthworms. All ratings are based upon results of two or more field trials in which the products were applied to Kentucky bluegrass turf at the highest labeled rate[1].

No Significant Toxicity	
Herbicides:	trichlopyr, dicamba, pendimethalin, prodiamine, dithiopyr, isoxaben
Fungicides:	triodimefon, fenarimol, propiconazole, chlorothalonil, iprodione, metaxayl, fosetyl-Al, cyproconazole, tebuconazole, myclobutanil
Insecticides:	isofenphos, cyfluthrin, fluvalinate, bifenthrin, azadirachtin, RH 5849 (an insect growth regulator), entomopathogenic nematodes *Steinernema carpocapsae* and *S. feltiae*.
Plant Growth Regulators:	mefluidide, flurprimidol
Moderate Toxicity (25-50% reduction)	
Insecticides:	trichlorfon, chlorpyrifos, isazofos,
Severe Toxicity (50-75% reduction)	
Fungicides:	benomyl, thiophanate-methyl
Insecticides:	diazinon
Very Severe Toxicity	
Insecticides:	fonofos, bendiocarb, ethoprop, carbaryl

[1]Based upon data from Potter et al. (J. Econ Entomol. 83: 2362-69) and from unpublished field tests conducted in 1992.



Heavy Roller Test Results

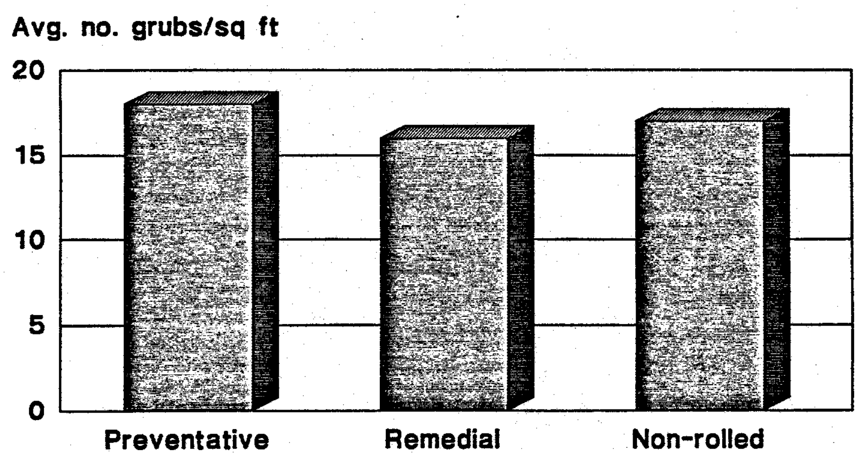


Fig. 1 Upper graph: Density of masked chafer and Japanese beetle grubs in non-irrigated plots vs. plots that were irrigated daily throughout the beetles' flight period. Asterisks denote significant differences ($P < 0.05$).

Lower graph: Densities of total grubs (masked chafer & Japanese beetle) in plots rolled prior to the beetles' flight period (preventative) or after grubs had reached the third instar (Remedial). There are no significant differences.

