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INTEGRATING NATURAL ENEMIES, CULTURAL CONTROL, AND PLANT RESISTANCE FOR SUSTAINABLE MANAGEMENT OF INSECT PESTS ON GOLF COURSES

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Executive Summary:

Objectives:

1) Evaluate the role of ants as beneficial predators in golf turf; determine the predominant species inhabiting golf courses; and develop tactics for managing mound-building pest ants on putting greens with reduced environmental risk or impact on beneficial species.
2) Investigate synergism between endophyte-enhanced, resistant turfgrasses and biorational insecticides for improved management of white grubs and black cutworms.
3) Examine the main and interacting effects of cultural practices (mowing height, irrigation, and N fertilization) on nutritional and defensive characteristics of creeping bentgrass, and on relative susceptibility to white grubs and black cutworms.
4) Identify the sex pheromone of northern and southern masked chafers (*Cyclocephala* spp.) and explore practical uses for the pheromone in golf courses settings.

Conservation of naturally-occurring biological controls is important for reducing need for insecticide usage on golf courses. Ants, the most abundant insects inhabiting turfgrass, are highly efficient predators on eggs and larvae of cutworms, grubs, and other pest insects. On golf courses, however, the positive aspects of ant predation must be weighed against the fact that some species build nests and mounds on putting greens and tees. This research seeks to identify beneficial and harmful ant species, document their significance, and develop effective means for controlling pest ants while conserving useful, predatory species.

Surveys of ants inhabiting roughs, fairways, tees, and putting greens of central Kentucky golf courses revealed that virtually all of the mound-building problems in close-cut creeping bentgrass are caused by one species, *Lasius neoniger*. *Lasius* appears to be the major nuisance ant on golf courses throughout much of the U.S. Surface insecticides usually won't eliminate these ants because they fail to reach the ground-nesting queen.

We evaluated two novel approaches for suppressing mounding activity on tees and greens. The first involved use of target-selective ant baits, some of which already have revolutionized ant control tactics used by the structural pest control industry. After testing seven candidate baits for acceptability to *Lasius*, we selected the three most attractive ones for evaluation on golf courses. These baits contained as active ingredients either avermectin (Advance® Granular Carpenter Ant Bait; WhitMire Micro-Gen, Inc.) hydramethylnon (Maxforce® granular ant bait; Clorox, Inc.), or spinosad (NAF-464; Dow AgroSciences). Each has a different, insect-specific mode of action, low mammalian toxicity, and favorable environmental characteristics. Advance® and MaxForce® already are labeled for use on turfgrass sites. Field trials on golf tees showed that use of these baits will provide rapid, 80-95% elimination of *Lasius* mounds and nests, either by broadcast, or by selective application from a shaker can. In another study, fipronil (Chipco Choice®, Rhone-Poulenc, Inc.) was found to be effective for season-long suppression of *Lasius* nests and mounds on putting greens. This novel phenyl pyrazole, characterized by low mammalian toxicity and very low use rates, is a promising candidate for ant management on golf courses.

Field experiments demonstrated that *Lasius neoniger* and other ant species are very important in suppressing other insect pests. In trial after trial on roughs, fairways, or putting greens, ants eliminated large numbers of eggs and young larvae of black cutworms, and eggs of Japanese beetle. This underscores the wisdom of selective, rather than fence-to-fence, management of nuisance ants where mound-building becomes a problem. Fortunately, our related work with halofenozide (Mach 2) and imidacloprid (Merit) has shown that these powerful new insecticides are compatible with preservation of beneficial species, including ants.

Our second objective concerns whether use of insect-resistant grasses in combination with reduced-risk insecticides can provide levels of control previously possible only with more broad-spectrum pesticides. In 1998, we studied possible synergistic or antagonistic interactions between endophytic perennial ryegrass and efficacy of *Bacillus thuringiensis* (Bt), *Bacillus popilliae* (milky disease bacteria), and spinosad (Conserve®). We sought to determine if the sublethal stress endured by pests feeding on endophytic grass might enhance the activity of these products. Dose-mortality studies with Bt and spinosad were conducted with black cutworms fed on either endophytic (E+) or non-endophytic (E-) grass. Even full label rates of Bt provided no suppression of cutworms, irrespective of endophyte level. Spinosad provided 100% control, even at 1/4 label rate, on both E+ and E- grass. Dose-mortality studies with milky disease and Japanese beetle grubs showed significant rate effects, but disease incidence was not affected by endophyte level.

