A PRACTICAL RESEARCH DIGEST FOR TURF MANAGERS

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Field Testing of Biological Pesticides

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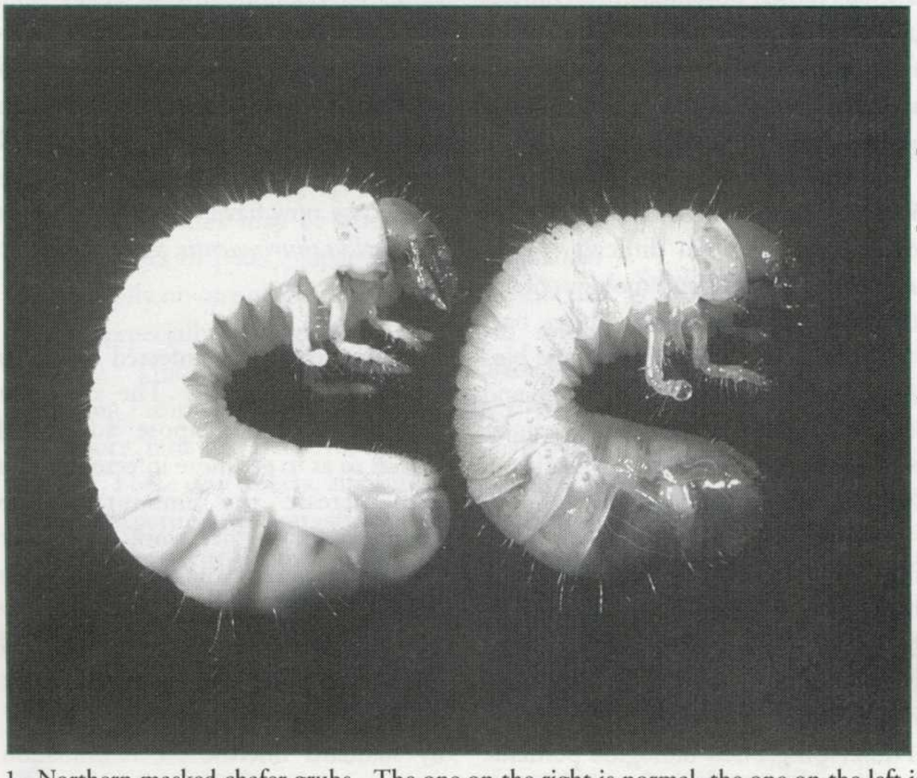
Over the last two decades, there has been a steadily increasing outcry for alternatives to standard, synthetic pesticides. Rachael Carson's "Silent Spring" was the first major alarm sounded pointing out that synthetic pesticides can often have widespread and undesired affects on animals and the environment. In the 1970s and 1980s, environmental groups, politicians and celebrities continued to decry the use of pesticides. Eventually, whether founded in fact or fiction, many people began to question the use of pesticides and sometimes attempted



All photos provided courtesy of Dr. David J. Shetlar

1. Northern masked chafer grubs. The one on the right is normal, the one on the left is infected with a milky disease. Notice the drop of blood at the end of the snipped leg. It is clear in the normal grub and milky in the infected grub.

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to get local regulations passed that would ban their use in domestic landscapes and on school grounds. Many home owners now are requesting that pesticides not be used unless absolutely necessary and many more are seeking alternatives to standard pesticides.

Entomologists have long been aware that most insect and mite pests have many predators, parasites and pathogens (lethal diseases) that can keep their populations below damaging levels. However, with the development of synthetic insecticides, much of the work on these biological controls was abandoned until the last two decades. This renewed interest in biological controls is in response, not only to the public outcry for alternatives, but because continued use of pesticides has resulted in the emergence of pests resistant to many pesticides and the uprising of "secondary pests." Secondary pests arise when their normal natural controls are killed by pesticides. Without these biological controls there is nothing to stop the increase of these pests to damaging numbers.

Because predators and parasites of insect pests are often difficult to rear and manage, many entomologists have turned to their pathogens - fungi, bacteria, virus and nematodes. These biological controls may be mass reared and are often applied as if they were regular pesticide sprays. This has given rise to the term "biological pesticide."