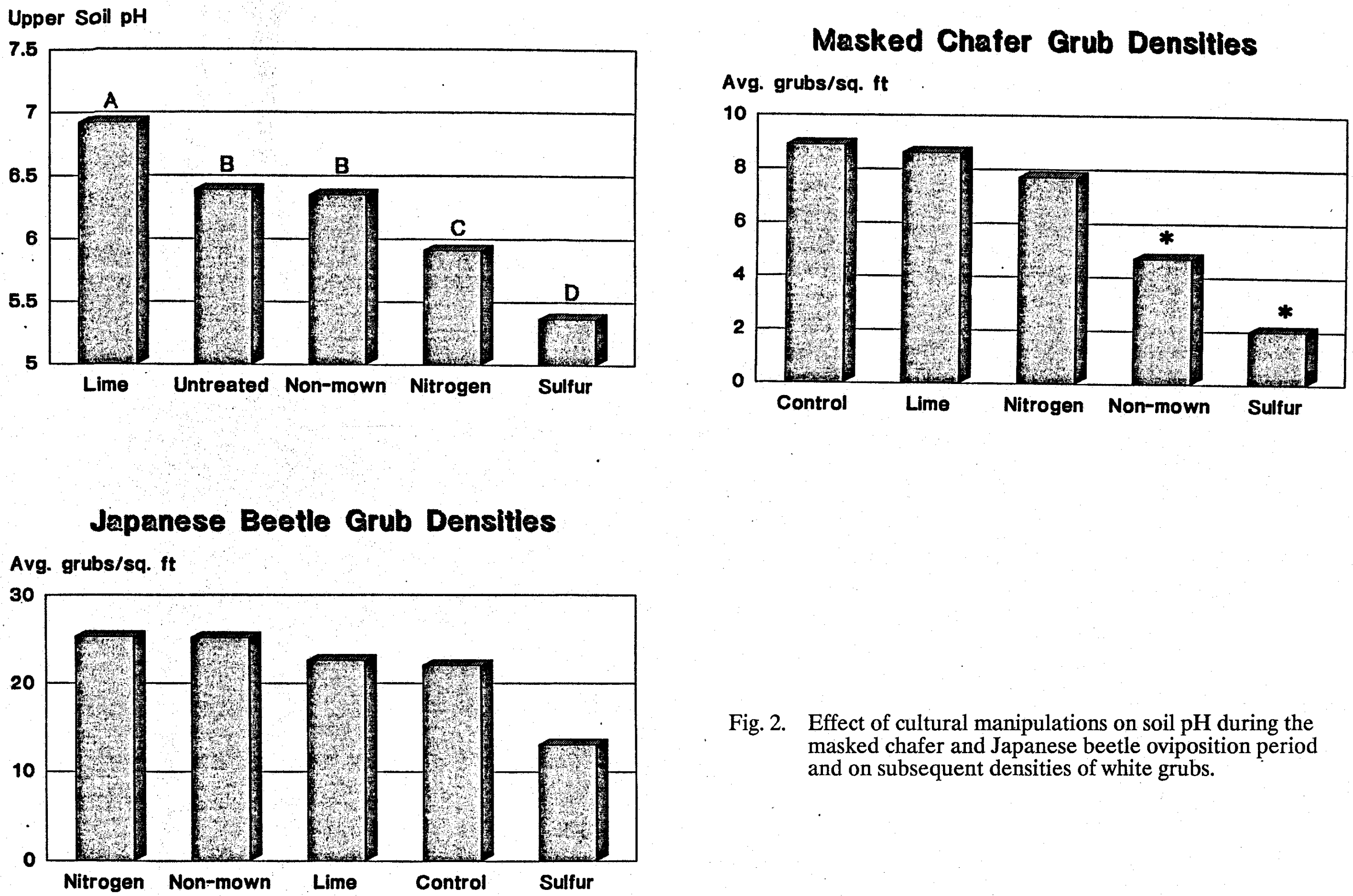


Fig. 2. Effect of cultural manipulations on soil pH during the masked chafer and Japanese beetle oviposition period and on subsequent densities of white grubs.

PUBLICATIONS

The following publications have resulted from work supported by this project (all papers include written acknowledgment of USGA support). Additional papers are in preparation.

A. Refereed Scientific Papers:

1. Potter, D.A. 1992. Pesticide and fertilizer effects on beneficial invertebrates and consequences for thatch degradation and pest outbreaks in turfgrass. Chapter 28, In: Fate and significance of pesticides in urban environments. K.D. Racke and A. Leslie (eds.). ACS Books, Washington.

2. Haynes, K.F., D.A. Potter,and J.T. Collins. 1992. Attraction of male beetles to grubs: evidence of evolution of a sex pheromone from larval odor. Journal of Chemical Ecology 18: 1117-1124.

3. Terry, L.A., D.A. Potter, C.G. Patterson, and P. G. Spicer. 1993. Insecticide impact on predatory arthropods and predation on Japanese beetle eggs and fall armyworm pupae in turfgrass. Journal of Economic Entomology. In Press.

4. Potter, D.A. 1993. Integrated insect management for turfgrasses: problems and prospects. International Turfgrass Society Journal. In Press. (this paper is the text of an invited Keynote Address to be presented at International Turfgrass Research Conference in 1993).