Finally, progress continued toward identifying the female sex pheromone of northern and southern masked chafers, the most destructive native grubs species in the U.S. A synthetic bait would be useful for monitoring, fine-tuning treatment schedules, or assessing local grub densities on golf courses. Earlier, we discovered that the pheromone also is present in the grubs. In 1998, ≈10,000 grubs were dug from Kentucky golf courses for pheromone collection. The grubs were rinsed in hexane to remove the pheromone; extracts were sent to collaborators (A. Attygalle, J. Meinwald, Cornell University) for analysis. The chemical peak representing the pheromone was pinpointed by gas chromatography and electroantennogram analysis, and its molecular weight was determined. Gas phase IR was used to further characterize the compound's structure. Hopefully, the identification can be completed this winter, so that field testing during beetle flights can begin in 1999.

OVERALL GOAL:

The long-term goal of this research is to better understand the factors determining the distribution and abundance of white grubs, cutworms, and other insect pests on golf courses, and to develop safer, more economical, and more effective methods for managing them with reduced use of broad-spectrum insecticides.

OBJECTIVE 1. *Evaluate the role of ants as beneficial predators in golf turf; determine the predominant species inhabiting golf courses; develop tactics for managing mound-building pest ants on putting greens with reduced environmental risk or impact on beneficial species.*

Ants (other than fire ants) are a mixed blessing on golf courses. Our work indicates that they are very important in suppressing insect pests, especially cutworms, armyworms, sod webworms, and white grubs by preying upon the eggs and young larvae. Probably no tactic that golf superintendents employ to manage insect pests is more important than the control exerted by these enormously abundant predators. Nevertheless, ants are a significant problem when they build mounds on putting greens and tees. Surface insecticides usually won't eliminate such ants because they fail to reach the ground-nesting queen.

Collection and identification of ants from ≈ 500 nests on six golf courses in central Kentucky revealed that virtually all problems on putting greens are caused by a single species, *Lasius neoniger*. This species reportedly is the major ant problem on putting greens in other areas of the U.S. as well (various turfgrass entomologists, *pers. communication*). Thus, further studies concentrated mainly on this pest species.

Two novel approaches to control of *L. neoniger* were tested in 1998. The first involved on-site evaluation of ant baits at Champions Golf Course, Lexington KY. Following discussions with experts on ant management in urban structures, we procured 7 candidate bait formulations for evaluation. Three of these already are registered in the U.S. for nuisance ant control, including nuisance ants in turf. Maxforce™ granular ant bait (Clorox, Inc.) contains hydramethylnon, which interferes with the ability of ants to convert food into energy, essentially starving the queen. Advance™ Granular ant bait and Advance™ Granular Carpenter Ant Bait (Whitmire Micro-gen, Inc.) contain avermectin, a natural compound produced by the soil microorganism *Streptomyces avermitilis*. Avermectin stimulates release of the inhibitory neurotransmitter GABA (gamma-aminobutyric acid), inhibiting signal transmission at the neuromuscular junction in target pests. It has selective toxicity for arthropods and an excellent safety margin for humans and nontarget species. In addition, we procured a fipronil-based ant bait (Nexa Lotte™, Rhone-Poulenc, Inc.) that is labeled for nuisance ant control in Europe, and three experimental ant baits containing spinosad (anchovy-, sugar-, and oil-based formulations) from Dow AgroSciences LLC.

Pairwise choice tests initially were performed to test relative acceptance of the baits by

L. neoniger. All pairwise combinations were tested, each with 25 different *L. neoniger* mounds. Advance Granular Carpenter Ant Bait [=Advance CAB], Maxforce bait, and the anchovy-based spinosad bait [NAF-464] were highly acceptable to *L. neoniger*, whereas the other baits were much less preferred or entirely ignored. Further work concentrated on the most acceptable baits, each of which has a different active ingredient and mode of action.

