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Volume 8, Number 8
April 15, 2009

PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 350 projects at a cost of $29 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Continuing the Search for Biological Control of White Grubs

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SUMMARY

Scientists at the University of Kentucky continue to explore methods of biological control of white grubs, the larvae of scarab beetles. Part of this investigation included conducting a sampling survey with 33 golf course superintendents throughout Kentucky to assess what parasites and bacterial, fungal, or nematode pathogens were present on the sampled grubs in an effort to identify new potential biological control agents. Survey results included:

- Masked chafer and Japanese beetle grubs accounted for 64 and 30%, respectively, of white grubs sent in from Kentucky golf courses, with May beetle, green June beetle, and black turfgrass ataenius comprising the rest.
- Grubs occurred in a wide range of Kentucky soils and pH ranges with no particular relationship between soil type and predominant grub species.
- Grub populations declined from about 18 per square foot in late August, to about 5 grubs per square foot in October, to less than 2 grubs per square foot the following spring despite no insecticides having been applied.
- In field studies, warm-season grasses, zoysiagrass and bermudagrass, had the highest densities of masked chafer grubs (about 7 and 4 grubs/0.1 m^2 [1 ft^2]) respectively, compared to 1.3 and 0.5 masked chafer grubs/0.1 m^2 in fairway-height bentgrass and ryegrass, and less than 1-2 grubs/0.1 m^2 in all rough-height grasses.
- Skunk damage was concentrated in fairway-height creeping bentgrass and perennial ryegrass, with almost none in the other grasses, including the zoysiagrass, bermudagrass, and tall fescue. The latter grasses, although they had plenty of grubs, develop thick thatchy mats and/or a dense root system that may be difficult for skunks to dig up.

White grubs, the immature stage of stout-bodied beetles called scarabs, are the most destructive insect pests of golf courses in the cool-season and transitional climatic zones (4). Grubs cause turf damage in two ways: by chewing off the roots resulting in dead patches that can be lifted or rolled back like a carpet, and by attracting skunks, raccoons, armadillos, and other varmints that dig up the turf to eat the grubs. Golf courses often sustain both types of injury. Many millions of dollars are spent each year to control white grubs in home lawns and on golf courses in the United States.

Golf course superintendents now rely almost exclusively on synthetic insecticides for grub control, applying a long-residual soil insecticide before egg hatch to intercept newly-hatched grubs, or spot-treating larger grubs in late summer if and when grub damage appears. Insecticides, however, are expensive and must be reapplied every year, and their overuse can impact the beneficial insects that help to buffer pest outbreaks.

Sole reliance on insecticides, especially of the same chemical class, can also lead to pesticide resistance. Resistance to pyrethroids was recently documented in annual bluegrass weevil populations on New England golf courses and for southern chinch bugs on St. Augustinegrass lawns in

