

**Feasibility of Using Non-Chemical Methods for Control of the European Chafer  
(*Rhizotrogus majalis*) in Turfgrass**

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## Executive Summary

In a preliminary research study funded by the CNLA and WCTA in 2003, the entomopathogenic nematode, *Heterorhabditis bacteriophora* showed promise in controlling second-instar chafer larvae in constructed grass plots. In 2004, first and second instar chafer larvae were collected from New Westminster lawns and placed in the same type of constructed grass plots. First instar chafer larvae were exposed to one of three treatments: a water control, *Steinernema carpocapsae*, or *Heterorhabditis bacteriophora*. Second instar chafer larvae were exposed to one of eight treatments: a water control, two rates of *H. bacteriophora*, two rates of *Steinernema kraussei*, and three rates of *S. carpocapsae*. The constructed grass plots were fragmented in October and the surviving chafer larvae in each plot were counted. The nematode *H. bacteriophora*, when applied at a rate of 1.5 billion per acre, considerably reduced the survival of first instar chafer larvae in the constructed grass plots. Second instar chafer survival was significantly reduced in plots treated with both low and high rates of *S. carpocapsae* (1.5 and 3 billion per acre), both low and high rates of *H. bacteriophora* (1.5 and 3 billion per acre), and a low rate of *S. kraussei* (1.5 billion per acre). *S. carpocapsae*, applied at the high rate of 3 billion per acre provided control of second instar chafer larvae as well, however results were inconsistent in response to rate. The most consistent control was with *H. bacteriophora*. Results obtained this year with other nematode species, would need to be verified by further work.

Nine residential lawns in New Westminster with chafer infestations were used for research trials in 2004. Each lawn was divided into three plots, with one plot treated with *H. bacteriophora* in late July to control first instar chafer, a second plot treated with *H. bacteriophora* in late August to control second instar chafer, and a third plot treated with water as a control. A rate of 3 billion nematodes per acre was used for both first and second instar treatments. The chafer population in ten 0.45 M<sup>2</sup> plots in each treatment of each lawn was assessed in October by rolling back strips of grass with a sod cutter. The number of chafer larvae still alive in the lawn in October was lowest in lawn areas treated with the nematode *H. bacteriophora* at a rate of 3 billion per acre in late July, when first instar larvae were present. Populations were reduced by half and in lawns with light or no visible damage, this level did not cause further lawn damage. Individual research lawns were categorized based on the management practices, the visible lawn condition when nematodes were applied in the summer, and the presence of chafer larvae in the untreated (control) area of the lawn when sampled in October. The results amongst the different lawns suggest that lawn care practices such as weekly mowing, thorough watering, annual fertilizing, liming, top dressing, power raking and aerating may make lawns less attractive to chafer adults. An application of the nematode, *H. bacteriophora* at a rate of 3 billion per acre in July to moderately chafer-infested lawns can effectively reduce the chafer population and the damage caused by chafer and chafer predators to lawns when used in combination with good lawn care practices. While the nematode was able to reduce the chafer population by a similar amount in heavily infested lawns, the number remaining was able to cause further damage. In heavily infested lawns therefore, nematode application alone will not be sufficient to allow the lawn to recover from damage. Improved lawn care practices in addition to a nematode application would be required.

## Background

The European Chafer, *Rhizotrogus majalis*, is a major pest of turfgrass in eastern North America. It was first found in 2001 in New Westminster, British Columbia, but was likely present in the area for a few years prior. As an invasive, non-native white grub, it has since caused considerable damage to lawns and boulevards in New Westminster and Burnaby. The European Chafer completes its life cycle in one year. Eggs hatch in mid-July, and the larvae progress through three instars over the summer and fall. In April they become pupae, with adults emerging in late May. Damage to turf grass is most severe in fall and spring, caused by the feeding of third-instar chafer larvae on the roots of grass. Secondary damage to lawns and boulevards is caused by skunks and crows, which dig through the grass to feed on the chafer larvae (Costello 2003). With more emphasis on non-chemical pest management, use of insecticidal drenches to control this pest in urban settings is not acceptable. In addition, commercial turf (pasture, turf farms, golf courses, sports fields) will all be seriously affected as this pest spreads from New Westminster to other areas of the Fraser Valley.

In a preliminary research study funded by the CNLA and WCTA in 2003, the feasibility of non-chemical products was studied for control of second and third instar chafer larvae in constructed grass plots. Of these biological control agents tested, the entomopathogenic nematode, *Heterorhabditis bacteriophora* showed promise for controlling second-instar chafer larvae at a rate of 3-12 billion nematodes per acre. Results indicated that this strain had good potential for controlling second instar chafer larvae in turfgrass, though the third instar larvae were not controlled at any rate. *H. bacteriophora* was not tested against first instar chafer larvae in 2003.