One of the confounding problems found, when developing a biological pesticide, is the field testing of these living organisms as if they were nonliving, chemical pesticides. The process of developing a new biological pesticide is difficult and expensive. In order to bring a biological pesticide to market, a company has to discover the pathogen, learn how to produce it, test it in small plots, test it in larger production settings, and market their new product.

How are biological pesticides discovered?

Location and identification

Many university and industry scientists are busy searching the globe for any new species or strains of diseases that attack harmful insects. These searches take several forms. Looking at native populations of the target pest may uncover significant natural controls, especially if the pest is an import to the US (i.e., Japanese beetle populations in Japan and China). Often, insects that are closely related to the target pest yield diseases with control potential (i.e., looking at sugar cane grubs for diseases that may attack turfgrass infesting grubs). Other scientists look for new forms of known pathogens (i.e., simply by taking soil and dust samples from around the world, we now have over 5,000 strains of *Bacillus thuringiensis*, a known insect pathogen).

In many cases, an infected insect is found and collected. The specimen may be used to expose additional insects so as to get more infected insects and increase the amount of the pathogen for further work. In other cases, especially if the pathogen looks like a genus of a known pathogen, the disease may be cultured on an artificial medium in order to get a larger sample. These samples are then "characterized" by standard microscopic examination (e.g., spore size and shape) or by molecular methods (e.g., protein characteri-

2. Japanese beetle grubs infected with *Beauveria* fungus.

zation, genetic makeup, etc.). Tests have to be performed to determine if the pathogen is, indeed, lethal to a target pest and if the new pathogen is an improvement over known pathogens. If the pathogen is sufficiently different from known examples and is an improvement, further development takes place and the pathogen is often "patented" in order to protect any future economic benefits.

Screening problems

Standard screening for new pesticides is usually targeted against the damaging stage of a pest while biological controls may be active only on specific stages or ages of pests. Japanese beetle adults can dine happily on one of the new BT strains while the larvae, especially young ones, are killed rather quickly. This brings up another problem with screening - using standard ages of the target. Many laboratory tests are performed on newly hatched insects. These tiny insects, all of the same age, serve as ideal experimental animals, but in nature, insect populations occur in mixed ages. As an example, several BTs will kill first instar sod webworms but little or no effect is obtained against the fourth, fifth and sixth instars. In cool-season turf in late June, there may be first through sixth instar bluegrass sod webworms present in any patch of turf. Therefore, standard screens using a uniform age-class of insects does not mimic field conditions.

Field testing - small plots

Figuring dose

Standard pesticide screens usually involve a range of concentration so that a dosage rating, usually the LD50, can be determined. However, with many biological pesticides a single spore or nematode can potentially kill the insect. Therefore, when varying concentrations of a pathogen yields no LD50, how does one determine the amount to use in the field? In many cases, simple guesses are made!

Benefits of small plot tests

Small test plots, usually in the range of ten by ten feet or less, are useful because small amounts of the pathogen can be used, the pest populations can be measured easily and are probably more uniformly present than in larger areas. Applications can also be determined more precisely by using highly calibrated equipment, and special environmental needs can be met, such as immediate watering. In these small plots, extreme ranges of the biological pesticide can be applied in order to better determine what the actual dosage has to be to perform adequately.

Small plot problems

A turfgrass stand is a complicated habitat. This habitat consists of the turf plants (leaves, stems, roots), thatch, soil of varying textures and chemical makeup, changing moisture levels, other microbes, insects and animals. This is obviously very different from the laboratory petri dish in which a target insect and pathogen have been placed together. Therefore, most initial small plot tests involve a bit of "just tossing it out" (the pathogen, that is) experimentation. If this general toss doesn't work, then further tests are needed to try and hold some of the turf habitat traits constant. Sites with and without thatch may be needed, varying soil pH and moisture may be needed and other, possibly competing, organisms will have to be measured or eliminated.