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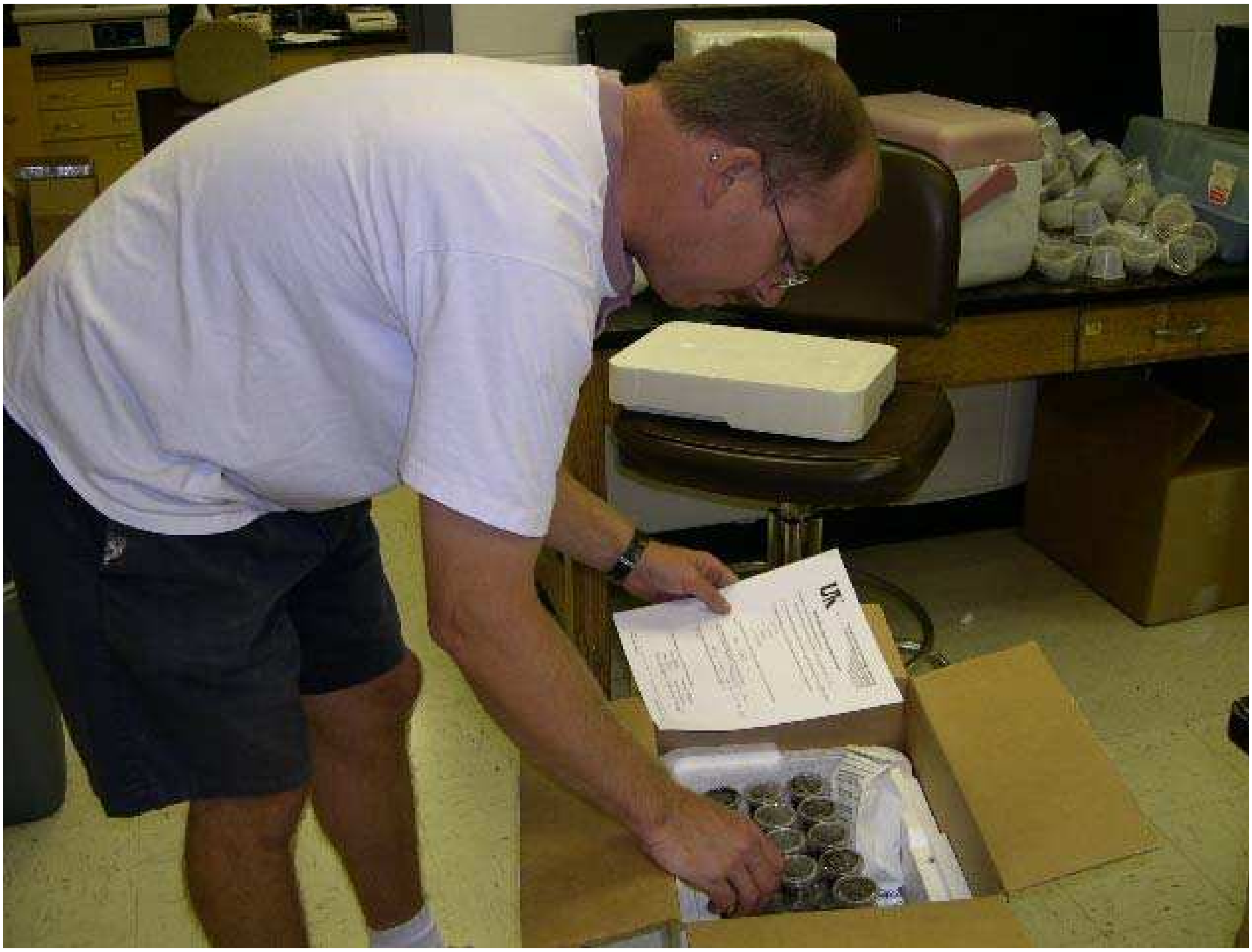
USGA Turfgrass and Environmental Research Online 8(8):1-8.
TGIF Record Number: 147049



Superintendents placed the white grubs in individual cups of peat moss and returned the kits by overnight mail. Grub samples were identified to species and examined for parasites and symptoms of disease.

Florida (1, 5). Because societal pressures to reduce use of synthetic insecticides, even reduced-risk products, likely will intensify, it is prudent to explore other approaches that can used to help reduce reliance on that lone chemical strategy.

Integrated pest management (IPM) uses a combination of tactics to keep pests below damaging levels while minimizing undesirable side-effects. Although synthetic insecticides will likely remain essential IPM tools for golf courses for the foreseeable future, integrating non-chemical controls into a management plan can help reduce pesticide dependence, as well as labor and costs. Integrating non-chemical controls also helps reduce the likelihood of pests developing resistance to insecticides.

Biological control, including use of microbial insecticides and conservation of natural enemies of pest species, is well-suited to perennial systems such as golf courses. At present, however, microbial insecticides constitute only a minis-

Kentucky superintendents sampled for white grubs in their most affected sites. Grubs occurred in a wide range of Kentucky soils and pH ranges with no particular relationship between soil type and predominant grub species.

cule fraction of the turf insecticide market (2), and not enough is known about predators, parasites, and pathogens of white grubs to effectively enlist their aid.

This project seeks new knowledge about the natural enemies of white grubs on golf courses in the transitional climatic zone. It includes the first survey of pathogens of masked chafers (*Cyclocephala* spp.), the most widespread and destructive native grub pests in the United States. We seek new control agents with potential as bio-insecticides for turf pests and better understanding of how site characteristics affect these agents for natural suppression of grub populations.

Sampling Surveys

In 2007, kits were sent to 33 golf superintendents throughout Kentucky who were asked to collect a sample of 30 grubs and soil during September from the worst grub site on their courses. Superintendents placed the white grubs in individual cups of peat moss and returned the kits by overnight mail. We identified each sampled grub to species, examined it for parasites or disease symptoms, and then held each one for 30 days to allow for any unseen disease to develop.

Each grub was dissected to determine the presence of bacterial, fungal, or nematode pathogens.

Tiphia pygidialis, a wasp that lays an egg on the back of masked chafer grubs and upon hatching its larva consumes the victims, was the most abundant parasite in autumn, killing 0 to 33% of the grubs at a given site.

Finally, each grub was dissected to determine the presence of bacterial, fungal, or nematode pathogens.