Entomopathic nematodes of the *Steinernematidae* and *Heterorhabditidae* families are generalist parasites of white grubs and have been used successfully as biological control agents of other turf pests in BC. *Steinernema carpocapsae* is currently the most commercially available nematode, while *Steinernema kraussei* is marketed for its cold tolerance. *S. carpocapsae* is a sit-and-wait or "ambush" forager which latches on to passing insects, while *H. bacteriophora* is a cruiser species, which seeks out lepidopterous and coleopterous insect larvae in the soil. In behaviour, *S. kraussei* is intermediate between these two. Simard *et al* (2001) tested *S. carpocapsae*, *H. bacteriophora* and *S. feltiae* against chafer larvae and found all strains to be ineffective at concentrations of 25-5000 nematodes/chafer larvae. However, because *H. bacteriophora* showed some control in our 2003 study against second instar chafer larvae in turf grass plots, it was the most promising treatment to examine further.

The entomopathic fungi, *Metarhizium anisopliae*, was also tested against second and third instar chafer in the 2003 preliminary study. *M. anisopliae* is a common pathogen of many insects including the Japanese Beetle, Black Vine Weevil, and termites, and has been experimented with to control the grass grub *Costelytra zealandica* in New Zealand pastures (Glare *et al.* 1995). In 2003, *M. anisopliae* had no effect on chafer survival in the constructed grass plots, however, the length of time larvae were exposed may not have been sufficiently long (2-7 weeks) as *M. anisopliae* showed efficacy in petri dish trials. Further research is needed to determine the ability of *M. anisopliae* to control first and second instar chafer when exposed for a greater length of time. Unfortunately, *Metarhizium anisopliae* could not be used as a treatment for first or second instar chafer in the 2004 research, as an import permit was required and the company (in Australia) was not co-operative about supplying product nor very optimistic that their product would be useful here due to the specificity inherent in fungal entomopathogens.

A new, softer insecticide, Merit (Imidacloprid), has recently received registration in some provinces for control of insect pests, including scarab beetles, in turfgrass. Merit is a systemic insecticide,

which when taken up by the plant, is toxic to scarab larvae as they feed on turfgrass roots. This insecticide is thought to have sufficient residual activity in lawns to control annual scarab grubs in the fall when it is applied the previous spring or summer at egg laying. The ability of Merit to control European chafer larvae in B.C. has yet to be studied. Merit was pending registration in B.C. and it was proposed as a treatment in the 2004 grass plot study, however since approval was not in place when required for this study, we were not able to buy it.

In this study, the methods to study chafer larvae developed in the preliminary trial in 2003 were used to study the efficacy of a broader range of non-chemical products against first and second instar chafer larvae in constructed grass plots. Third instar larvae were not included as no product controlled this stage of larva in the preliminary trial. In addition to the plot study, nine lawns belonging to homeowners in New Westminster were treated with *Heterorhabditis bacteriophora* against first and second instar chafer larvae and compared to a water control.

## **Materials and Methods**

### Preparation of Grass Plots

On June 10, 2004, one hundred and thirty-five grass “plots” were constructed from sod placed over parent soil in individual 22” x 14” plastic tulip boxes, lined with permeable landscape cloth. The sod was cut with sod-cutter from the soil-based Stadium Field in Queens Park, New Westminster. The tulip boxes varied in depth between 7” and 9”, and were filled three-quarters full with soil and sod. This sod and soil was believed to be free of chafer.

All 135 tulip boxes were taken to the fenced tree nursery in Queens Park, New Westminster. The tulip boxes were arranged in an 11X11 block, allowing a complete randomized block design. The additional 14 tulip boxes were placed at one end of the block to be kept as spares. Each sod-filled tulip box was sunk into the woodchip substrate with a 40cm space between each box to provide easy walking access between plots and an adequate buffer between treatments.

On June 11<sup>th</sup> all tulip box plots were covered with Remay©, a lightweight woven synthetic fabric which allowed air, light, and water through to grass, but would exclude adult chafer from entering and laying their eggs in the plots. The Remay was anchored down around the edges of the block with earth staples.

The grass plots were watered by sprinkler for 15 minutes daily. On June 25<sup>th</sup>, the sprinkling time was increased to 25 minutes per day, as weather was hot and the grass plots appeared to need more water.

On July 5<sup>th</sup>, the Remay was removed from the plots, as adult chafers were no longer flying. The grass in the tulip boxes was cut by hand with clippers on July 9<sup>th</sup>, July 16<sup>th</sup>/21<sup>st</sup>, and August 19<sup>th</sup>.

