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Volume 7, Number 15
August 1, 2008

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 450 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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Insights into Managing Annual Bluegrass Weevils

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SUMMARY

Turf scientists from across the country are collaborating in the Northeast Regional Hatch Project 1025 to study the management of two important pests of annual bluegrass, anthracnose disease and annual bluegrass weevil (ABW). This article reviews ABW's biology, and points include:

- The annual bluegrass weevil, *Listronotus maculicollis*, (formerly called Hyperodes), principally feeds as larvae on annual bluegrass.
- With 2-3 generations per year, this weevil can build to astonishing populations (small patches may reach 1,200 larvae per square foot) that can stress or kill annual bluegrass in greens and fairways.
- The wide-spread use of pyrethroid insecticides has left many courses with adult weevils that are now virtually impossible to kill with any pyrethroid. Superintendents should be aware that the physiological changes due to pyrethroid resistance may also make some other insecticides less effective.
- When pyrethroid-resistant annual bluegrass weevil adults are exposed to piperonyl butoxide, their susceptibility to pyrethroids is restored.
- Among the non-pyrethroid products being confirmed as effective against ABW are trichlorfon (Dylox®), spinosad (Conserve®), chlorantraniliprole (Acelepryn®), and indoxacarb (Provaunt®).
- A study conducted over a three-year period on untreated fairways of three golf courses in New Jersey demonstrated that two species of entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) regularly infect ABW stages from the third larval instar through newly hatched adults. The low susceptibility of adults even under ideal laboratory conditions suggests that entomopathogenic nematodes are not likely to replace preventive chemical pesticides for adult control.

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To help superintendents meet the challenges of maintaining annual bluegrass, *Poa annua*, turf scientists from across the country are collaborating in the Northeast Regional Hatch Project 1025 to study the management of two important pests of annual bluegrass: anthracnose disease and annual bluegrass weevil (ABW). The scientists authoring this article are contributing to a better understanding of annual bluegrass weevil biology and its control.

The annual bluegrass weevil, *Listronotus maculicollis*, (formerly called Hyperodes), principally feeds as larvae on annual bluegrass. Adults mostly overwinter in protected areas along the edge of woods or in the rough. During the spring, adults migrate onto golf courses where they feed on grass blades before mating and laying eggs within the stem of *P. annua* (Figure 1). Eggs hatch into the first-instar larvae, which feed within the grass stem, where they complete two additional larval stages. Third-instars eventually exit the stem, whereupon the fourth and fifth instars continue feeding on *P. annua* root crowns while living at the surface of the soil. After completing this feeding, the larvae transform into pupae, and then into adults.

With 2-3 generations per year, this weevil can build to astonishing populations (small patches may reach 1,200 larvae per square foot) that can stress or kill annual bluegrass in greens and fairways. Where *P. annua* is considered a weed as it invades other grasses, annual bluegrass weevil feeding may be perceived as beneficial because the weevil acts as a biological control agent. However, in older courses with extensive populations of *P. annua* in greens and fairways, this grass can form an acceptable playing surface and the goal is then to maintain its health. Scattered observations reveal that annual bluegrass weevil larvae can also feed on bentgrass, and this complicates its status as a pest. To date, however, nothing is known about annual bluegrass weevil