Masked chafer and Japanese beetle grubs accounted for 64 and 30%, respectively, of white grubs sent in from Kentucky golf courses, with May beetles, green June beetle, and black turfgrass ataenius comprising the rest. European chafer, Oriental beetle, and Asiatic garden beetle, invasive grub species that are expanding their ranges beyond the northeastern United States, evidently are not yet established in Kentucky. Grubs occurred in a wide range of Kentucky soils and pH ranges with no particular relationship between soil type and predominant grub species. Masked chafer accounted for approximately 80% of the grubs in samples sent in from 13 of the courses, Japanese beetle grubs dominated at three courses, and on 17 courses the sampled areas had a mixture of Japanese beetle and masked chafer grubs.

Nematodes found infecting white grubs were given wax worms to infect. The color of the cadavers aids in identifying the nematode strains. One of the nematode strains was noticeably larger than commercial strains and may have potential as a new bio-insecticide especially suited for white grubs in the transition zone.

Biological Control Agents

Various biological control agents were found. *Tiphia pygidialis*, a wasp that lays an egg on the back of masked chafer grubs and, upon hatching, its larva consumes the victims, was the most abundant parasite in autumn, killing 0 to 33% of the grubs at a given site. *Paenbacillus popilliae* bacteria, causal agent of milky disease, were the most abundant pathogens. Infection rates averaged 12 and 20% for Japanese beetle and masked chafer grubs, respectively, across all sites, but some sites had as many as 31% of the Japanese beetle and 73% of the masked chafers infected with this lethal disease. Other pathogens included a greenish fungus (*Metarrhizium* sp.), *Serratia entomophila*, a bacterium that causes amber disease which causes infected grubs to stop feeding, and several strains of insect-parasitic nematodes.

Masked chafer grub naturally infected with amber disease caused by *Serratia* bacteria. Infection by this bacterium causes the grub to quit feeding.

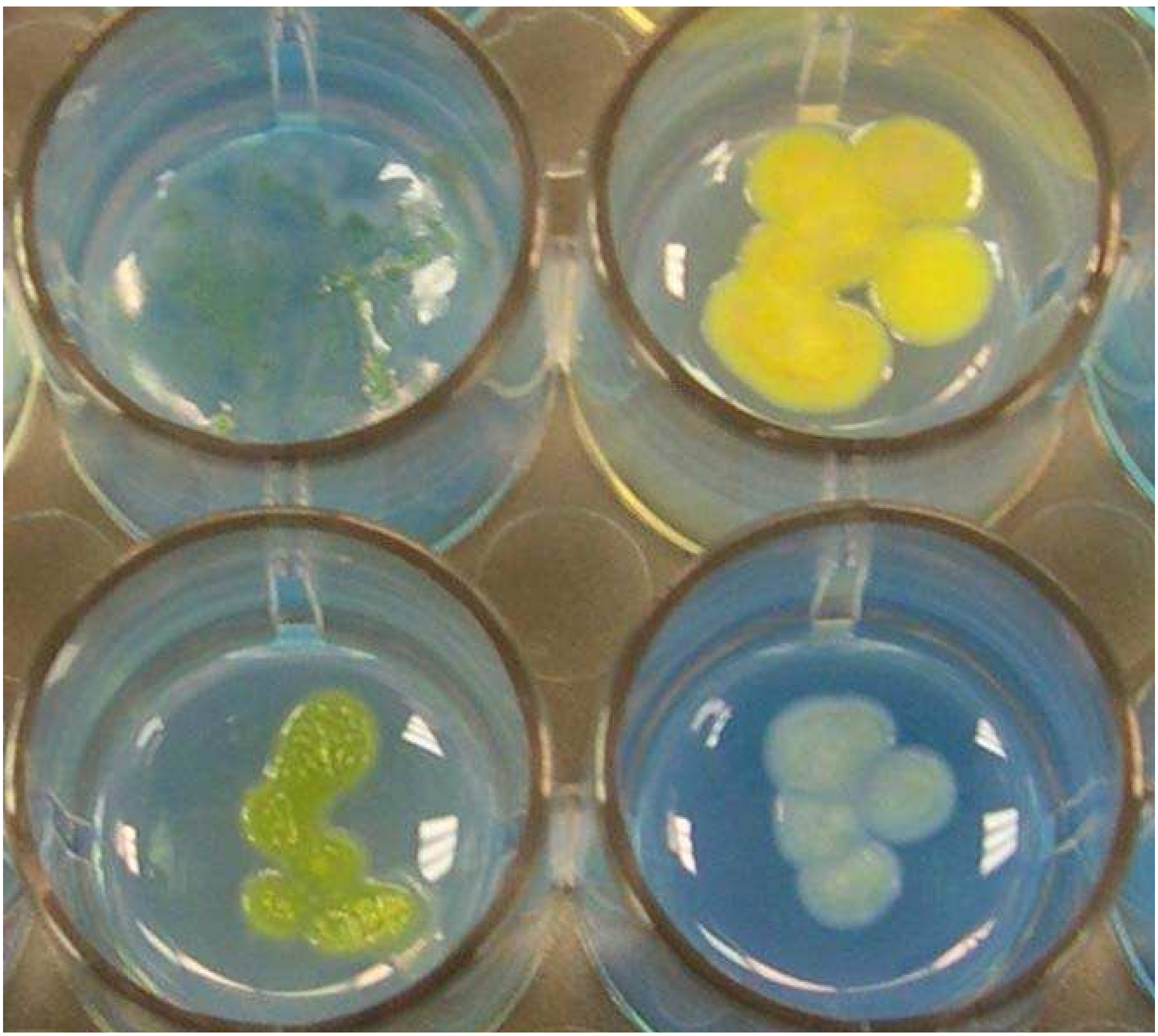
Ovavesicula sp., a protozoan that causes reduced fertility in adult beetles (3), was uncommon in Kentucky. Gregarines (*Stichtospora* sp.), tapeworm-like parasites, were uncommon in autumn but infected as many as 27% of Japanese beetle grubs in the spring. Gregarine-infected