5. Potter, D.A. and K.F. Haynes. 1993. Field-testing pheromone traps for predicting masked chafer grub density in golf course turf and home lawns. Journal of Entomological Science. In Review.

B. Industry-Oriented Papers:

1. Potter, D.A. 1991. Earthworms, thatch, and pesticides. U.S. Golf Assoc. Green Section Record, Sept./Oct. issue

2. Potter, D.A. 1992. Natural enemies reduce pest populations in turf. U.S. Golf Assoc. Green Section Record. In Press

APPENDIX

Attached are copies of reviews of our most recent paper (Terry et al.....) indicating acceptance for publication, a of Dr. Meinwald's letter expressing interest in collaborating on pheromone identification.

Entomological Society of America

Reviewer No. 1

DATE: 6/1/92

MS. NO.: J92-157

AUTHOR(S): L.A. Terry, D.A. Potter, et. al.

RECOMMENDATION:
- ☐ Accept without change
- ☒ Accept with minor revision
- ☐ Accept with major revision
- ☐ Reject

TITLE: Insecticide Impact on Predatory Arthropods and Predation of Japanese Beetle ...

GENERAL AND SPECIFIC COMMENTS (Use additional pages if necessary):

✓ MS is well presented
only minor editorial suggestions

✓ Excellent research, good design
and outstand results that
should impact a large chemical
using industry

✓ Results immediately applicable

✓ Author should keep up the good
and timely contributions.

[redacted]

Please sign original only.

Return original and one copy.

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Entomological Society of America

Reviewer No. 2

DATE: 6/1/92

MS. NO.: J92-157

AUTHOR(S): L.A. Terry, D.A. Potter, et. al.

RECOMMENDATION:
- [X] Accept without change
- [ ] Accept with minor revision
- [ ] Accept with major revision
- [ ] Reject

TITLE: Insecticide Impact on Predatory Arthropods and Predation of Japanese Beetle ...

GENERAL AND SPECIFIC COMMENTS (Use additional pages if necessary):

- Well documented, and well written paper. Good work. The use of manuscript paper is appreciated.
- According to CIBA-GEIGY, the correct spelling is "isazofos", not "isazophos" as in your manuscript.

Please sign original only.

Return original and one copy.

Signature of Reviewer

Date:

Address:

Journal of Chemical Ecology, Vol. 18, No. 7, 1992

ATTRACTION OF MALE BEETLES TO GRUBS: EVIDENCE FOR EVOLUTION OF A SEX PHEROMONE FROM LARVAL ODOR

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(Received November 12, 1991; accepted February 28, 1992)

Abstract—Females of the scarabaeid beetle *Cyclocephala lurida* produce a volatile sex pheromone which attracts conspecific males. Field experiments demonstrated that larvae of both sexes also emit volatile chemicals that stimulate similar responses in adult males, including attempts by the attracted males to mate with the nonreproductive immature stage. Significantly more adult males were caught in traps baited with conspecific male or female larvae or adult females than in blank control traps. Hexane extracts of both male and female grubs were at least as effective as live larvae in trapping male adults, demonstrating that the behavioral responses are mediated by volatile chemicals. Sensory and behavioral responses of males to sex pheromones emitted by adult females are part of the functional communication system. However, their response to grubs is not functional, because grubs are normally temporally and spatially inaccessible to mate-seeking males. In theory, the evolution of a communication system is problematic because it requires the development of a signal in one sex and the sensory and behavioral attributes to respond to that signal in the other sex. The ontogeny of sex pheromone communication in *C. lurida* suggests a partial solution to this evolutionary problem. We propose that this sex pheromone communication system is probably derived from noncommunicative volatile chemicals that are lost in adult males and retained by adult females.

Key Words—*Cyclocephala lurida*, Coleoptera, Scarabaeidae, sex pheromone, chemical communication, evolution of signals.

*To whom correspondence should be addressed.