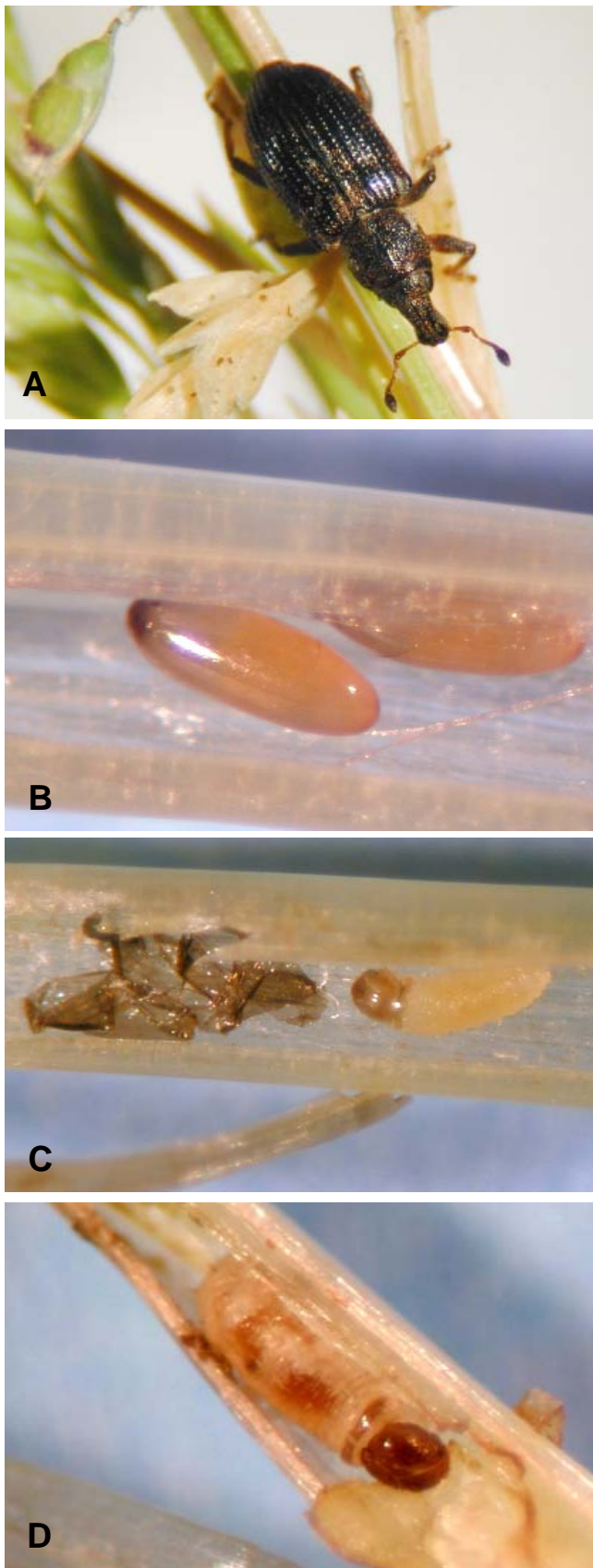


Figure 1. Some development stages of annual bluegrass weevil, *Listronotus maculicollis*: (A) adult, (B) eggs inserted in the grass stem, (C) newly hatched larva within the stem, (D) second-instar larva.

in association with bentgrass.

Over the past few years, annual bluegrass weevil has become one of the most difficult insect pests to manage on golf courses. During the previous decade, superintendents could be assured that a well-timed pyrethroid spray in the spring would prevent damage for the remainder of the season. The strategy was to apply a pyrethroid spray to fairways, or even just their perimeter, at the time that forsythia (*Forsythia intermedia*) reached the half-green half-gold late stage of bloom, or when downy serviceberry (*Amelanchier arborea*) was in bloom. Adult weevils feeding at that time would encounter a lethal dose of insecticide before they started laying eggs, and the life cycle would be interrupted. The situation has changed, so that this approach no longer works on some courses.

We will explain the underlying causes for control failures and ways in which superintendents may effectively respond to this challenge. Changes in strategies to combat this pest are an immediate need where control practices have failed and may prevent similar failures in the remaining locations.

Targeting Annual Bluegrass Weevil

Some challenges for managing annual bluegrass weevil are related to targeting. Any intervention tactic, chemical insecticide or otherwise, has to hit the target in both space and time. "In space" means understanding and predicting where the insect populations will reach damaging levels. One issue is whether perimeter applications of insecticides are sufficient since these are the areas where damage is most prevalent, or whether wall-to-wall fairway applications are required because potentially damaging populations are spread out over a wider area. "In time" means understanding and predicting when the insect appears and when the life stages are present that are susceptible to control measures.

Recent studies describe annual bluegrass weevil movement in the golf course habitat, ultimately refining our targeting ability. For instance, field studies have led to a new conceptual model

about how annual bluegrass weevil adults might migrate between overwintering sites (in protected areas off the course) and developmental sites (susceptible turf on golf course playing surfaces) (2). Ongoing studies will continue to test new ideas that (a) adults may rely on walking to invade fairways in the spring, but rely on flight to disperse back to protected overwintering sites in the fall, and (b) overwintering is largely done away from the fairway, primarily along defined tree lines up to 180 feet away, to which flying adults orient in the fall.

Other studies are taking a detailed look at seasonal population fluctuations. These are revealing the extent to which populations vary from site to site and year to year in terms of abundance, timing, synchrony, and number of generations. From these studies we will be able to gauge how much we can generalize about annual bluegrass weevil population patterns. For instance, from 2004 to 2006, annual bluegrass weevil populations in western New York completed either two or three generations, but in each case the last one was relatively small or only partially com-

pleted. Furthermore, in some years, arrival of adults to highly maintained turf is rapid and synchronous, which provides ideal circumstances for effective targeting with a well-timed insecticide application.

In other years, however, adults migrate in two waves or stretch out over a longer period of time. As a result, these populations are difficult to target effectively with a single insecticide application. Adult migration, population development, and the number of generations completed may depend on growing-degree-days. A temperature-driven model is currently being developed and validated to determine how it could accompany plant phenological indicators as a tool for superintendents to time intervention tactics or scouting activities.