### First Instar Larva Trial in Grass Plots

On July 22<sup>nd</sup> and 23<sup>rd</sup>, 550 first-instar European chafer larvae were harvested from the grass at City Hall in New Westminster. These larvae were collected in containers with soil. Larvae collected on July 22<sup>nd</sup> were held in a refrigerator overnight. Only those larvae appearing healthy and active were used in the experiment. On July 23<sup>rd</sup>, the first-instar larvae were inserted 15 per plot into 33 of the tulip box plots in Queens Park. Individual chafer larvae were inserted into 2” deep holes that had been cut into the turf with a trowel. After dropping a larva in, each hole was again filled with soil

and the turf patted back down. The first-instar chafer larvae were given a week to acclimatize in the plots before treatments were applied.

On July 26<sup>th</sup>, all plots were given extra water by hand as the grass was becoming dry due to the very hot weather.

On July 29<sup>th</sup>, between 5-7pm, treatments were applied to the grass plots containing first-instar chafer larvae. Treatments were replicated eleven times (See Appendix, Figure 1). These included:

1. Water control
2. *Steinernema carpocapsae* (nematode)
3. *Heterorhabditis bacteriophora* (nematode)

All plots were watered by sprinkler for 30 minutes prior to treatment.

For the control treatment, 3 litres of water were applied by watering can to the surface of each plot.

*Steinernema carpocapsae* was applied at a rate of 3 billion per acre (150,000 nematodes per plot). An initial 3.75 million *S. carpocapsae* were diluted into 10 litres of water. Of this solution, 400mL were taken and mixed with 600mL of water in a watering can, which was then applied to the surface of a plot. An additional 2 litres of water (equivalent to 1cm of irrigation water) was applied to each plot by watering can, to help wash the nematodes down into the soil.

*Heterorhabditis bacteriophora* was applied at a rate of 1.5 billion per acre (75,000 nematodes per plot). An initial 3.75 million were diluted into 10 litres of water. Of this solution, 200mL were taken and mixed with 800mL of water in a watering can, which was then applied to the surface of each plot. Two litres of water followed to help wash the nematodes down into the soil.

The plots were watered by sprinkler for one hour immediately following the first-instar treatments.

The first instar chafer larvae were left in the grass plots for 8 weeks following treatment. To determine the results of the treatments, each tulip box plot was dissected individually. The tulip crate was emptied onto a table, and the soil/sand and grass contents were sifted through by hand. For each plot, all live and dead larvae, and fragments of larvae, were picked out of the soil and placed into a labelled plastic cup and held at room temperature. Numbers of dead and live chafers found in each plot were recorded.

### Second Instar Larva Trial in Grass Plots

On August 18<sup>th</sup> and 19<sup>th</sup>, 1400 second-instar chafer larvae were collected from two residential lawns in New Westminster. These second-instar larvae were placed in containers with soil. Larvae collected on August 18<sup>th</sup> were kept in a refrigerator overnight while those collected on Aug. 19 were placed in plots the same day. Only those larvae appearing healthy and active were used in the experiment. On August 19<sup>th</sup>, the second-instar larvae were inserted 15 per plot, into 88 of the tulip box plots in Queens Park. As was done with the first-instar larvae, individual second-instar chafer larvae were inserted into 2” deep trowel holes that had been cut into the turf. After dropping a larva in, each hole was filled with soil and the turf patted back down. The second-instar chafer larvae were given 5 days to acclimatize in the plots before treatments were applied.

The second-instar chafer larvae were exposed to one of eight treatments. Treatments were replicated eleven times (see Appendix: Figure 1). The treatments included:

1. water control,
2. low rate of *H. bacteriophora* (1.5 billion/acre)
3. high rate of *H. bacteriophora* (3 billion/acre)
4. low rate of *S. kraussei* (1.5 billion/acre)
5. high rate of *S. kraussei* (3 billion/acre)
6. low rate of *S. carpocapsae* (1.5 billion/acre)
7. high rate of *S. carpocapsae* (3 billion/acre)
8. extra high rate of *S. carpocapsae* (6 billion/acre)

All plots were watered by sprinkler for 30 minutes prior to treatment. The water control was carried out as for the first-instar treatment. *H. bacteriophora*, *S. kraussei* and *S. carpocapsae* were applied at a low rate of 1.5 billion/acre (75,000 per plot), and a high rate of 3 billion/acre (150,000 per plot). An additional, extra high rate of *S. carpocapsae* of 6 billion/acre (300,000 per plot) was applied, as this nematode is currently commercially available to home gardeners, and less costly than Heterorhabditid species. In addition, neither the strain of *H. bacteriophora* used in these trials, nor *S. kraussei* are available commercially at this time (though Becker Underwood intends to make the *H. bacteriophora* strain available for 2005).

To apply the *H. bacteriophora*, an initial 25 million nematodes were diluted into 20 litres of water. For the low rate, 60mL of this solution were mixed with 940mL of water in a watering can, which was then applied to the surface of a plot. For the high rate, 120mL of nematodes were mixed with 880mL of water in a watering can before being applied to the surface of a plot. An additional 2 litres of water was applied to each plot to help wash the nematodes down into the soil.