The first, full-scale field test was conducted on heavily-mounded tees at Champions Golf Course, Lexington, KY, in late May. Twenty-eight nests, each averaging ≈ 6 mounds per nest, were treated with each bait by manually spreading ≈ 3 g of bait around each mound. More than 900 individual mounds were mapped, blocked by pretreatment count, and treated. A granular, non-bait fipronil formulation (Choice™) was applied in identical manner for comparison. Results evaluated after 4 days were highly encouraging (Figure 1).

The next test was conducted on tees at Champions Golf Course in mid-late June. In this test, baits (Advance CAB, Maxforce, NAF-464) were broadcast at labeled or manufacturer-suggested rates (0.028 kg AI/ha). Plots, which consisted of whole small tees or portions of large tees, averaged 230 m^2, with ≈ 40 mounds per plot. Treatments were blocked by pretreatment counts. Baits were sprinkled evenly throughout the plots by hand. Three insecticide treatments were included for comparison. These were 1) Scimitar™ (Lamda cyhalothrin, a pyrethroid) sprayed at label rate (0.067 kg AI/ha), 2) granular fipronil (Choice™) broadcast on the tee at 0.28 kg AI/ha, or 3) the same rate of fipronil applied as a perimeter treatment (2.5 m wide band) around each plot, the plot itself being left untreated. Ant mounds were counted at 1 week after treatment. Suppression of mounding was as follows: Maxforce, 85.7%; Scimitar, 83.1%, fipronil broadcast, 77.8%, and Advance CAB, 76.8%; with no significant difference among those treatments. Effectiveness of the baits is especially impressive given that >5 cm of rain fell within 2 hours after the last replicate was treated. As rain or irrigation usually greatly diminishes attractiveness of ant baits, this shows that these products are so attractive to *L. neoniger* that only a relatively brief exposure to foraging workers will kill a nest. Thus, use of these baits should not require that irrigation be withheld for very long. The spinosad bait (NAF-464) and fipronil perimeter treatments were less effective (29.4 and 23.1% suppression, respectively).

A third test was conducted in late July to evaluate efficacy of selective treatment by applying smaller amounts of bait (≈ 1 g/mound) from a shaker can with a perforated lid. Baits tested were Advance CAB, Maxforce, and NAF-464, with 5 replicates (nests) randomly assigned to 20 plots among 15 different tees at Champions Golf Course. Percentage reductions after 1 week were: Maxforce, 95.6%; Advance CAB, 88.3%; and NAF-464, 82.4%.

A different approach was tested by evaluating season-long suppression of ants by fipronil (Chipco Choice™). Fipronil is a phenyl pyrazole, a new class of insecticides that act as GABA inhibitors in insects. Presently labeled against fire ants and mole crickets, it offers advantages of long residual, very low use rates, and low mammalian toxicity. Efficacy and persistence were compared with Scimitar, a commonly-used pyrethroid.

The study was conducted on a large, newly-seeded, sand-based creeping bentgrass putting green at the University of Kentucky Turfgrass Research Facility. Infestation by *L. neoniger* was severe, averaging >150 mounds per 58 m^2 (25 x 25 ft) plot at the start of the test. Fipronil was applied at three rates, 0.0069, 0.014, or 0.028 kg AI/ha; Scimitar was tested at label rate (0.067 kg AI/ha). There were four replicates (58 m^2 plots) plus untreated controls. Following the one-time application on 4 June, whole-plot mound counts were counted at 7, 14, 30, and 60 days.

Scimitar and the two higher rates of fipronil provided 73.4, 72.0, and 79.9% suppression of mounds after 7 days. By 60 days, however, ant populations in the Scimitar-treated plots had recovered (only about 10% reduction compared to controls), whereas medium and high rates of fipronil continued to give 81.2 and 86.5% suppression of mounding, respectively.