INTRODUCTION

Sex pheromone communication involves release of a chemical signal by individuals of one sex and specialized sensory receptors for, and behavioral responses to, that signal in members of the other sex (Payne et al., 1986). The evolution of chemical communication and systems relying on other sensory modalities is problematic because of the disparate requirements for effective communication in the signaler and receiver (Krebs and Dawkins, 1984; Otte, 1974; Ryan and Wilczynski, 1988; Smith, 1977). The initial evolution of chemical communication may be facilitated by modification of existing cues and/or biosynthetic pathways for signal production. For example, in the crab *Pachygrapsus crassipes*, the female's excretion of crustecdysone, a hormone that regulates molting, stimulates search and display mating behaviors in males and thus takes on a pheromonal function in this context (Kittredge and Takahashi, 1972). In scolytid bark beetles, biosynthetic modification of host-tree compounds is often involved in the production of species-specific aggregation pheromones (see references cited by Vanderwel and Oehlschlager, 1987). The ontogeny of the sex pheromone signal in the southern masked chafer, *Cyclocephala lurida* (Coleoptera: Scarabaeidae), suggests another, previously undescribed evolutionary route for the development of chemical communication, in which sex pheromones are derived from larval odors.

As is typical of other scarabaeid beetles and many other insects (Tamaki, 1985), adult females of *C. lurida* produce a sex pheromone which attracts conspecific males (Potter, 1980). Release of this pheromone is behaviorally controlled by the female beetle, which emerges from the soil beneath pasture or turfgrass shortly after sunset, in close daily synchrony with the emergence and flight of conspecific males. Unmated females remain on the ground or climb grass blades. Males fly close to the ground, zigzagging upwind toward females. Mating follows, often with several males attempting to copulate with a single female (Figure 1A). Male beetles will show a similar response to hexane or ether extracts of adult females, demonstrating the chemical nature of this communication system (Potter, 1980). Mated females quickly burrow into the soil, often before the male has disengaged. After the mating period, which lasts from dusk until about 11 PM (EST), the remaining beetles burrow into the soil. If unmated females are held aboveground in traps after this mating period, they are attractive to a closely related sympatric scarabaeid species, *C. borealis*, which normally mates after midnight (Potter, 1980). This provides evidence that effective pheromone emission is behaviorally controlled by emergence of the female beetle from the ground. There is one generation per year, mating flights generally occurring during late June and early July (Potter, 1981).

Initially we observed male beetles attempting to mate with conspecific grubs, which if developing normally would not be present when males are active.

FIG. 1. (A) Normal mating behavior of *Cyclocephala lurida*. Male beetles are attracted to a sex pheromone emitted by the female. (B) Males are attracted to and attempt to mate with the nonreproductive grub. Males will mount grubs and probe the grub with their aedeagus.

These grubs were naturally infected with milky disease [*Bacillus popilliae* (*Cyclocephala* strain)], a bacterial disease that delays their development. For an unknown reason a few infected individuals were found on the ground surface. In this report, we provide evidence that both healthy male and female larvae produce a volatile chemical cue that attracts adult male beetles and stimulates them to attempt to copulate with the nonreproductive immature stage. This response of males to the immature stage is clearly not an adaptive feature of sexual communication, because the root-feeding grubs are not ordinarily present during late June and July when males are active, nor are they normally above-

FIG. 1. Continued.

ground. However, the response suggests that sex communication in this species may have evolved by loss of the signal in males and specialization of the males' sensory system to detect the same signal from the adult female.

METHODS AND MATERIALS

Last-instar grubs were collected in May by digging up grass sod and examining the root systems for the feeding larvae. Grubs were stored in sod at 18°C to delay development. Grubs were separated by sex, based on well-defined sexual characteristics (Tashiro, 1987). Adult male and female beetles displaying characteristic precopulatory behavior were collected on a golf course fairway the night before an experiment. Adult females can be distinguished from males by their behavior, the structure of their tarsal claws, and other morphological differences (Potter, 1980; Tashiro, 1987). When an individual was collected, its sex was determined and it was placed with other members of the same sex in a paper carton with moistened paper towels. The beetles were stored until the next day at 8°C.