To apply *S. kraussei* and *S. carpocapsae*, an initial 50 million nematodes were diluted in 20 litres of water. For the low rate, 30mL of the nematode solution was added to 970mL water, and watered into a plot. For the high rate, 60mL of the nematodes solution was added to 940mL of water and watered into a plot. For the extra high rate of *S. carpocapsae*, 120mL of the nematode solution was added to 880mL of water, and watered into a plot. An additional 2 litres of water was applied to each plot to help wash the nematodes down into the soil.

It was raining during and after the treatment of the grass plots, so the plots were not given additional water by sprinkler following the second-instar treatments.

The second instar larvae were left in the grass plots for 5 weeks following treatment. To determine the results of the treatments, each tulip box plot was dissected individually. The tulip crate was emptied onto a table, and the soil/sand and grass contents were sifted through by hand. For each plot, all live and dead larvae, and fragments of larvae, were picked out of the soil and placed into a labelled plastic cup and held at room temperature for at least 3 days. Numbers of dead and live chafers found in each plot were recorded.

#### First and Second Instar Larva Trial in Lawns

In early July 2004, lawn owners in New Westminster who had notified the city about chafer infestations in their lawns in 2003 were contacted about participating in the research trial in 2004. Lawns were visited and ten were chosen across the city for their suitability for chafer research. A letter of agreement was drawn up for the lawn owners, outlining the details of the research and our

expectations. The lawn owners were asked to water their lawns twice weekly on their designated watering days and to record all watering activities. They were informed that the nematode treatments would be applied to their lawns during the week of July 26-30 and August 23-27, and they were asked to water the lawn for 3 hours on the evening prior to treatment, the morning of the treatment, immediately before treatment in the evening, and immediately after the treatment. They were also asked to avoid insecticides for the duration of the trial. Permits were obtained from the City of New Westminster to allow extra watering on the day of application for lawns not allowed to be watered on that day.

Each lawn was measured and its dimensions and the physical characteristics of the soil and turf were noted. A map was drawn for each lawn, and on it three 12m<sup>2</sup> “plots” were outlined to be used for the three treatments. One plot was to be treated with *H. bacteriophora* in July to control 1<sup>st</sup> instar chafer, a second plot on the same lawn in late August to control 2<sup>nd</sup> instar chafer, and a third plot in the same lawn to be treated with water only. The plots were randomly assigned within each lawn. In summary, the treatments were:

1. water control
2. *H. bacteriophora* applied in July for 1<sup>st</sup> instar Chafer, at a rate of 3 billion/acre
3. *H. bacteriophora* applied in late August for 2<sup>nd</sup> instar Chafer at a rate of 3 billion/acre

At each lawn site, the plots were only staked with flags during the treatments. All measurements were taken from permanent fixtures such as sidewalks and walkways, and no trace of the plot divisions were left after treatments had been applied.

On July 28<sup>th</sup> and 29<sup>th</sup>, the *H. bacteriophora* and control treatments were applied to two of the plots in each lawn for first-instar chafer. For the control plot, two watering cans full of water were evenly applied to one 12m<sup>2</sup> area. For the treated plot, an initial 90 million *H. bacteriophora* were mixed into 10 litres of water. Of this solution, 500mL were mixed into each of two watering cans full of water. These two watering cans of *H. bacteriophora* were applied to the second 12m<sup>2</sup> area.

On August 25<sup>th</sup> and 26<sup>th</sup>, the *H. bacteriophora* treatment was applied to the remaining plot in each lawn for second-instar chafer. The control plot was treated with water again at this time. In one residential lawn, the nematodes could not be thoroughly watered in following treatment because the owners were away and the water supply had been turned off. This lawn was eliminated from the trial, leaving 9 participating lawns.

The lawns were left for 8 weeks following the first instar treatment, and 4 weeks following the second instar treatment, before the results of the treatments were assessed. On September 20-23, the 9 lawns in New Westminster were sampled to assess the results of the nematode and water treatments.

A sod cutter was used to cut three 1.5 ft X 10 ft strips through each lawn. One strip was cut in the plot treated with *H. bacteriophora* in July, a second strip was cut in the plot treated with *H. bacteriophora* in August, and a third strip was cut in the control plot. The sod cutter cut to a depth of approximately 5cm, cleanly separating the layer of turf from the soil below.

The 10 ft sod strips were then further divided into 1 ft sections. Each 1 ft X 1.5 ft section was rolled back, and the number of chafer larvae found in the soil below and grass roots above was recorded.

All chafer larvae found in the plots were removed and drowned. The instar stage of the larvae found within each plot was noted.