The importance of ants as agents of biological control on golf courses also was studied. Five series of experiments were conducted; results are briefly summarized below:

Predation by ants on black cutworm larvae. Multiple nests of four different ant species, *Lasius neoniger*, *Formica schaufussi*, *Formica subcericea*, and *Formica pallidifulva* were located in lawns on the University of Kentucky campus (these same species are found on golf courses). Ten, 3rd-instar black cutworms (BCW) were exposed to each nest every 4 hours for 24 hours by dropping the larvae into the turf beside the nest and observing the ants' response. This was repeated on five dates. *Formica schaufussi* was the most aggressive species, showing explosive recruitment of nestmates whenever a foraging worker came into contact with the prey. *Formica subcericea* also was voracious. Individual nests of these two species killed as many as 45 BCW larvae in 24 h. Even *L. neoniger*, a relatively small species, took as many as 31 larvae per nest in 24 h. It was especially active at night, when activity of BCW normally occurs.

Another test with small BCW larvae was conducted on creeping bentgrass putting greens at Spindletop Farm in late July 1998. First instars were dropped near *Lasius neoniger* mounds and response of the ants and larvae was observed. Two observers exposed a total of 1600 larvae over several days. If a larva was not attacked within 5 minutes it was scored as a failure by the ants. Out of 1600 larvae exposed, 1052 were attacked and dragged down into the ant nests. Of these, 640 were attacked and taken by one ant, and 412 were taken by 2-3 ants. Another 123 larvae were attacked and escaped. Single ants were able to overcome only the smallest BCW larvae. Older first instar BCW, which are slightly larger than *L. neoniger* ants, become aggressively defensive, so that 2-3 ants were required to subdue them, and some of them managed to escape.

Predation by *Lasius neoniger* on BCW eggs over 24-h cycles. BCW moths were allowed to lay eggs on creeping bentgrass turf cores. Grass blades bearing eggs were marked on their tips with orange turf paint. Six cores were implanted into putting greens on

Champions Golf Course, and at Spindletop Farm, on each of two dates in mid- to late summer. Observations were made every 2 hours for 24 h. Within 1 day, ants consumed 1360/1820 (74.7%) of the total eggs exposed at Champions, and 1483/1624 (91.3%) of those exposed at Spindletop. All observed egg predation was by *Lasius neoniger*.

Predation on BCW and Japanese beetle eggs on fairways and roughs. Paired cores of creeping bentgrass bearing BCW eggs (as described above) were implanted into fairways and untreated roughs at Champions and Idlehour golf courses in late summer 1997. Cores (12 per golf course) were set in late afternoon and recovered before fairways were mowed the following morning. Japanese beetle (JB) eggs were simultaneously exposed to predation by setting small, open petri dishes containing soil and 10 eggs under each grass core. At Champions, predation on BCW eggs averaged 84% in one night in roughs, as compared with 27.4% in fairways. Predation on JB eggs averaged 43.3% in roughs, versus 43.3% in roughs. At Idlehour, predation rates on BCW eggs in roughs and fairways were 62.6 and 2.9%, respectively, and predation on JB eggs averaged 55% versus only 1.7%. Significantly lower rates of predation in fairways were correlated with lower abundance of ants.

The aforementioned test was repeated with BCW eggs in May 1998, except that cores were inspected every 2 hours during the exposure periods to identify which predators were responsible for disappearance of the eggs. Results were similar to the previous year: 84% versus 27.4% predation in roughs and fairways at Champions, and 62.6 versus 5.1% predation in roughs and fairways at Idlehour. Almost all of the observed egg predation was by *Lasius neoniger*.

Consequences of elimination of ants. Paired 10 m^2 plots were established in roughs beside three different fairways on both the Champions and Idlehour golf courses. One plot within each pair was treated with fipronil in early July to suppress ants. Any ant mounds remaining after 2 weeks were individually baited with Advance Carpenter Ant Bait. Pitfall traps confirmed a sharp reduction of ant activity in treated plots. Then, two turf cores (15 cm diameter) with several hundred BCW eggs were set into each plot and egg predation was monitored every 2 hours for 24 hours. Only *L. neoniger* was observed taking the eggs.

