



Turf Research &
Advisory Board

Argentine Stem Weevil Control using Entomopathogenic Nematodes (ENs)



Introduction

The Victorian Golf Association Turf Research and Advisory Board funds trial work of potential benefit to Victorian golf clubs, and the wider turf industry. A representative survey of Victorian golf courses in 1998 indicated there was pressure on clubs and their Superintendents to reduce pesticide use and investigate the new non-pesticide and biological control products coming onto the market. One such product is the Entomopathogenic Nematode (EN) formulation developed by Dr. Robin Bedding, CSIRO Entomology, Canberra. ENs have provided excellent kills on scarab pests in turf but had not proven their efficacy on Argentine Stem Weevils prior to this trial. The results from a trial plot at Tullarmarine Golf Club showed a 90% kill of larvae demonstrating that the EN product can be considered a mainstream Stem Weevil control product.

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SEPTEMBER 2000

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Argentine Stem Weevils

The Argentine Stem Weevil (*Listronotus bonariensis*) is a member of the order Coleoptera (Beetles). Its former name was *Hyperodes bonariensis*. It is a major pest of cool season turfgrasses in Australia, particularly Bent or *Poa annua* greens. In New Zealand the insect is known as the Ryegrass Stem Borer and is primarily a pest of ryegrasses and *Poa annua* in pastures. In the US and Canada a similar pest is known as the Hyperodes Weevil or the Annual Bluegrass Weevil (*Listronotus maculicollis*).

The mature adult weevil is a grey/brown hard bodied insect around 3mm long. The female lays up to 300 eggs over a number of weeks. The eggs are laid in the grass leaf sheath from which the tiny maggot-like larvae hatch. The larvae tunnel and mine their way into the grass stems until they are too large for the stem diameter (this will depend on the grass species they are attacking). After this stage the larvae will drop out of the stems and move into the soil/thatch layer to feed on the roots. The larvae have a creamy white body with a tan coloured head (see Photo 1). They don't have legs (unlike the cockchafer group). They grow to around 5mm long before pupation.



Photo 1: Larva of Argentine Stem Weevil showing discolouration due to infection from entomopathogenic nematodes.

The pupae are immobile in the soil and metamorphose over several weeks to the adult form. The new adults are light brown in colour and rest in the soil until their exoskeleton hardens and turns dark. Adults emerge to mate and lay more eggs. They can fly for short distances in calm weather but usually don't if there is enough food at the original site.

New Zealand research indicates a high level of variability in life cycle from the North Island to the South Island so one would expect even greater differences in biology under Australian conditions. There are several generations per season which

depends on the climate at a particular location. The rate of insect development is temperature dependent so entomologists frequently use a Degree-Day model to estimate the time required to complete a full life cycle. This model tallies the cumulative average daily temperatures until a pre-determined number is reached. The New Zealand research concludes a full life cycle takes around 360 Degree-Days - this could be as little as 4 weeks in Victoria's summer climate. It is suspected that there are three to four generations per year from around August to May each year, so a small initial population can build up over the summer if uncontrolled. Quite often the major damage is caused in March because of the high numbers that have built up.

Adults that emerge in the autumn overwinter in the thatch layer. With the onset of warmer temperatures and longer daylength in the following spring they lay the first eggs of the next year's cycle.

The larval stage does all the damage, both from stem-boring and from root feeding. Despite their small size, larval numbers as low as 100 per square metre can do substantial damage, especially when combined with heat and drought stress. The damage appears as indistinct patches of yellowing turf, followed by browning and death (see Photo 2). Weevil damage is often mistaken for heat or drought stress - conversely, drought stress is often mistaken for weevil damage. Only careful inspection of turf samples, or use of the Pyrethrum detection method (see Appendix 2) can determine the actual cause.