The following evening, traps were baited with male or female adults or male or female grubs and placed on golf course fairways or adjacent roughs. Experiments were conducted at the Lexington Country Club Golf Course, Lexington, KY, in late June 1991. We used standard metal Ellisco Japanese beetle traps placed on the ground and supported by wire stands. Traps were at least 5 m apart and were arranged in a randomized complete block with five replications. Three individuals were placed in the bait well of each insect-baited trap. One trap per replicate was left unbaited to serve as a control. The position of traps was rerandomized within each block at 15-min intervals. After the nightly flight period, traps were retrieved and beetles were counted. Data were analyzed using a two-way (treatments and experimental blocks) analysis of variance on log-transformed data [log $(x + 1)$]. Means of insect-baited traps were compared to blank traps using the LSD procedure for preplanned comparisons.

In the second experiment, grubs were collected at field sites in May and brought back to the laboratory to be extracted. The extraction procedure followed a protocol that has proved to be successful in extracting pheromone from adult females (Potter, 1980; Haynes and Potter, unpublished data). Seventeen male and 19 female grubs were rinsed with 5 ml of hexane in separate 50-ml flasks. Approximately 4 ml of the hexane was recovered, and the extract was then diluted to 3 grub equivalents/ml. At sunset, traps were baited with hexane extracts of male or female grubs by dispensing 1 ml of these solutions into a 3.5-cm-diameter aluminum dish. Other traps were baited with aluminum dishes containing three male or three female grubs. Blank traps contained an aluminum dish treated with 1 ml of hexane. Other procedures were identical to the first experiment.

RESULTS AND DISCUSSION

Both male and female grubs attracted large numbers of adult male beetles (Figure 1B). Traps baited with larvae of either sex caught significantly more male *C. lurida* than blank traps (Figure 2A). Adult female *C. lurida* also attracted conspecific male beetles as expected. In contrast, traps baited with adult males and blank control traps caught very few beetles. Thus, males become unattractive to other males as adults, while females retain their attractiveness to males.

Attraction of males to grubs is mediated by a hexane-extractable chemical or chemicals. Hexane extracts of male or female grubs attracted significantly more male *C. lurida* than hexane alone (blank traps) (Figure 2B). Male or female grub extracts were as attractive as live grubs.

Attraction of other adult arthropods to signals from conspecific immature stages is not without precedent (Thornhill and Alcock, 1983). However, in each of the previously documented examples this attraction represents an adaptive