Pyrethroid Resistance

Recent studies demonstrated that there are dramatic differences in the susceptibility to pyrethroid insecticides of annual bluegrass weevil populations found in CT and the greater NY met-



With 2-3 generations per year, this weevil can build to astonishing populations (small patches may reach 1,200 larvae per square foot) that can stress or kill annual bluegrass in greens and fairways.

ropolitan area (DR, unpublished data). Whereas susceptible weevils succumb when exposed to 0.8 nanogram (one billionth of a gram) of the active ingredients of Talstar® (bifenthrin) or Scimitar® (lambda-cyhalothin), weevils from courses that have experienced intensive prior use of pyrethroids are only killed at a 30- to 200-fold greater exposure. These results point to the evolution of resistance to pesticides, a problem also commonly encountered with fungicides. Repeated use of effective pesticides selects for individuals with genetic traits that allow them to survive. Their offspring, in turn, will be more difficult to kill than the previous generations.

In the case of annual bluegrass weevil, selection with pyrethroids has left many courses with adult weevils that are now virtually impossible to kill with any pyrethroid. What has occurred is called cross-resistance, in which selection with one product allows the insect to withstand another, usually closely related insecticide. Recent investigations into the genetics and biochemistry of insecticide resistance in other insects demonstrated that a large suite of traits can simultaneously evolve to cause insecticide resistance, whereby each trait contributes different yet complementary roles allowing the insect to cope with toxic chemicals (3, 4).

As we have no reason to believe that the genetics or physiology of annual bluegrass weevil is different from other insects, superintendents should be aware that the physiological changes due to pyrethroid resistance may also make some other insecticides less effective. Although insecticides with new modes of action may provide additional options for managing pyrethroid-resistant weevils, new and old chemistries alike are jeopardized by the development of pyrethroid resistance.

One category of resistance is metabolic detoxification in which enzymes degrade insecticide molecules before they reach target sites in the nervous system. Several families of these enzymes exist, the most important of which are the cytochrome P450 system, carboxyesterases, and glutathione transferases, all of which confer some general detoxification capabilities.



Full bloom of *Rhododendron catawbiense* coincides with optimum nematode application timing, when the first generation of ABW larvae are leaving grass stems and starting to feed on the root crown.

Laboratory tests with adult annual bluegrass weevil demonstrated that the cytochrome P450 system is involved with resistance to pyrethroids. Unfortunately, other resistance traits are also involved, but these other traits have not yet been characterized (DR, unpublished data).

The cytochrome P450, and to some extent the carboxyesterase enzymes, can be blocked with a synergist called piperonyl butoxide (PBO), commonly found in household insecticide aerosols, and available in registered products (e.g., Exponent and Prentox PBO-8) intended for use in tank mixes. Although non-toxic by itself, piperonyl butoxide enhances the toxicity of the pyrethroid. When pyrethroid-resistant annual bluegrass weevil adults are exposed to piperonyl butoxide, their susceptibility to pyrethroids is restored. This capability can be exploited for diagnosing pyrethroid-resistant weevils in the laboratory or with diagnostic test kits. While piperonyl butoxide restores pyrethroid toxicity in the laboratory, it decomposes quickly when exposed to sunlight. Therefore, we do not know how well it might perform against populations of annual bluegrass weevil in the field. Field trials are underway to determine the potential for synergists such as piperonyl butoxide to restore the toxicity of pyrethroids to annual bluegrass weevil.