Stem Weevils are usually treated with insecticides especially where the turf has a history of damage. Preventative applications are often applied in spring and several follow up applications may be required through the year. Stem Weevil control accounts for the highest use of insecticides on Australian golf greens in an average year. Up until the EN technology there has never been a biological control product for Stem Weevils available for the turf industry.

New Zealand is having success with two biological control agents, a parasitic wasp (*Microctonus hyperodae*) and a fungal pathogen (*Beauveria bassiana*). The status or potential of these agents in Australia is unknown.

Photo 2: The site in front of the 5th Green, Tullamarine Country Club. The degree of damage can clearly be seen. The turf being rolled here was taken to the lab for intensive counting.



Dr. Robin Bedding, of CSIRO Entomology (Canberra), first developed parasitic nematodes in the 1960's to control Sirex wasp in pine trees. His work then led to using the rather different EN control of many insects across a wide range of crops, not only in Australia but overseas as well. Basically, the Entomopathogenic Nematodes are beneficial in that they survive by feeding on insects. They are not able to feed off plants. They can only feed off a narrow range of target insect pests. The EN locates its host from carbon dioxide emissions (following a CO₂ gradient) after it has been stimulated by host movements. It enters the host (usually the larval stage) through natural openings such as spiracles, the mouth or the anus, or in the case of *Heterorhabditis* species by burrowing through interskeletal membranes. Once inside the EN releases a bacteria that multiplies in the host insect, quickly killing it. The bacteria also preserves the cadaver, so an infected larva might be dead for several weeks but remains largely intact (Photo 1). The internal body fat of the insect larva becomes very stringy after being infected by *Heterorhabditis* species, so infected larvae can be identified after being pulled apart (see Photo 3).



Photo 3: An infected larva is stringy when pulled apart

The EN feeds off the bacteria that has multiplied in the host, reaching quite a large size (3mm or so - see Photo 4). These large female nematodes reproduce releasing hundreds of infective juveniles, which can move into the soil searching for further hosts. The survival of the nematodes depends on the availability of a food source. When practical control of an insect pest has been achieved, the nematode itself will probably only survive sporadically. Low soil temperatures also diminish their activity.

Dr. Bedding's research identified several EN species, each with its preferred range of target insect pests. Our early work this summer found two EN species within the genera *Heterorhabditis* and *Steinernema* that are highly infective of Stem Weevils in the lab.

Dr. Bedding's research has also developed production techniques that allow the breeding of massive numbers of ENs and the ability to store these in a dormant state. This means the EN products have a reasonable shelf-life and are not excessively expensive. This makes ENs a practical option for Golf Course Superintendents to use in a pest control program. Great success has been achieved already in the control of various cockchafer (including African Black Beetle, Black Headed Cockchafer, Argentine Scarab and billbugs). A bonus for Superintendents is the ability to control several pest species with the one EN product.

Until this trial no work had been done on the control of Argentine Stem Weevils using EN products and our aim this summer was to determine their effectiveness on this important pest.



Photo 4: Large females (*Heterorhabditis zealandica*) found in larvae and pupae when they are cut open. These nematodes are 2 - 3 mm long.



Summary of Stem Weevil trial work, Summer 1999/2000

Two sites were selected, both with a known Stem Weevil history. The first site, the 15th green at Maryborough Golf Club, was the site of major devastation the previous summer and we were confident they'd reappear this summer. The second site was Tullamarine Golf Club which suffers Stem Weevil damage to virtually the whole course. It was a matter of wait and see at Tullamarine and move in as soon as a good outbreak occurred. The Superintendent and committee at both these clubs were prepared to risk Stem Weevil damage so we could run our trial plots and we are grateful for their contribution to the trial work.

A summary of the summer's investigation is listed below, culminating in the final application at Tullamarine which provided the successful result:

1. 30th September: Adult Stem Weevils were trapped at Yowani Country Club, Canberra, and supplied to Dr. Bedding (CSIRO, Canberra).