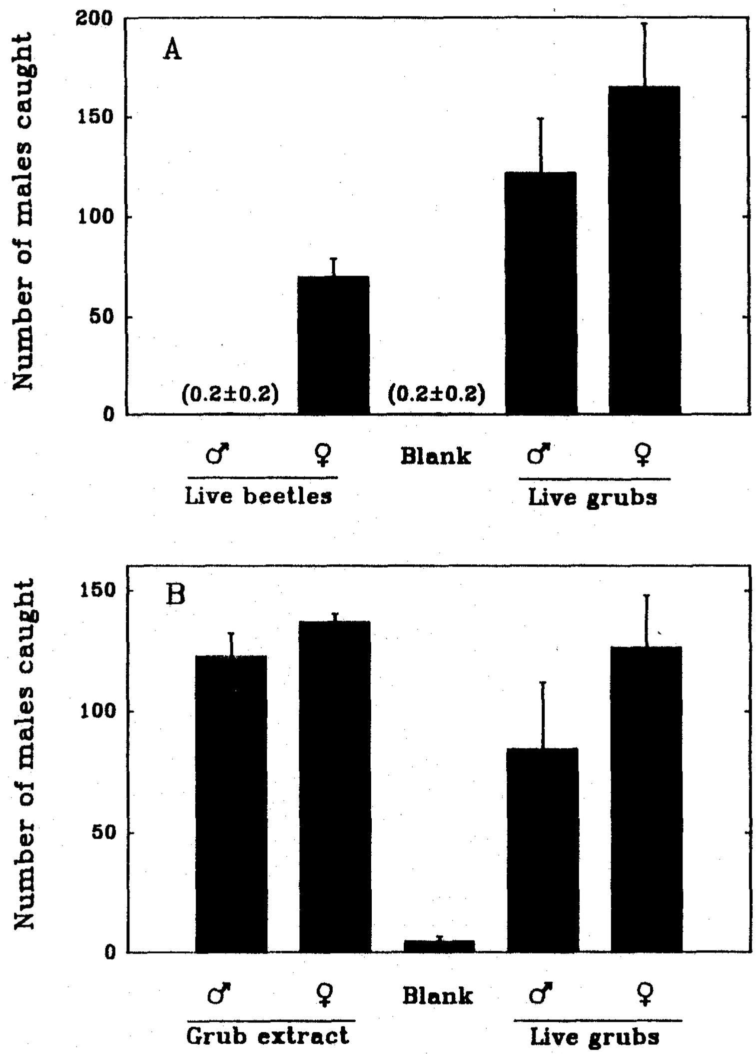

FIG. 2. (A) Attraction of adult male *Cyclocephala lurida* to live adults and grubs. Traps baited with adult females, male grubs, or female grubs caught significantly more males than blank control traps (LSD test after two-way analysis of variance; $P < 0.001$, df = 4,16, $F = 183.5$). Error bars show 1 SE. (B) Extracts of both male and female grubs caught significantly more adult male beetles than blank control traps (LSD test after two-way analysis of variance; $P < 0.001$, df = 4,16, $F = 31.5$).

feature of reproductive behavior with the immature individual close to maturation. For example, male spider mites are attracted to quiescent female deutonymphs, which they guard until emergence and mating (Potter et al., 1976). The pupal stage of some heliconiid butterflies is attractive to males, with mating usually occurring on the pupal case as the female emerges (Gilbert, 1976).

In normal circumstances grubs and reproductively active adult male *C.*

lurida do not co-occur. Activity of grubs is confined to underground feeding on grass roots, while mating activity occurs aboveground. The combined prepupal and pupal period lasts 15–21 days. This species has a single generation per year, with adult activity confined to a few weeks (Potter, 1981). Thus, there would normally be little or no temporal overlap between adult males and grubs. Because chemically mediated sex attraction of adult males to larvae of holometabolous insects is unexpected, it may not have been investigated before. This phenomenon may be common to scarabaeid beetles (a family which includes many species of economic importance) or may even be phylogenetically widespread in species in which adults and larvae do not co-occur in the same habitat.

The evolution of communication systems is complicated by the fact that signaler and receiver must coevolve. De novo and simultaneous evolution of both signal and receptors is unlikely. The attraction of male *C. lurida* beetles to the immature grub stage suggests that it may be possible for the evolution of sex pheromone communication to begin with odors common to all developmental stages. Apparently, males lose the attractive components during metamorphosis.

An alternative explanation of the lack of attraction to adult males could be that they produce a repellent. This possibility is not supported by observations or experimentation. It is common to find clusters of males surrounding a single pheromone-releasing female (Potter, 1980). Traps baited with females captured large numbers of males. When extracts of males and females are combined, there is no decrease in the number of males captured (unpublished data).

In summary, these experiments have demonstrated that adult females and both male and female larvae produce a volatile sex attractant. This observation appears to be unprecedented for *C. lurida* or for any other insect species. We hypothesize that the evolution of the communication system in this species involved sex-specific loss of this chemical cue in the male, combined with specialization of the adult male's sensory receptors to detect that cue.

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July 27, 1992

Dr. K.R. Haynes
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Dear Dr. Haynes:

I enjoyed your recent publication on sex pheromone evolution, *J. Chem. Ecol.*, **18**, 1117-1124 (1992), very much. This is a problem that has been of interest to me for some time, and the idea that larval odors may take on a special meaning is not an obvious one.

I wonder whether you have pursued chemical work on the attractive hexane extracts. Clearly the chemical details of what compound(s) your beetles are using would be very interesting, and even a demonstration that the active component(s) from grubs and females are chemically identical would provide firm evidence that females really do retain a larval chemical trait. I can imagine that you may have this sort of work in progress, but if this is not the case, I would be delighted to pursue the chemical aspects of this problem in collaboration with you. It is a most attractive problem for a number of reasons, and it should culminate in a synthesis of the pheromone and some analogs, which might even prove of some practical use.

Please let me know if a collaboration of this sort might interest you. We have, of course, very extensive experience in this field, and we could undertake the chemical studies as soon as extracts are available.

Sincerely yours,

Jerrold Meinwald

Jerrold Meinwald

JM/crj

Telephone: 607/255-3301 FAX: 607/255-4137