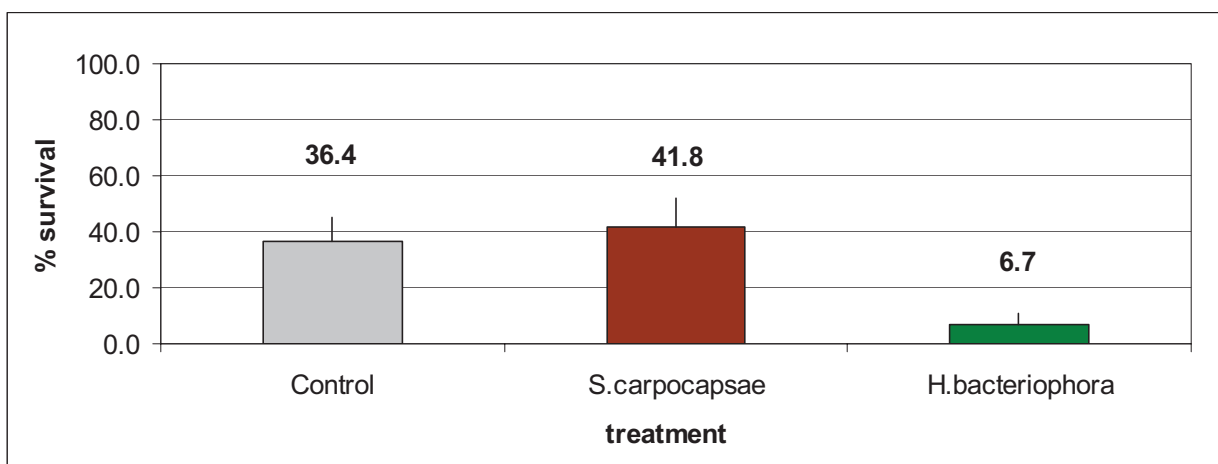
In the fall, lawn owners were surveyed in person and by telephone regarding their lawn care practices in 2004.

## Results

### First Instar Larva Trial in Grass Plots

First instar chafer survival was significantly reduced in the plots treated with *H. bacteriophora* at a rate of 1.5 billion per acre. Compared to the control, *H. bacteriophora* reduced chafer populations by 82%.

Only 7% of the first instar larvae survived this *H. bacteriophora* treatment ( $P < 0.001$ ), compared to a 42% survival rate in the *S. carpocapsae* plots, and a 36% survival rate in the control plots. *S. carpocapsae* did not provide significant control of first instar chafer larvae when applied at a rate of 3 billion per acre under our experimental conditions. The results of the first instar treatments are graphed in Figure 1. Complete statistical results can be found in Table 1 of Appendix A.



**Figure 1. Survival of first-instar chafer larvae in tulip box plots treated with water (control), *Steinernema carpocapsae*, and *Heterorhabditis bacteriophora*. The error bars are equal to half the standard deviation of the mean**

### Second Instar Larva Trial in Grass Plots

Second instar chafer survival was significantly reduced in plots treated with low and high rates of *S. carpocapsae* ( $P < 0.001$ ), low and high rates of *H. bacteriophora* ( $P < 0.001$ ), and in those plots treated with a low rate of *S. kraussei* ( $P = 0.012$ ). The extra high rate of *S. carpocapsae* and the high rate of *S. kraussei* did not significantly alter the second instar chafer survival. Complete statistical results for treatment of second instar larvae in plot trial can be found in Table 2 of Appendix A.

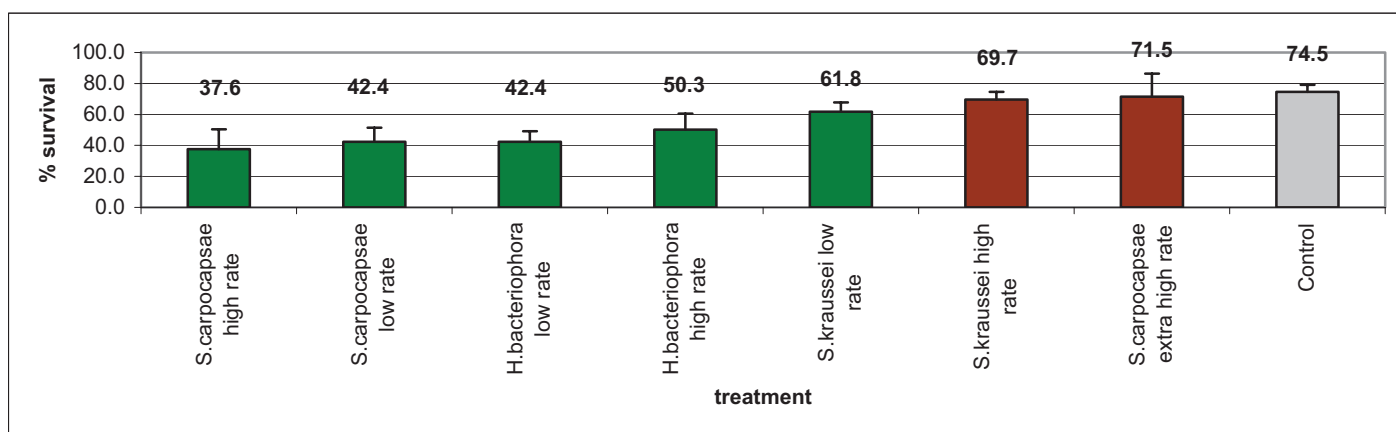
There was no significant difference between the low and high rates of *S. carpocapsae*, nor between the low and high rates of *H. bacteriophora* in their effectiveness to control second instar chafer