2. 27th October: infested turf sods cut from surrounds of 5th Green at Tullamarine Golf Club and assessed for Stem Weevil. Around 10 m² of this turf was sent to Dr. Bedding for bioassay work. Dr. Bedding reported that larvae were highly susceptible to a couple of EN species in the laboratory bioassay.

3. 9th and 10th December: a replicated trial was applied to the 15th Green at Maryborough Golf Club. The treatment plots consisted of two rates (full rate and half rate) of *Steinernema* and *Heterorhabditis*, as well as the industry standard pesticide (fipronil, sold as Chipco Choice®) and a control plot (nothing applied). The treatments were applied at dusk and watered in. These plots were assessed several times over the summer and although adult Stem Weevils could be detected on the green there was no significant damage that would warrant taking turf samples for larval counts.

4. 18th January: ENs were applied again to the 15th Green at Maryborough. Follow up assessments still revealed no stem weevil damage, even in the untreated areas. The green was carefully managed and the lack of damage corroborates the common perception that Stem Weevil damage is usually associated with Moisture Stress and Heat Stress on turf, except possibly where insect numbers reach plague proportions.

5. 16th February: EN plots were applied to the 10th tee at Tullamarine Golf Club. The tee was badly damaged by Stem Weevils (probably in tandem with some moisture stress). These plots were assessed on 9th March (22 days later). Intensive soil counts revealed only a few live larvae (around 3 larvae per 0.25 m²), leading to the conclusion that the damage was mainly due to moisture stress. Our later work also showed that the plots had been left too long before assessment as any killed larvae would have rotted away by then.

6. 23rd March: Another site was treated at Tullamarine, this time a well irrigated area in front of the 5th green. There was no doubt that the Stem Weevil numbers were substantial and that the damage to the turf was directly due

to their high numbers rather than moisture or heat stress. In this case a single square of turf (15m x 15m) was treated with the full rate of *Heterorhabditis*. An adjacent untreated area was to act as the control. Turf was cut for assessment on 30th March - half this turf was immediately used for an intensive soil count on that day (7 days after application) and the other half stored in the glasshouse for a second week count on 6th April (14 days after application). The results of these counts are shown in Appendix 1.

Results - Tullamarine Trial

Our replicated trial work over the summer had been a failure due to lack of Stem Weevil numbers. By March our chance of achieving a result was remote but a late opportunity arose at Tullamarine, where an outbreak of Stem Weevil was confirmed. Our only objective at this stage was to see if the ENs could find, infect and kill Stem Weevils in the field.

The trial commenced on 23rd March, consisting of a single 15m x 15m plot treated with *Heterorhabditis* applied at a rate of 200,000 nematodes per square metre. An area immediately adjacent to the treated area was to act as the control. After 7 days turf rolls were cut with a turf cutter for counting in the laboratory. These rolls were sub-sampled to provide 0.225m² sections for intensive counting. In Week 1 (7 days after application), three treated and three untreated sections were counted. In Week 2 (14 days) two treated sections and one control section were counted.

This counting revealed Stem Weevil numbers (including larvae, pupae and adults) varying in density from 17 per 0.225m² sample up to 96 per 0.225m². Because of the variability in numbers and because the treatment plots weren't replicated, no statistics have been done other than averaging the counts.