Japanese beetle grubs developed into normal sized beetles, but their emergence was delayed about two weeks. Overall, none of the individual pathogens accounted for greater than 20% mortality, but, collectively, about 48% of sampled masked chafer and 33% of Japanese beetle grubs were infected with one or more debilitating or lethal pathogens.

Insect-pathogenic nematodes we isolated from Japanese beetle and masked chafer grubs are currently being identified using genetic markers. One of the strains from masked chafers is noticeably larger and more robust than commercially-available nematodes which originally were isolated from grubs other than masked chafer. If the identifications confirm it as new, future studies will determine if it is more infective than commercial nematodes against masked chafer. This could lead to a nematode-based bio-insecticide especially suited for white grubs in the transitional climatic zone.

Follow-up Surveys

We also repeatedly sampled 18 sites on six central Kentucky golf courses in late August, mid-September, and early October 2007, and again in early May 2008, to track grub density and incidence of natural enemies over time. Masked chafer grubs predominated, as they had in the

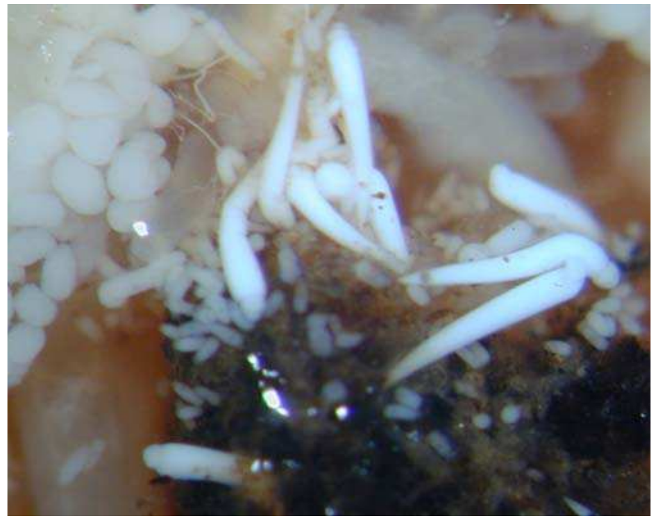


Diagnostic test for identifying *Serratia* bacteria from infected white grubs by the color of the bacterial colonies.

Kentucky state survey. Interesting, grub populations declined from about 18 per square foot in late August, to about 5 grubs per square foot in October, to less than 2 grubs per square foot the following spring despite no insecticides having been applied. The incidence of the natural enemies patterned the state survey and no doubt contributed to the grub population declines.

The Japanese beetle continues to aggressively expand its range into the Great Plains, Great Lakes, and central United States, and is an ever-present threat to become established in California, Oregon, and Washington. Historically, Japanese beetle reaches outbreak levels when it first invades new areas, followed by gradual decline and stabilization. We sought to determine if buildup of diseases might explain that pattern.