larvae. The percent reduction in the chafer population from each species and rate of nematode tested is shown in Table 1. The results of the second instar treatments are graphed in Figure 2.

**Table 1. Percent reduction in 2<sup>nd</sup> instar chafer larvae populations when exposed to various nematode treatments**

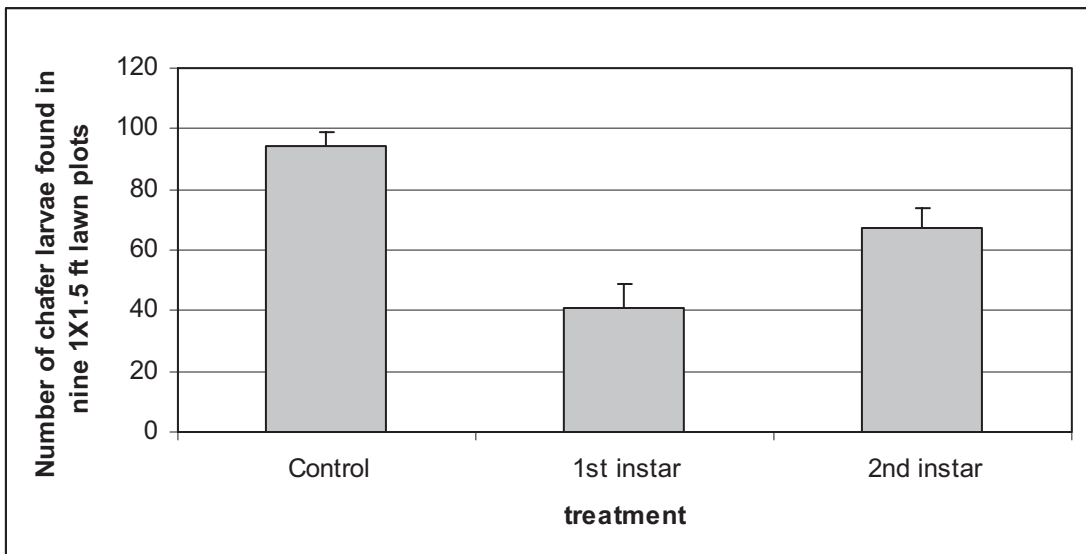
Treatment	Reduction in 2 <sup>nd</sup> instar Chafer Larval Population
<i>S.carpocapsae</i> low rate	43 %
<i>S.carpocapsae</i> high rate	49 %
<i>S.carpocapsae</i> extra high rate	4 %
<i>H.bacteriophora</i> low rate	43 %
<i>H.bacteriophora</i> high rate	32 %
<i>S.kraussei</i> low rate	17 %
<i>S.kraussei</i> high rate	6 %



**Figure 2. Survival of second instar chafer larvae in tulip box plots treated with water (control), two rates of *Steinernema kraussei*, two rates of *Heterorhabditis bacteriophora*, and three rates of *Steinernema carpocapsae*. The error bars are equal to half the standard deviation of the mean**