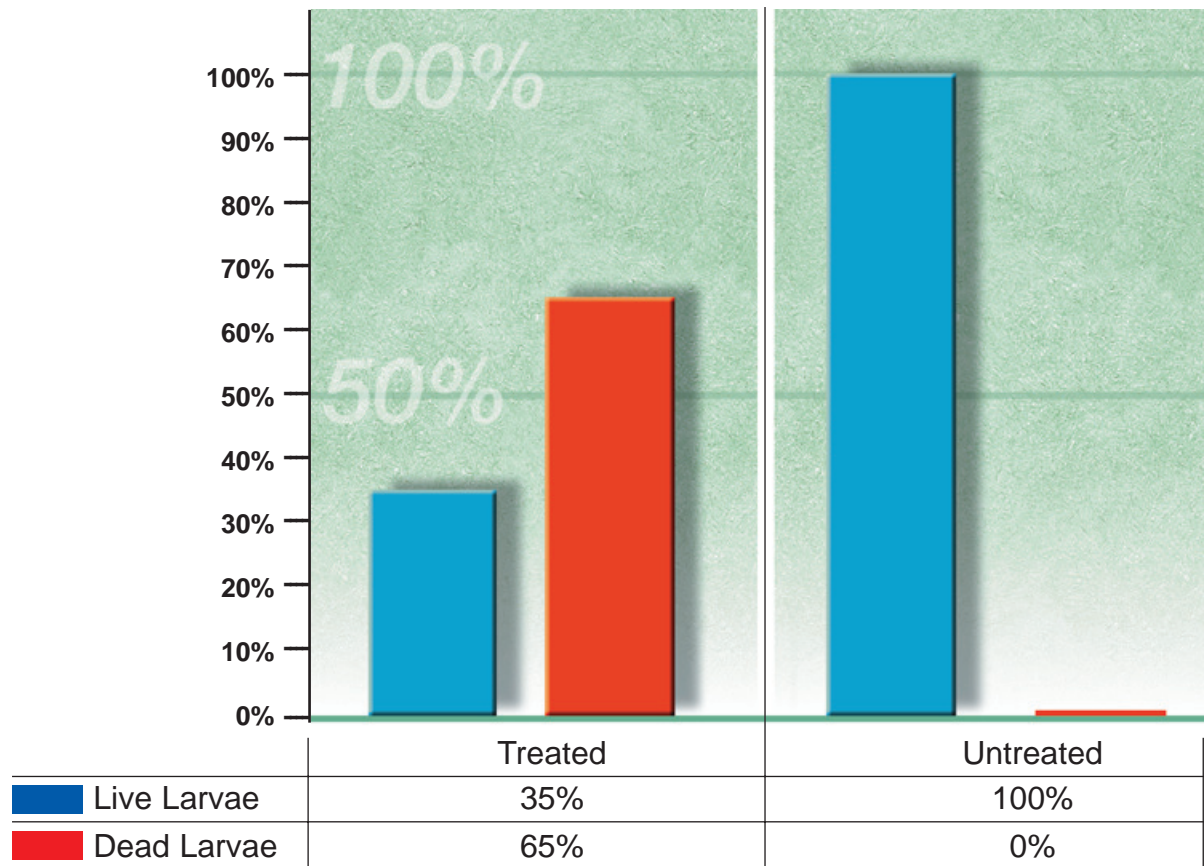
The actual numbers of dead and live larvae and adults and pupae are shown in Appendix 1. Live larvae were quite active so it was easy to distinguish between living and dead larvae. Pupae were not as active and it was only possible to determine if they were infested with the EN parasite by cutting them open under a dissecting microscope (x 30 magnification). Time did not permit this degree of examination but nearly all of the 20 or so pupae from the treated plot that were inspected microscopically were shown to be infected with ENs. The nematodes find their target by following movement and CO₂ emissions from the host. Pupae are normally quite inactive so the fact the ENs could find pupae was significant in itself.

In Week 1 the dead larvae were intact and easily identifiable by their inactivity and their grey colour, in contrast to the active, creamy white live larvae. Dead larvae were also very stringy when pulled apart as shown in the photo. By Week 2 the dead larvae were starting to decompose - it would be impossible to do a Week 3 count as these dead larvae would have disappeared altogether.