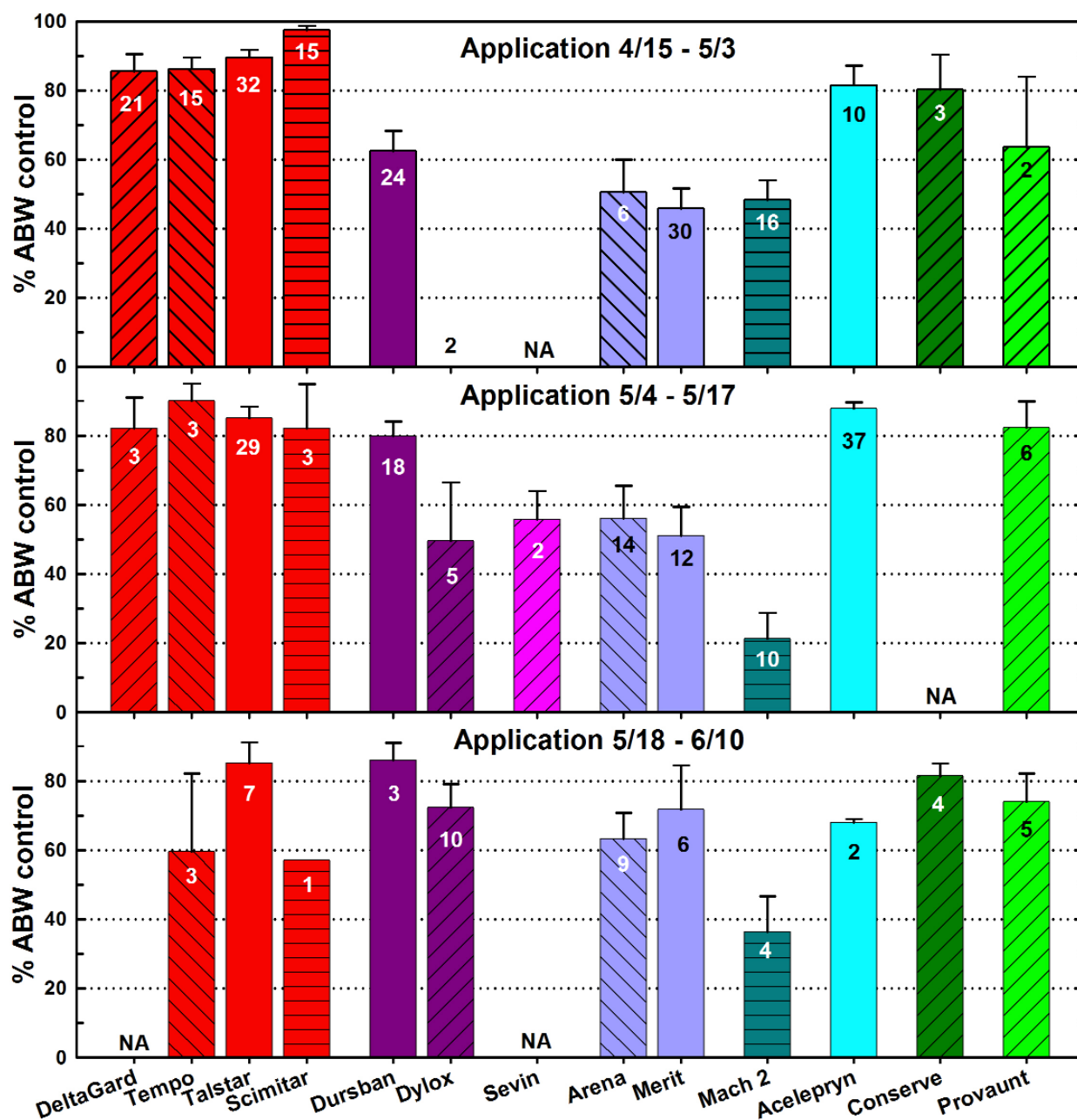


Figure 2. Efficacy of insecticides targeting annual bluegrass weevil as immigrating adults (applications 4/15 - 5/3, forsythia full to late bloom / dogwood full bloom), early stage larvae feeding within the grass stem (application 5/4 - 5/17, forsythia late bloom / dogwood full to late bloom / first anthesis of *Poa annua*) or late-instar larvae feeding on the crown within the soil (application 5/18 - 6/10, *Rhododendron catawbiense* full to late bloom). Insecticides are grouped by chemical class: red, pyrethroid; purple, organophosphate; magenta, carbamate; blue, neonicotinoid; teal, ecdysone agonist (insect growth regulator); aquamarine, anthranilic diamide; green, no class name assigned. The trials were conducted over the geographical range of all the collaborating authors, over the past several years. Dates are adjusted to the NY metropolitan area. The number of trials from which the mean and standard errors were calculated is given within the bar. Percent control is the percent reduction of larvae and pupae recovered relative to untreated check plots.

The continued use of pyrethroids, even when combined with piperonyl butoxide, should be approached with caution as there are additional mechanisms that could allow annual bluegrass weevil to circumvent insecticides. Besides meta-