Grub collection kits were sent to 20 cooperators throughout the eastern and central United States who sent us samples 30 Japanese beetle grubs from local golf courses and other turf sites. Samples of grubs and soil were sent in from eastern states (New Hampshire, Maryland, Rhode Island, New York, Pennsylvania, New Jersey, North Carolina, and Georgia) where Japanese beetles have been established for more than 50 years (it was first found in the USA in 1916, near Riverton, NJ), to central states (Michigan, Ohio, Kentucky, Illinois, Tennessee)) to states into which Japanese beetle more recently has spread



Grub infected by whitish carrot-shaped gregarine pathogens (*Stichtospora*).

(Wisconsin, Nebraska, Oklahoma, Arkansas, Iowa, Minnesota).

The samples confirmed that Japanese beetle grubs can handle a wide range of soil types and pH levels. Overall pathogen load, as predicted, was highest in the eastern states. Milky disease infection, for example, averaged about 7% (range 0-27%) and combined infection by *Ovavesecula* and gregarines averaged 6.1% (range 0-17%) in the East, whereas *Ovavesicula* was absent and gregarine infection was less than 1% in grubs sent in from the Great Plains and Great Lakes states. Less than 1% of the Japanese beetle grubs shipped to us were infected with pathogenic nematodes.

Although the pathogen loads in our samples seem too low to convincingly explain why Japanese beetle populations decline over time, the data from each location represent just a snapshot in time, so the cumulative toll of pathogens from egg hatch in mid-summer to beetle emergence the following year likely is much higher. Indeed, we saw dramatic declines in grub densities at the same sites on the six Kentucky golf courses from August to May of the following year.

Grub whose excretory organs (similar to kidneys) show whitish nodules indicative of protozoan (*Ovavesicula*) infection.

Field Studies

In a separate study, we established two stands of replicated irrigated cool- and warm-turfgrasses that are used on golf courses in the transition zone. The first stand was mowed at fairway


Grub killed by greenish *Metarrhizium* fungus.

height (1.6 cm, or 5/8 inch) and contains creeping bentgrass, perennial ryegrass, zoysiagrass, and bermudagrass. The other stand is mowed at rough height (6.4 cm, or 2.5 inch) and has turf-type tall fescue, Kentucky bluegrass, perennial ryegrass, and a tall fescue/ Kentucky bluegrass mix.

The plots were sampled for grubs in September 2008 to determine if masked chafer or Japanese beetle favor certain grasses over others. All grubs were held and dissected to see if grass type affects incidence of pathogens. Twenty masked chafer grubs were introduced into 0.1 m^2 (about 1 ft^2) enclosures in each plot to assess parasitism by *Tiphia pygidialis*. We also rated each plot for damage resulting from skunks digging for grubs.

Interestingly, the warm-season grasses, zoysiagrass and bermudagrass, had the highest densities of masked chafer grubs (about 7 and 4 grubs/0.1 m^2 [1 ft^2]) respectively, compared to 1.3 and 0.5 masked chafer grubs/0.1 m^2 in fairway-height bentgrass and ryegrass, and less than 1-2 grubs/0.1 m^2 in all rough-height grasses. Masked chafer grubs also suffered the highest rates of milky disease and parasitism by *Tiphia* in zoysiagrass (24 and 10%, respectively). Japanese beetle grubs were at least twice as abundant in rough-height perennial ryegrass. tall fescue, and mixed tall fescue/Kentucky bluegrass than in rough-height Kentucky bluegrass or any of the fairway-height grasses.

Skunk damage was concentrated in fairway-height creeping bentgrass and perennial ryegrass, with almost none in the other grasses, including the zoysiagrass, bermudagrass, and tall fescue. The latter grasses, although they had plenty of grubs, develop thick thatchy mats and/or a dense root system that may be difficult for skunks to dig up. Thus, so long as some grubs are present, skunk foraging may be dictated more by ease of digging up the turf than by relative grub density. Parasitism rates of Japanese beetle grubs by the spring-active *Tiphia vernalis* will be evaluated in May.

Conclusions

This ongoing study is providing new insight about natural enemies of white grubs on golf courses in the transition zone. The pathogens isolated, especially from masked chafers, may provide raw material for bio-insecticides better suited for controlling the types of grubs that predominate on courses in the Midwest, Southeast, and Great Plains states. Our data also provide a start toward understanding why grub populations vary from year to year and where on golf courses particular species of grubs are most likely to occur.

Acknowledgements

The authors wish to thank USGA's Turfgrass and Environmental Research Program for its support of this project and all of the cooperating Kentucky golf course superintendents and university colleagues who sampled and shipped us grubs from their respective sites.

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