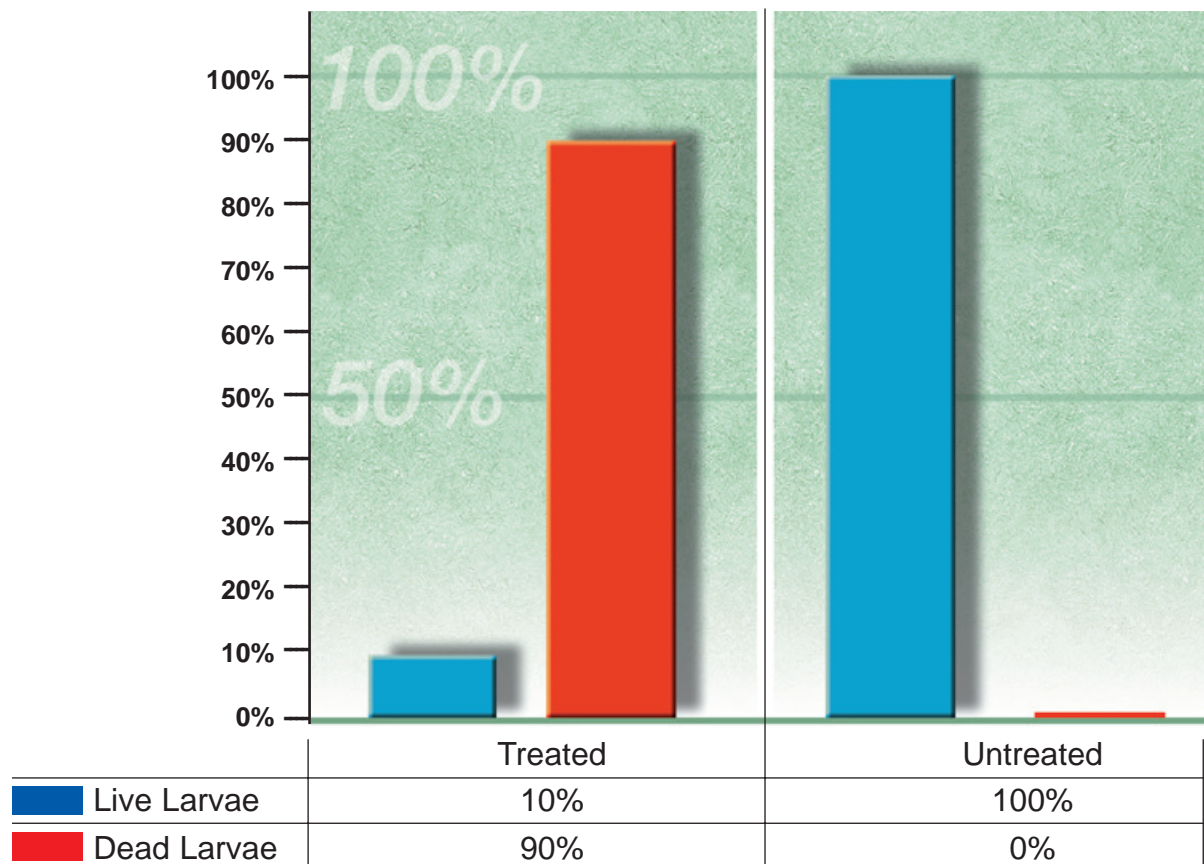
For convenience the proportions of live larvae to dead larvae are shown for the Week 1 count (7 days after application) and Week 2 count. These proportions are the average of counts from several sub-samples.



Week 1 Kill



Week 2 Kill





Conclusion

The critical piece of trial work that yielded these results was not replicated and didn't include a standard industry insecticide for comparison. Added to this the Stem Weevil numbers in the trial area were extremely variable. This meant statistical analysis was impossible, but despite this the results were clear and conclusive:

1. Regardless of Stem Weevil population numbers, the critical measure in a trial such as this is the degree of control achieved. This is well expressed as a % Kill.
2. The EN application resulted in a % Kill of around 65% after 7 days and around 90% after 14 days.
3. The EN formulation is cost effective (in comparison with pesticide alternatives) and practical to purchase, store and apply. It has no human or off-target hazard.
4. The EN formulation is the only biological control product for Stem Weevils available in the Australian turf industry.
5. Given that Stem Weevil control accounts for the highest use of insecticides on greens in an average year, adoption of the EN product has the potential to dramatically cut the use of insecticides in the turf industry.