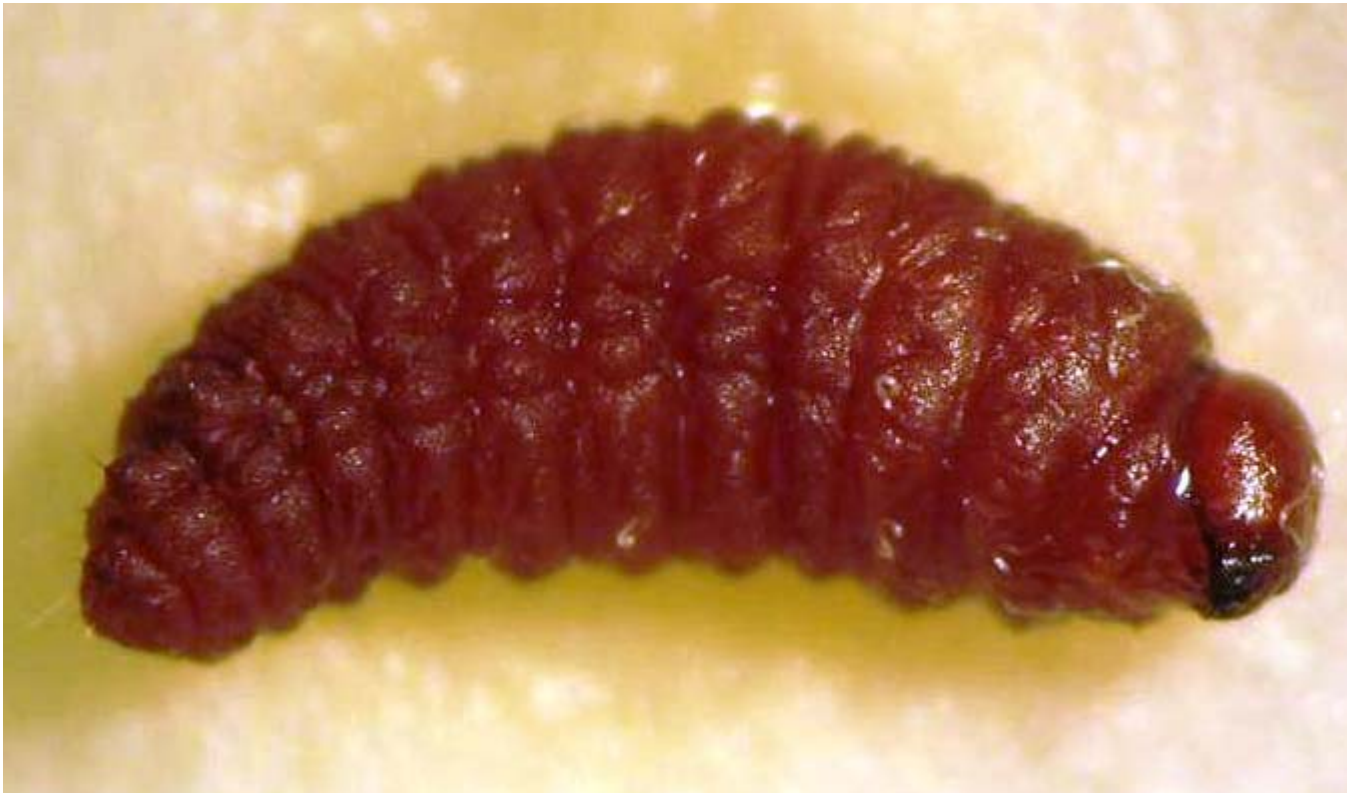
bolic degradation (which is currently taking place), the most important is target site insensitivity. Pyrethroids bind to sodium channels of nerves, which are their "target site," like a key fitting into a lock. If the shape of the lock changes

so that the key no longer fits, then the insect gains resistance to that class of insecticide. A few years of intensive selection with pyrethroid + piperonyl butoxide combinations could result in resistance due to target site modification, which could even make synergized pyrethroids ineffective. Continuing studies will determine whether target site insensitivity (along with detoxification) is contributing to poor performance of pyrethroids against adult annual bluegrass weevil.