Measuring efficacy

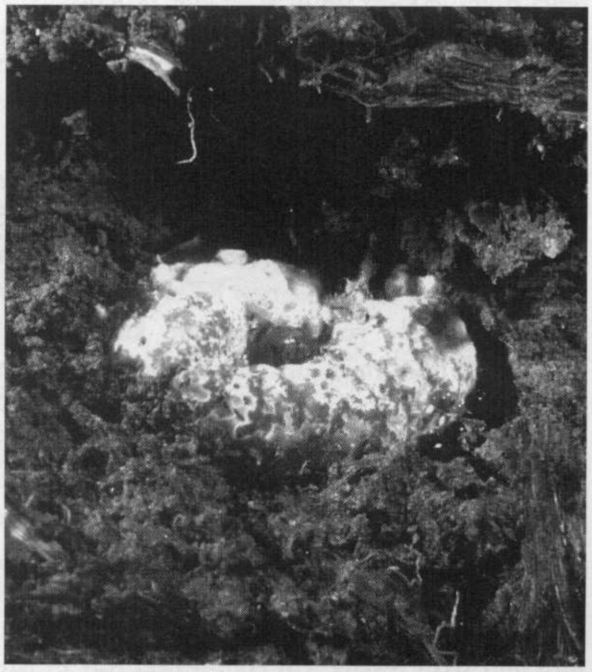
In standard chemical insecticide tests, the chemical is applied and after a short period, usually a week to a month, the insect "kill" is measured. This is usually based on the number of live insects remaining in the treated plots compared to "control" or "check" plots that were not treated. Many biological pesticides take considerably longer to act or they may do unexpected things to the insect. When white grubs become infected with the milky disease bacterium, *Bacillus popilliae,* they usually stop feeding immediately but they may remain mobile for several weeks to months before actually dying. Likewise, when caterpillars pick up certain strains of BT, they don't die within minutes or days, but may take one to two weeks before they expire.

Another problem with evaluating biological pesticides is choosing a "standard" for comparison. In chemical tests, this standard is often the top selling insecticide or a known insecticide within the same general chemical category. In many cases, biological pesticides are compared to these same standard pesticides. Standard pesticides have immediate effects while biological pesticides, especially ones involving living microbes, may be progressively lethal over time. Insects that became infected this week may not die until next season. However, they were eventually eliminated from contributing to the next generation.

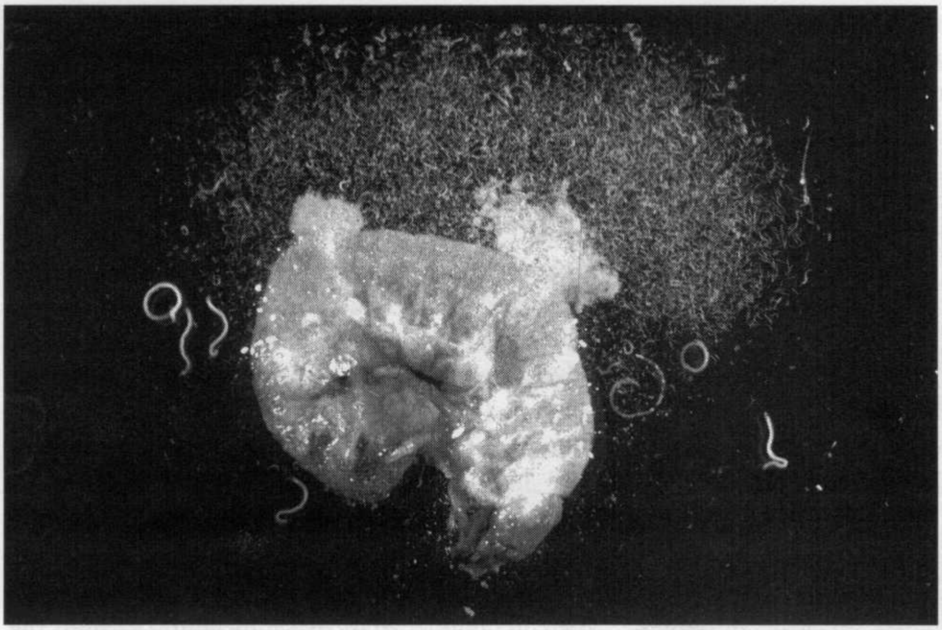
Scaling up

Production

Once a biological pesticide has been successfully isolated, laboratory tested and small plot tested, the next major hurdle is to produce sufficient quantities of the pathogen to perform large plot or commercial sized applications. For many pathogens, this means moving from "counter top" production (production of small quantities in petri dishes or flasks), to medium fermentor production (perhaps ten to 100 gallons at a time). At this stage, many biological pesticides suddenly run into problems. In the larger production setting, the pathogen may lose its virulence or activity. Many bacteria and fungi seem to become "lazy" in the larger fermentor setting. They may lose their toxins, or the amount of toxin produced may be reduced. They may lose their viability or survivability. Therefore, constant testing for quality control must be performed in order to ensure that the cultured pathogen remains as active as the original organism.