To study consequences for predation on Japanese beetle (JB) eggs, two 15 cm PVC cylinders were implanted into each plot. Each cylinder was infested with 15 pairs of adult beetles and then covered with a screened lid. Sassafras leaves were provided as food. The cylinders were removed after 1 week. After 2 more weeks, cores where the JB had been confined were removed and examined for JB grubs. A total of 97 JB grubs was found in plots without ants, compared to only 9 grubs in plots with abundant ants.

OBJECTIVE 2. *Investigate synergism between endophyte-enhanced, resistant turfgrasses and biorational insecticides for improved management of white grubs and black cutworms.*

Earlier studies in our laboratory showed that endophytic grasses may have sublethal effects on grubs and cutworms (e.g., delayed development, stunted growth) rather than killing them outright. This is important because stressed insects often are more susceptible to pathogens or insecticides. Thus, it is possible that use of endophytic grasses in combination with reduced-risk insecticides could broaden the spectrum of control with these products, at reduced rates. Alternatively, control might be compromised if entomopathogens are sensitive to the same endophyte-produced alkaloids that the insects must cope with. Three separate studies were conducted in summer/fall 1998 to evaluate these potential interactions.

Flats of 'Assure' perennial ryegrass, either endophyte-free (E-) or infected with the *Neotyphodium* endophyte (E+), were grown in walk-in growth chambers. In the first experiment, we evaluated two formulations (Thuricide® and Dipel®) of the microbial insecticide *Bacillus thuringiensis* subsp. *kurstaki* (Bt) against black cutworms feeding in E+ or E- grass. Bt is labeled for cutworm control in turf, but there is little information regarding it's efficacy. Each formulation was applied to separate flats of E- or E+ grass at 0, 1/4, ½, and full label rate. Spray residues were completely dry before clippings were cut and fed to the larvae. There were four replicates of 10 second instar larvae for each rate/grass combination. To ensure exposure to fresh residues, the grass was retreated every other day. Larvae were weighed at 3 and 6 days, and the experiment was terminated after 1 week. Surprisingly, neither Bt product provided *any* control of cutworms on either grass, even at full label rate.

A second, similar experiment was conducted with spinosad (Conserve®) applied to E+ or E- turf at 0, 0.25, 0.5, or full label rate. Spinosad, which is labeled for cutworm control on golf courses, is a naturally-occurring mixture of two closely related macrocyclic lactones produced by the actinomycete *Saccharopolyspora spinosa*. Clippings were harvested after spray residues had dried and fed to second instar larvae for 7 days. Weights and survival of cutworms were monitored. Spinosad was highly effective at all rates (e.g., >80% mortality 0.25 label rate) on both grasses. There were no main effects or interactions with endophyte, perhaps because the treatments were so effective irrespective of grass. We hope to repeat this test with even lower rates to test for possible synergism by endophyte.

Finally, we compared infectivity of *Bacillus popilliae*, causal agent of milky disease, against Japanese beetle grubs feeding on roots of E+ or E- perennial ryegrass. Grasses were grown in greenhouse flats with dividers that provided multiple 2 x 2" cells. Milky disease spore powder, obtained from M. Klein (USDA Horticultural Insects Lab), was incorporated into the soil at planting. Four rates, ranging from 0 to 4 x 10^9 spores/gram of soil, were tested with each grass type, using four replicates of 16 grubs (64 grubs) per grass-rate combination (512 total grubs). Grubs were introduced as early third instars. Surviving grubs were counted, weighed, and inspected for macroscopic symptoms of milky disease every 7 days for 45 days. Milky disease infection rates ranged from 43 to 90%, with a significant rate effect. There were no main effects or interactions with endophyte, suggesting that infectivity of *B. popilliae* is neither enhanced or impeded by endophyte. Another analysis of grub weight and survival in the absence of *B. popilliae* showed no negative effects of the endophyte at ant sample date.

OBJECTIVE 3. *Examine the main and interacting effects of cultural practices (mowing height, irrigation, and N fertilization) on nutritional and defensive characteristics of creeping bentgrass, and on relative susceptibility to white grubs and black cutworms.*

Work was not initiated on this objective because the work on Objectives 1 & 2 required full-time effort of available personnel. I hope to recruit a graduate student to work on this question next year.