#### First and Second Instar Larva Trial in Lawns

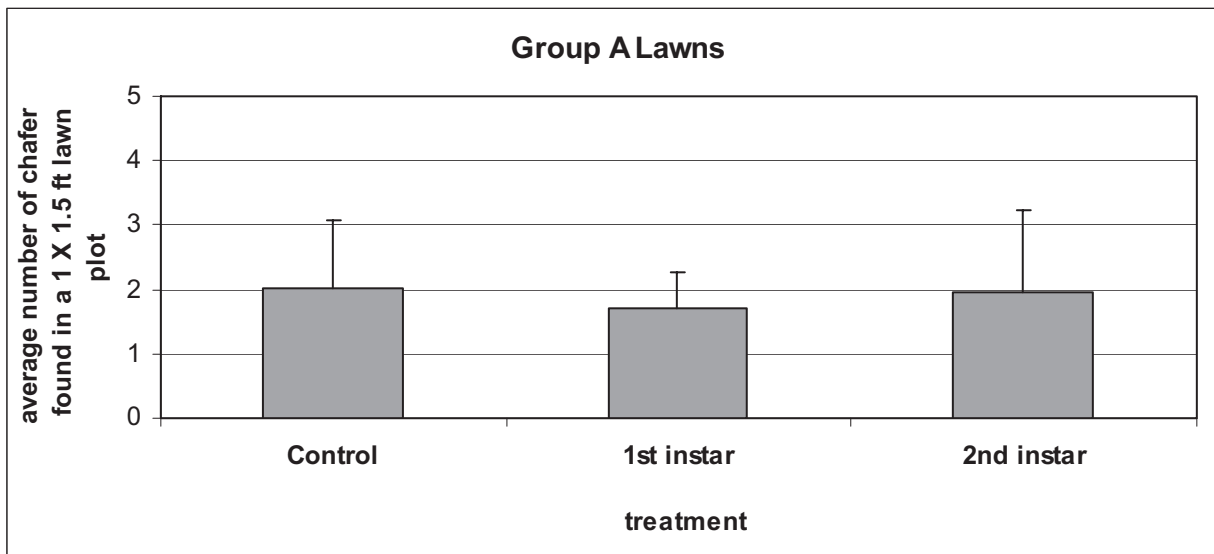
Overall, chafer populations were significantly lower in lawn areas treated with *H. bacteriophora* against first instar larvae in July ( $P < 0.001$ ) and in lawn areas treated with *H. bacteriophora* against second instar larvae in August ( $P < 0.001$ ) than in control lawn areas (see Figure 3). Chafer populations in first instar treated lawn areas were also significantly lower than second instar treated lawn areas ( $P < 0.001$ ). Compared to the control lawn areas, first instar chafer populations were reduced by 57%, while second instar chafer larvae populations were reduced by only 29%.



**Figure 3. Average number of chafer found per lawn in the nine 1X1.5ft plots treated with water (control), *H. bacteriophora* in July for first instar, and *H. bacteriophora* in August for second instar. The error bars are equal to half the standard deviation of the mean**

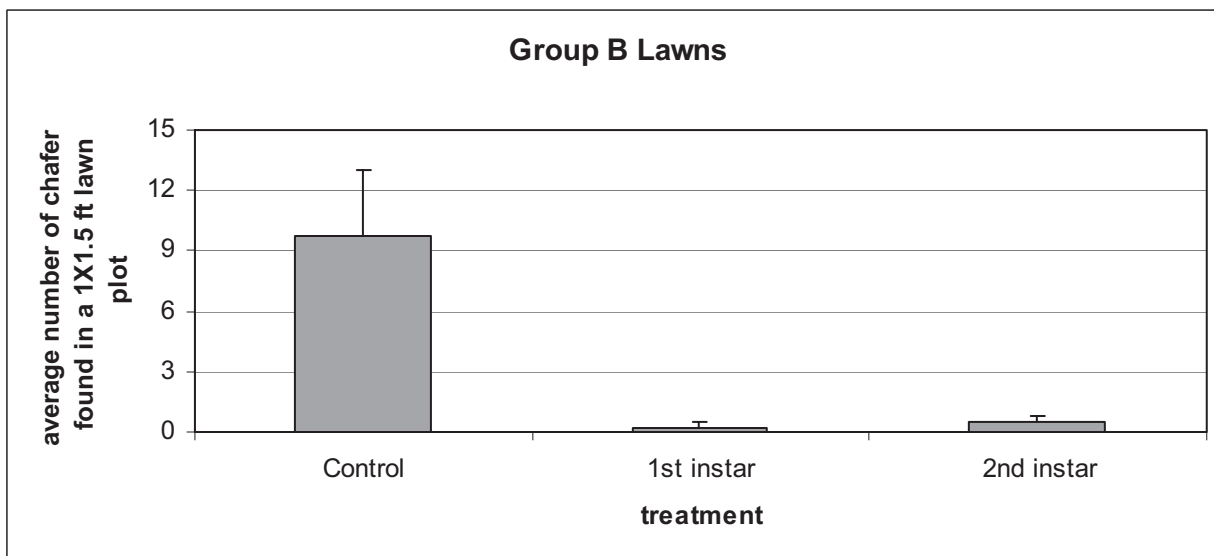
The individual research lawns were divided into three categories based on management practices, visible lawn condition at treatment and the presence of chafer larvae in the untreated (control) area of the lawn when sampled in October. Group A lawns (lawns #1, #2 and #3) were in excellent condition in July and August when nematodes were applied, and very few chafer larvae were found in the untreated control when sampled in October. Group B lawns (lawns #4 and #5) were in excellent condition in July and August, but chafer larvae were present in the untreated section when sampled, in numbers sufficiently high to cause damage. Group C lawns (lawns #6, #7, #8 and #9) were in poor condition at the time of nematode application, either because of consecutive years of chafer damage, or due to a minimal lawn care practices, and many chafer were present in the untreated sections at sampling.