3. A northern masked chafer grub infected with *Metarhizium* fungus in the process of producing its greenish spores (the darker patches within the white mycelia).



4. A Japanese beetle grub broken open to show the infection with *Steinernema carpocapsae* nematodes. The larger white curled nematodes are the adults while the "halo" around the body consists of hundreds of the new infective juviniles.

Formulation and packaging

In small plots, it is fairly easy to deal with unusual small containers of liquids containing a biological pesticide. However, if a gallon of the original material only covers a thousand square feet, then about 44 gallons will be needed to cover an acre. When compared to standard insecticides that may require one or less gallons to cover an acre, the weight incurred in shipping becomes a real, and expensive, problem. Many biological pesticides have limited shelf life. Most entomopathogenic nematodes can be kept viable for six months if not exposed to extreme heat. Many bacteria form resistant spores that can remain active for several seasons.

Commercial testing: making a fit

Once the small plot tests have yielded the specifics about application that allow the biological pesticide to perform at its best, fitting the new control product into the existing cultural system can be a real difficulty. Turfgrass is managed with a variety of fertilizers, herbicides, fungicides and insecticides. In some cases, these chemicals may be lethal to the biological pesticide, especially in tank mixes. Therefore, if a broadleaf herbicide is lethal to a bacterium being applied at the same time to kill black turfgrass ataenius adults, the applicator will often opt for a standard insecticide that will not require two separate applications.

Training the user

Expectations

Based on past experience with standard insecticides, most people making their first application of a biological pesticide expect the same things to happen - rapid, and often visual, kill of the target pest. Golf course superintendents "expect" to see a "body count" of cutworms within hours after applying a standard insecticide. However, if a nematode or spinosad (a pesticide derived from a bacterium) is used, no cutworms appear on the surface, within hours or days.

A case study

Entomopathogenic nematodes

Attempts to use two groups of insect killing nematodes have occurred since the 1930's. These are now in the genera, *Steinernema* and *Heterorhabditis*. As previously mentioned, applied field work on these organisms was abandoned when modern synthetic pesticides were discovered.

In the late 1970's, interest in these nematodes was renewed in academic circles and a small "biotech" firm was established in California (now Biosys, Columbia, MD). With some infusion of venture capital, this firm spent considerable time learning how to produce moderate numbers of nematodes, in vivo (growing them in living insects), then large numbers of nematodes, in vitro (growing on artificial media), and finally massive numbers in thousand gallon fermentors.

At first, only one nematode, *S. carpocapsae,* appeared to be "cooperative" and readily adapted to in vitro production. Numerous tests were performed in the laboratory, in petri dishes and small containers containing target insects. There appeared to be few insects that *S. carpocapsae* could not kill in this manner. However, when university entomologists were given this nematode, they soon observed that field applications were not working or the nematodes only worked once in a while.

My evaluations of this nematode began in 1986. Tests in the laboratory demonstrated that this nematode could easily kill sod webworms, black cutworms, Japanese beetle grubs and northern masked chafer grubs. However, in small plot tests on a golf course, the grub control was sporadic and marginal. Realizing that these nematodes were applied in the microscopic, infective juvenile stage, we suspected that the standard application techniques may be killing the nematodes if immediate irrigation did not follow the application. Sure enough, if the nematodes were immediately watered into the turf with a minimum of $\frac{1}{4}$ inch of water, efficacy greatly increased (Shetlar et al. 1988). This has been reconfirmed through work by Downing (1994) and Yeh and Alm (1995).

Subsequent to this finding, we began larger scale treatments of entire lawns. Again, even with irrigation, the nematodes seemed to fail. Fortunately, we had saved some of the nematode material that was used. Under the microscope these nematodes appeared alive and healthy. However, when given a chance to kill insects in petri dish tests, nothing happened! Apparently, the nematodes had lost their ability to kill the insects. Was this a case of a "lazy" pathogen, created by the *in vitro* process? Some rapid investigations by the Biosys scientists found that the active nematodes had non-pathogenic bacteria in their storage organs. The nematodes don't actually kill their host directly but they regurgitate a lethal bacterium in the insect's body cavity. The bacterium quickly kills the insect and the nematodes begin to reproduce while feeding on these bacteria. In our case, the bacterium had lost its virulence in culture. The nematodes didn't know the difference between lethal bacteria and nonlethal bacteria. The result, the nematodes were "shooting blanks"!