OBJECTIVE 4. *Identify the sex pheromone of northern and southern masked chafers (Cyclocephala spp.) and explore practical uses for the pheromone in golf courses settings. (Note: this objective is a carryover from the previous grant)*

Identification and synthesis of this pheromone could provide a tool for monitoring beetle flight, allowing better timing of treatments. Masked chafers have localized flights, so there should be a good correlation between local beetle activity and subsequent grub densities. Superintendents could employ simple traps to target "high-risk" sites.

Northern and southern masked chafers, the grubs most destructive to golf courses throughout most of the U.S., are morphologically indistinguishable as larvae. Thus, little is known about their relative importance within golf course habitats (e.g., roughs, tee banks, fairways, or particular grass species). The species co-occur throughout much of the central U.S. Because they respond to the same pheromone, a synthetic lure would provide a means for studying their distributions and habitat preferences. If the species differ in susceptibility to insecticides, being able to distinguish between them could be important in management.

Identification of the sex pheromone is proving to be particularly challenging for two reasons. First, active fraction is present in very minute amounts, making it difficult to get enough material for analysis. It is, however, extremely potent. Second, the structure seems to be unique among known insect pheromones. Nevertheless, there were significant breakthroughs in 1997-98. During this period, we collected, extracted, and shipped about 12,000 masked chafer grubs to collaborators at Cornell University. The chemical peak representing the active compound was pinpointed by simultaneous gas chromatography and electroantennogram analysis. The active compound has Kovat's indices of 1790 and 2356 on nonpolar and polar columns, respectively. The compound was purified and subjected to infrared and mass spectroscopy. The molecular weight is 246. Gas phase IR confirmed that the compound is an aldehyde, not conjugated, and without terminal =CH2 groups (Attygalle, *pers. communication*). This greatly narrows the possibilities. With this information in hand, a chemical structure for the molecule can be determined. Once identified, the molecule will be purchased if it is available from a commercial source (e.g., Sigma, Aldrich) or else synthesized by the Cornell group. We hope to begin testing the synthetic pheromone on golf courses in 1999.

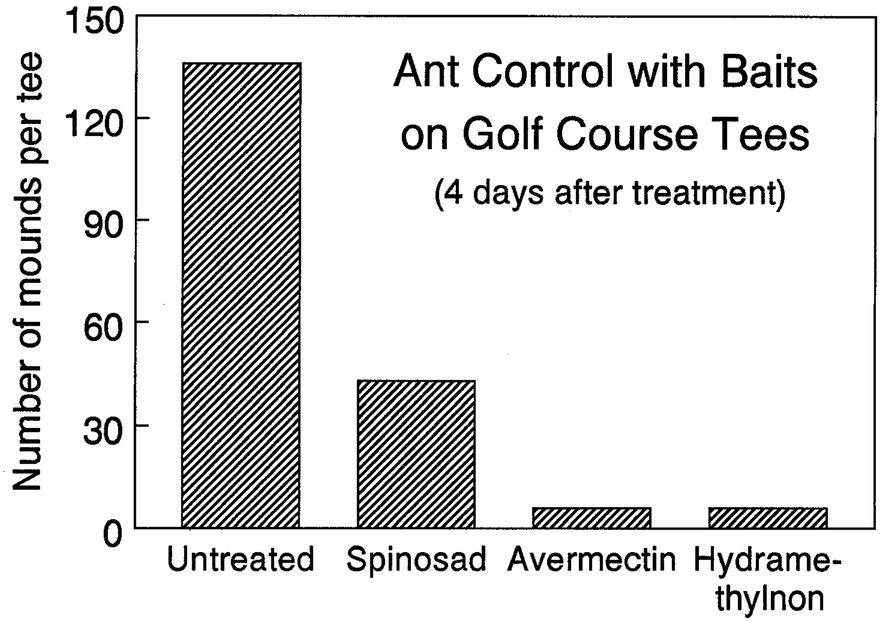

Figure 1. Suppression of *Lasius neoniger* ant mounds on tees 4 days after application of granular ant baits containing spinosad, avermectin (Advance® Granular Carpenter Ant Bait), or hydramethylnon (Maxforce® Granular Ant Bait).