The recommendations in this report are based on a set of trials and conditions as laid down within this report and should not be taken as a decisive or conclusive recommendation.

Each club's circumstances are different and it is hoped that this research assists clubs and superintendents to make relevant decisions that are best suited to their club's particular needs.

Future Work

Future Work

The trial work has demonstrated that Stem Weevils are highly susceptible in the field to one EN species of the genus *Heterorhabditis*. In fact their susceptibility raises the possibility that cheaper effective control might be obtained using reduced EN rates. This is a potential topic for future trials.

A lot of work is also required on the timing of EN applications. The successful application at Tullamarine was put on after substantial Stem Weevil damage had already occurred - obviously clubs would be looking at effective control before damage occurs. It is possible and practical to monitor Stem Weevil numbers in the field using the Pyrethrum method (see Appendix 2) and one strategy might involve application of ENs when a certain Action Threshold has been reached (possibly around 5 adults/m²).

Acknowledgement

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The Victorian Golf Association Turf Research and Advisory Board wishes to thank Maryborough Golf Club (Superintendent Damian Truslove) and Tullamarine Country Club (Superintendent Scott Kempster) for their co-operation in this trial work. We would also like to thank Ecogrow for the supply of ENs and Dr. Robin Bedding for his assistance over the summer.

The Association also records its thanks to the members of the Turf Research & Advisory Board and, in particular, to Mr Phillip Ford who has designed and managed the project and prepared this report. In addition, thanks are due to the Victorian Golf Foundation which funded the publication of this report.

Appendix 1: Numbers from the Tullamarine plots

Week 1 Number per 0.225m² of turf

	Treated			Control		
Plot	1	2	3	1	2	3
Live Larvae	7	42	13	17	8	8
Dead Infected Larvae	37	35	25	0	0	0
Pupae (total)	26*	19*	9*	22	8	7
% Kill of Larvae	84.1%	45.5%	65.8%	0 %	0%	0%
Adults (live)	0	0	0	1	1	2

Summary: an average of 65% larvae killed after 1 week

* most pupae were dead or infected, but it was too hard to distinguish between dead and alive ones.



Appendix 1: Numbers from the Tullamarine plots

Week 2 Number per 0.225m ²			
	Treated		Control
Plot	1	2	1
Live Larvae	4	3	9
Dead Infected Larvae	31	33	0
Pupae (total)	5*	12*	9
% Kill of Larvae	88.6%	91.7%	0 %
Adults (live)	0	0	1

Summary: an average of 90% larvae killed after 2 weeks

* Most pupae infected as checked under microscope

Appendix 2: Pyrethrum method

The Pyrethrum method uses a weak, irritating dose of insecticide to bring Stem Weevils to the surface where they can be counted. Usually it is only the adults that are mobile enough to crawl up to be counted, but occasionally larvae will emerge from stems and can be counted. It is a monitoring technique that indicates the general presence and numbers of Stem Weevil and should be done on a program basis (eg: once per week on three selected greens). The technique is as follows:

1. Make a mix in a watering can of 5 litres of water plus 20 ml of Pyrethrum (other insecticides can be used too).
2. Drench around 1 litre of this mix onto 0.25m² of turf (a 0.5m x 0.5m square). Do another area close by.

3. Sit and observe these areas intensively for at least 10 minutes. Catch any adult weevils that emerge. Count the total and convert to numbers per square metre.

4. Through experience, develop an "Action Threshold" to determine when control measures are required. The AT number is probably around 3 adults per square metre for *Poa annua* greens, particularly if hot weather is forecast. The number is higher for bent greens - possibly around 7 adults per square metre.

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