Armed with this new information, Biosys and many university researchers performed bioassays with the nematodes before using them in the field. This is now a standard procedure during the "quality control" process of nematode production. Finally, armed with nematodes that worked and the knowledge that irrigation and avoidance of direct sunlight improved nematode survival, Biosys wanted to begin selling their nematodes on a commercial basis. While working cooperatively with university researchers, several larger scale applications of nematodes were used by golf course superintendents for management of their black cutworms on greens and tees. It soon became evident that, while effective when used according to directions, the nematodes were not easy to mix and apply, when compared to standard insecticides. The nematodes arrived in jars containing a screen or sponge and these had to be thoroughly rinsed out in clean water. Many superintendents balked at the prospect of rinsing and washing five to 20 of these containers. Golf course superintendents also like to apply fungicides at the same time that they apply insect control. Many of these fungicides, herbicides and previously applied insecticides can be lethal to the nematodes.

The end result, golf course superintendents only used the nematodes if they had no choice. Some superintendents felt that being able to say that they had eliminated the use of insecticides to manage one or two pests was worth the extra effort. Most, however, wanted a biological control but something less difficult to use.

Subsequently, Biosys pioneered a new formulation, a water disbursable granule. The only draw back was that the granule had a shorter shelf life (less than one year) and fewer nematodes per unit volume could be contained. Through some inventive marketing, this product ended up being best suited for the home owner trade. In these markets, home owners only want small amounts to treat special problem areas of their landscapes. When flea larvae and pupae were found to be susceptible to these nematodes, the market increased dramatically.

Also, during the development of *S. carpocapsae* for cutworm and sod webworm management, different nematodes were being discovered and tested for other insect targets. University of Florida researchers soon found a nematode, now *S. scapterisci*, that appeared to be superior to any other at locating and killing mole crickets. This nematode was eventually licensed to a firm in Florida under the trade name of Proactant-Ss™. Soon thereafter, *S. riobravos* was described and this species also was good at attacking mole crickets. It is sold under the trade name Vector-MC™. Both products have enjoyed an increase in sales as golf course superintendents become more comfortable with the idea that these nematodes need immediate irrigation, applications should be made in the late afternoon to avoid direct sunlight, and dead mole crickets do not appear on the surface the next morning.

On other fronts, species of *Steinernema* that appear to be more suitable for white grub control have been reared in quantities sufficient to make them marketable (Selvan et al. 1994). Likewise, the *Heterorabditis* species, which have been difficult to rear in mass, are now also being grown in commercial quantities. *H. bacteriophora* and related species have always been among the best agents for control of white grubs.

In retrospect, field evaluation and development of biological pesticides requires constant fine tuning of handling, mixing and application techniques. After becoming complacent to the sameness of using standard pesticides, we have had to find all the weak links in delivering a biological pesticide and find solutions. For the end users, this will also require rethinking their ways of applying and utilizing control products.

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Bacteria

Several bacteria produce toxins that affect insects or cause an infection that kills the pests. Most bacteria produce a spore that can survive harsh environmental conditions and many bacteria can be grown in artificial media, therefore reducing the cost of their production.

Bacillus thuringiensis (commonly called "BT") has numerous strains that produce a toxin that affects the gut lining of specific insect groups. Affected insects stop feeding and die within a few days.

BT variety *kurstaki* - is registered under several trade names (Dipel Dust™, Sod Webworm Attack™, Bactosphene™, etc.) and is registered for sod webworm. Laboratory tests indicate good efficacy against first and second instar larvae but poor activity against larger larvae. The BT var. *kurstaki,* strain 'Spodoptera' (Javelin™) has shown good activity against the tropical sod webworm in Gulf States.

BT variety *israelensis* - is registered under several trade names (VectoBac™, Bactimos™, etc.) and is effective against mosquito larvae in water as well as black fly larval control in streams.