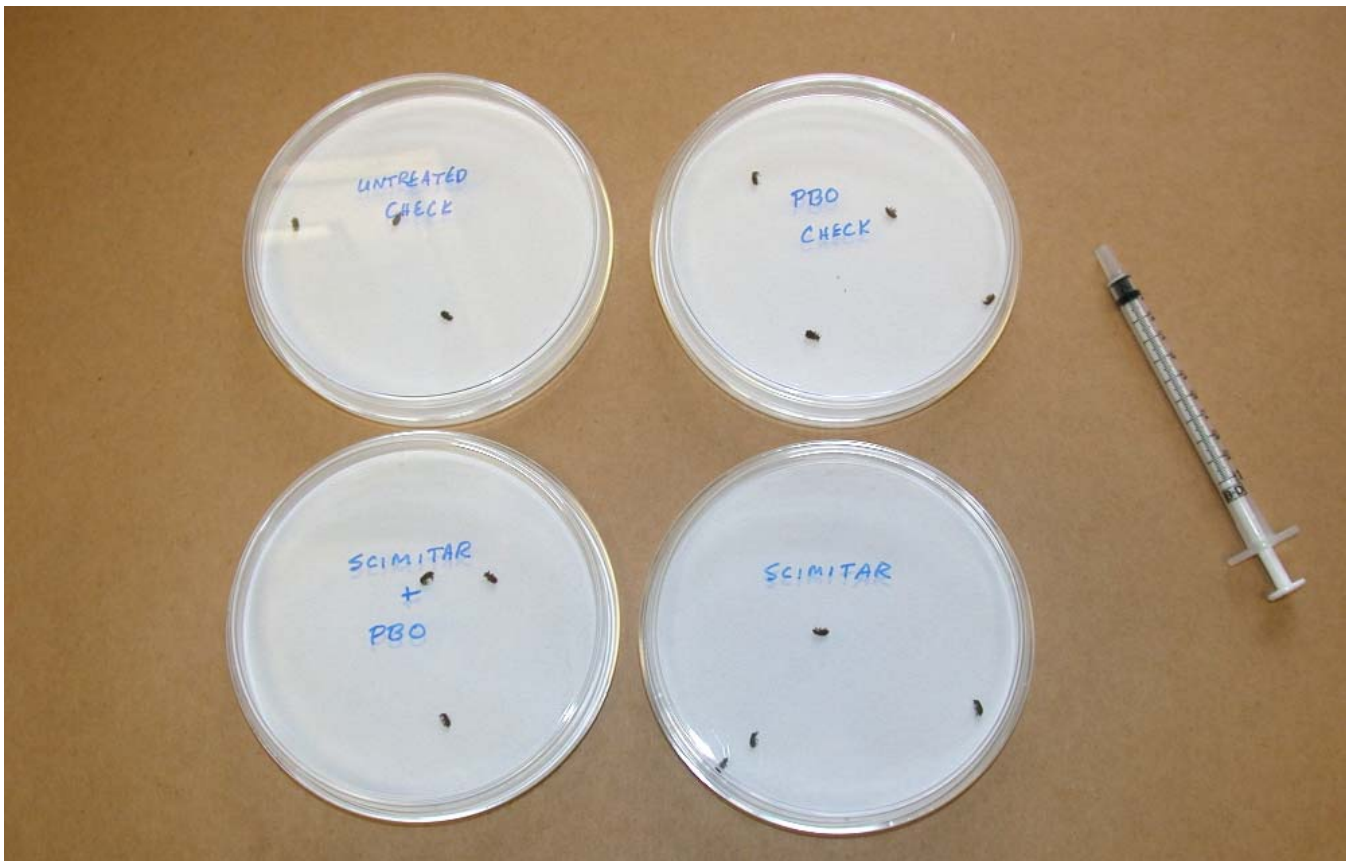
Another approach for managing existing resistant populations is to use insecticides in combinations with each other. Insecticides affecting different functions of nerves sometimes synergize each other. Essentially, it is like hooking up two amplifiers in series: each toxin causes the nerves to fire more often, and so the combination of two poisons will multiply their effects. Two companies are introducing insecticides containing a pyrethroid (bifenthrin) in combination with a neonicotinoid. Aloft® (Arysta) combines clothi-

anidin with bifenthrin, while Allectus® (Bayer) combines imidacloprid with bifenthrin. Neither clothianidin nor imidacloprid by themselves have reliable activity against annual bluegrass weevil; however, they do work together with bifenthrin to provide better effect (Cowles, unpublished data). Ongoing studies will further test prospects for these synergistic combinations, as well as those which do not include pyrethroids.

The basis for making intelligent pest management decisions is accurate information. A very simple diagnostic test kit is now available that will allow a superintendent to determine whether annual bluegrass weevil populations on a course are resistant to pyrethroids (Cowles, unpublished data). The test requires at least 24 adult weevils, which can be obtained with a soapy irritant drench or can be picked directly off the turf. Those adults are added to four disposable plastic dishes or zippered plastic bags along with a piece of moistened filter paper. That paper was previously dosed with



Probably the most effective way to counteract insecticide resistance is to rely more on biological control and less on insecticides. A study conducted over a three-year period on untreated fairways of three golf courses in New Jersey demonstrated that two species of entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) regularly infect ABW stages from the third larval instar through newly hatched adults. Shown above is ABW infected with *H. bacteriophora*.



When pyrethroid-resistant annual bluegrass weevil adults are exposed to piperonyl butoxide, their susceptibility to pyrethroids is restored. This capability can be exploited for diagnosing pyrethroid-resistant weevils in the laboratory or with diagnostic test kits. The kits usually provide an easily interpreted all-or-nothing response: weevils from susceptible populations all die on exposure to pyrethroid or pyrethroid + PBO, whereas ones from resistant populations only die when exposed to the pyrethroid + PBO.

the field rate (on an area basis) of a pyrethroid insecticide, and then kept at room temperature. The weevils are then rated as being alive or dead 2 days after dropping them onto the treated filter paper. The kits usually provide an easily interpreted all-or-nothing response: weevils from susceptible populations all die on exposure to pyrethroid or pyrethroid + PBO, whereas ones from resistant populations only die when exposed to the pyrethroid + PBO. Although 24 weevils can provide statistically valid results, using twice as many weevils is advisable.