BT variety *japonensis,* strain 'buibui' - has recently been tested for control of white grubs with good success. Registered products are expected within a couple of years.

Bacillus popilliae is called the milky disease of white grubs. The bacterium causes infected insects to stop feeding and their body fluids to turn a characteristic white color. Infected insects may take weeks or months to die, even though they have stopped feeding. Numerous strains have been identified that attack certain species of white grubs. Only the Japanese beetle strain is in commercial production under several trade names (Milky Spore™, Doom™, Japademic™) by two firms: Fairfax Labs in New York (914)266-3705, and St. Gabriel Laboratories in Gainesville, VA (800)801-0061. Field studies in New England States have yielded 30 to 50% infection. Tests in Ohio and Kentucky have resulted in 20% infection or less.

Serratia entomophila is called the amber disease of white grubs. The bacterium causes infected insects to stop feeding and their body fluids to turn a honey-amber color. Affected insects turn flaccid within a few weeks and soon rot. A commercial product, Invade™, is being used in New Zealand for white grub management in pastures but no products are currently registered in the United States.

Fungi

In general, fungi usually require high moisture and are relatively intolerant of sunlight. Though they can often be cultured on artificial media, creating the right conditions for spore formation is usually the major problem in commercial production.

Beauveria bassiana is called the white fungus of insects. Infected insects become sluggish and eventually stop all activity. Within a few days or weeks the fungus sporulates by forming a dense white, cottony mass over the insect exterior. Chinch bugs and billbug adults are commonly attacked during periods of rainy, warm weather. A recent product, Natuaralis-T ™, has been registered for use against a variety of agricultural pests as well as turf infesting mole crickets and chinch bugs. Sufficient replicated field tests of this material have not been performed against these turf pests.

Metarhizium anisophiae is called the green fungus of insects. Infected insects become sluggish and stop all activity. Fungal sporulation begins as a white coating but the blue green spores soon coat the exterior of infected insects. Though several strains are being developed by foreign and U.S. companies for management of white grubs, no commercial products are yet available.

Entomopathogenic Nematodes

These nematodes are specialized roundworms that carry a bacterium which is lethal to insects. The juvenile nematodes usually enter insects through the mouth, anus or breathing pores though some species may be able to penetrate through the insect cuticle. Once inside the insect, the nematode regurgitates its specific bacterium. The bacteria multiply, killing the insect and preventing other bacteria from colonizing the cadaver. The nematodes feed on the bacteria, mature and reproduce. These nematodes are not harmful to animals other than insects and they can not enter plant tissues.

Steinernema nematodes are commercially available under several trade names (Biosafe™, Vector™, Savior™, Scanmask™, etc.). *S. carpocapsae* is the most commonly produced species because of the ease with which juveniles can be grown in large fermentation tanks. *S. carpocapsae* is most useful for management of cutworms, sod webworms, billbugs and fleas. However, nematodes are very susceptible to desiccation, can not tolerate direct sunlight, and they may be killed by other insecticides or fungicides commonly applied to turf. *S. feltiae* and *S. glaseri* are also marketed for surface insect and white grub management. Steinermatid nematodes, in general, have not performed well for management of white grubs. *S. riobravos* (Vector-MC™) and *S. scapterisci* (Proactant-Ss™) are species registered for control of mole crickets and properly made applications have produced satisfactory control.

Heterorhabditis bacteriophora nematodes are commercially available but generally from smaller suppliers. Recently, Ecogen has begun larger scale production of this nematode under the name of Curiser™. This nematode has generally been the best performing species for control of white grubs.

Terms to know:

LD50 - the lethal dose of a pesticide or chemical required to kill 50% of a group of exposed plants or animals.

Entomopathogenic - literally insect killing disease. Entomopathogenic nematodes are microscopic nematodes that enter insects and release an insect killing bacterium.

In vitro - outside the body, to grow something in an artificial medium.

In vivo - inside the body, to grow something within a living organism.

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Erratum

In our most recent survey of golf course superintendents, under the question: "Which other publications do you ***read***?" we erroneously included *Golf and Environment* in the list of alternatives. We should not have done that as *Golf and Environment* is a **video** magazine.

The results published were not accurate because respondents were unsure of whether *Golf & Environment* referred to the popular video magazine, or a print publication they never heard of.

We regret any confusion this may have caused and apologize for the error.