It is unknown whether larvae from populations where the adults have developed resistance to pyrethroids are also resistant. Based on insecticide tests (Figure 2), pyrethroid applications not only kill annual bluegrass weevil adults, but they also kill larvae traveling from stem to stem or feeding as late instars on the plant crowns. Therefore, pyrethroids have been effective when

applied when many life stages of weevils were present. If adult weevils are resistant to pyrethroids but the larvae are not, then a change of application date -- from targeting the adults to targeting the third through fifth instars--may still allow these products to be useful.

Non-pyrethroid Alternatives

The results of several years of insecticide trials targeting annual bluegrass weevil are summarized in Figure 2, in which the efficacy of many newer insecticides are contrasted with pyrethroids and older chemicals. Research trials conducted by most of the authors have confirmed that effective alternatives to pyrethroids do exist for combating annual bluegrass weevil.

Rather than relying on pyrethroids to intercept and target adults immigrating onto fairway from overwintering sites, more emphasis may



Over the past few years, annual bluegrass weevil has become one of the most difficult insect pests to manage on golf courses.

have to be placed on targeting the larvae slightly later in the season. An advantage of this approach is the opportunity to spot treat areas in a curative fashion, i.e., once scouting has indicated where populations are localized and determined whether thresholds have been surpassed. That degree of fine tuning is not possible with the standard preventive approach. Along with savings from unnecessary insecticide applications, spot treatments could reduce the total area treated and the proportion of the weevil population being selected with insecticides, thereby reducing selection for resistance.

Among the non-pyrethroid products being confirmed as effective against ABW are trichlorfon (Dylox®), spinosad (Conserve®), chlorantraniliprole (Acelepryn®), and indoxacarb (Provaunt®). Although we know the optimal timing for using Dylox, more studies are needed to make reliable suggestions for the use of the other chemistries.

As an older chemical, researchers have had the longest experience with Dylox. Dylox is a contact insecticide and is most effective when used as a curative or rescue treatment that targets larvae after they have exited the grass stem. While younger (smaller) larvae live within their host as stem borers, as they mature and get larger they move out of the stem and reside at the soil surface where they feed externally on crowns.

Because Dylox is an organophosphate, superintendents must be aware of this insecticide's heightened human and environmental toxicity.

Acelepryn acts systemically, entering the plant and becoming ingested by the insect. This insecticide can be applied from peak emergence of adults to the appearance of the first young larvae in the stems. After eggs are laid and larvae emerge, the insecticide is in place within the plant to target the young larvae feeding within the stem. However, more trials are necessary to study the efficacy of earlier applications and especially of curative applications.

Provaunt and Conserve have each demonstrated some activity against both annual bluegrass weevil adults and larvae. Intriguingly, indoxacarb is made more toxic to the insect through the action of one family of enzymes (carboxyesterases) implicated in resistance to pyrethroids. Therefore, Provaunt may have special value for targeting pyrethroid-resistant ABW populations. Both Conserve and Provaunt are



Damage from annual bluegrass weevil often is found along the edges of fairways and putting green collars.

known from agricultural systems to be less toxic to beneficial predators and are overall much less toxic to the applicator, golfers, and the environment than Dylox or pyrethroids.

Probably the most effective way to counteract insecticide resistance is to rely more on biological control and less on insecticides. Predators and pathogens are the perfect countermeasure to insecticide resistance because they kill the survivors of insecticide treatments. Older insecticide chemistries such as pyrethroids and Dylox are highly toxic to predators and parasitic insects. Newer chemistries such as Conserve, Provaunt, and Acelepryn will probably cause less collateral damage to populations of natural enemies, and thereby move us closer to an integrated system where insecticides and predators can work in concert.

Since annual bluegrass weevil is often continuously present from mid-summer to autumn, superintendents need to be aware that insecticide applications targeting other turf pests (e.g., white grubs and cutworms) can cause inadvertent selection of annual bluegrass weevil populations. Adoption of newer insecticides with reduced impact on beneficial insects will help the overall turf insect management system.

Biological Control with Nematodes

We are currently investigating entomopathogenic nematodes (EPNs) as a biological control option to suppress annual bluegrass weevil



After ABW larvae have completed feeding on annual bluegrass, larvae transform into pupae (shown above), and then into adults.

populations. Entomopathogenic nematodes are microscopic roundworms found in the soils of most ecosystems. They attack insects by entering through natural openings or some instances directly through the insect's cuticle. Once inside the insect's body cavity, entomopathogenic nematodes release symbiotic bacteria that assist in killing the insect (usually within 48 hours).

The bacteria break down the insect's internal tissues and provide a substrate for entomopathogenic nematode reproduction. After one to three reproductive cycles within the insect (usually one to two weeks) thousands to hundreds of thousands of juvenile nematodes exit the insect cadaver in search of new hosts. Their ability to cause rapid death to the insect and their high reproductive capabilities make them ideal candidates for biological control of soil dwelling turf-grass pests.

A study conducted over a three-year period on untreated fairways of three golf courses in New Jersey demonstrated that two species of entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) regularly infect ABW stages from the third larval instar through newly hatched adults (5). The impact of entomopathogenic nematodes on annual bluegrass weevil was variable, ranging from 0 to 50% mortality within annual bluegrass weevil generations. Entomopathogenic nematodes were found during all months that annual bluegrass weevil stages were detected on fairways (early April to mid-October), yet their densities were shown to fluctuate dramatically with annual bluegrass weevil densities and environmental conditions. Entomopathogenic nematodes are sensitive to extreme moisture and temperature conditions. Not surprisingly, entomopathogenic nematode populations crashed during excessively hot conditions of the 2005 and 2006 summers.

The variable annual bluegrass weevil mortality and sensitivity to environmental extremes suggest that resident populations of entomopathogenic nematodes are unlikely to reduce annual bluegrass weevil populations consistently to the low thresholds for damage imposed by most golf course operations. However, the ability of natural

populations to infect a wide range of annual bluegrass weevil stages and cause moderate generational mortality suggest that there is potential in using entomopathogenic nematodes as inundative, curative controls against the damaging soil dwelling stages of annual bluegrass weevil.

We are testing the virulence of five commercially available entomopathogenic nematodes (*S. carpocapsae*, *S. feltiae*, *S. kraussei*, *H. bacteriophora*, *H. megidis*) and two entomopathogenic nematode isolates collected from naturally infected annual bluegrass weevil cadavers (*S. carpocapsae*, *H. bacteriophora*) against different ABW stages. In laboratory assays, adults collected as they emerged on fairways in April 2007 had similarly low susceptibility to entomopathogenic nematodes as fall-collected adults. *S. carpocapsae*, the top performing species, only provided 50-60% mortality after 12-day exposure to 250 nematodes per weevil. No differences were observed between our locally isolated entomopathogenic nematodes and their commercial counterparts. The low susceptibility of adults even under ideal laboratory conditions suggests that entomopathogenic nematodes are not likely to replace preventive chemical pesticides for adult control.

Virulence of entomopathogenic nematodes to ABW fourth- and fifth-instar larvae was assessed in field-infested turf cores in the laboratory. Fourth-instar control ranged from 65 to 100% with 97% for *S. feltiae* and 100% for the field-isolated *S. carpocapsae*. Fifth-instar control was overall lower but reached 90% for *S. feltiae*. This suggests that entomopathogenic nematode applications should be targeted against the early fourth instars to maximize control and minimize the potential for turf damage.

Field trials using one endemic and five commercial entomopathogenic nematode strains at two rates indicate that high levels of control can be achieved with well-timed applications against first generation soil stages of annual bluegrass weevil. Our applications have been timed to follow the peak in third-instar densities (the last stage typically found boring within the plant), before a majority of the annual bluegrass weevil stages have entered the soil.

In 2006, entomopathogenic nematodes provided 62 to 92% control of annual bluegrass weevil when applied to moderate infestations (~25 annual bluegrass weevils/ft²) at the rate of 1 billion entomopathogenic nematodes per acre. *S. feltiae* (92% control) and the endemic strain of *H. bacteriophora* (85% control) provided the greatest benefit. More variable control (0-87%) was observed in the 2007 field trials and is likely attributable to high annual bluegrass weevil densities in the plots (> 70 annual bluegrass weevils/ft² in the untreated controls). *S. carpocapsae* (1 billion/acre) provided the most consistent control (70%). However, split applications of *H. bacteriophora* (87%) and a mixed species treatment (*H. bacteriophora* + *S. carpocapsae*) (82%) provided the greatest reductions in annual bluegrass weevil densities. Both of these treatments were able to reduce densities below damaging threshold in the field (< 40 annual bluegrass weevils/ft²).

We will continue to investigate the potential of entomopathogenic nematodes for annual bluegrass weevil management with the most consistent candidates (*S. feltiae*, *S. carpocapsae*, *H. bacteriophora*). In 2008 we are addressing the effect of timing and application rates on the level of annual bluegrass weevil control.

Acknowledgments

The authors would like to thank USDA Hatch Regional Hatch Project NE-1025, USDA Northeast Regional IPM Project #2007-34103-18124, GCSAA, USGA, GCSANJ, LIGCSA, Keystone AGCS, Pennsylvania Turfgrass Council, and donations from Arysta Corporation, Bayer Environmental Science, DuPont, FMC Corporation, and Syngenta for supporting this work. Graduate students Maria Diaz and Masanori Seto, technicians Chuck Dawson, Danny Kline, and Alan Rollins, and numerous student assistants contributed greatly to this research. The authors would also like to thank the participating golf superintendents and their clubs for allowing us to do research on their courses.

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