Group A lawns did not show significant differences in chafer larvae numbers between treated and control areas. Chafer populations were very low, frequently zero, in the treated and control 1 ft X 1.5 ft replicate samples of lawn. Results of chafer survival in Group A lawns are graphed in Figure 4.



**Figure 4. Average number of chafer found in 1X1.5ft lawn plots in Group A lawns treated with water (control), *H. bacteriophora* in July for first instar, and *H. bacteriophora* in August for second instar. The error bars are equal to half the standard deviation of the mean**

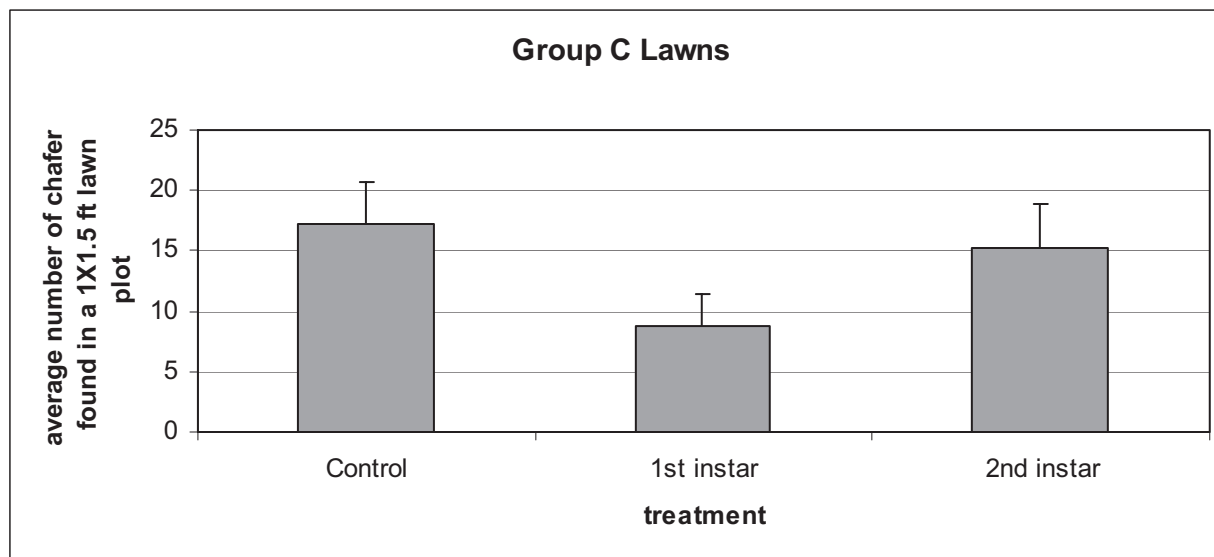
Group B lawns had significantly lower chafer populations in the areas treated with *H. bacteriophora* against first instar larvae in July than in the control areas ( $P < 0.001$ ). The chafer populations were also significantly lower in the areas treated with *H. bacteriophora* against second instar larvae in August than in the untreated areas of the lawns ( $P < 0.001$ ). Results of chafer survival in Group B lawns are graphed in Figure 5.



**Figure 5. Average number of chafer found in 1X1.5ft lawn plots in Group B lawns treated with water (control), *H. bacteriophora* in July for first instar, and *H. bacteriophora* in August for second instar. The error bars are equal to half the standard deviation of the mean**

Group C lawns showed mixed results from the *H. bacteriophora* treatments. Lawns #6, #7, and #8 showed significant control of first instar chafer in areas treated in July ( $P = 0.003$ ,  $P = 0.004$ ,  $P < 0.001$ , respectively), but insignificant control of second instar chafer larvae in areas treated in August.

Lawn #9 showed insignificant control of first instar chafer in the area treated in July, but significant control of second instar chafer in the area treated in August ( $P < 0.001$ ). This was likely due to variation in chafer populations in these highly infested lawns. Results of chafer survival in Group C lawns are graphed in Figure 6. Complete statistical results for all lawns appear in Table 3 of Appendix A.



**Figure 6. Average number of chafer found in 1X1.5ft lawn plots in Group C lawns treated with water (control), *H. bacteriophora* in July for first instar, and *H. bacteriophora* in August for second instar. The error bars are equal to half the standard deviation of the mean**

In summary, the treatment of all lawn types with *H. bacteriophora* in July was the most effective means of reducing first instar chafer populations. Table 2 summarizes the percent reduction in chafer populations in the three lawn types when treated with *H. bacteriophora* in July for first instar control, and in August for second instar control.

**Table 2. Percent reduction in 1<sup>st</sup> and 2<sup>nd</sup> instar chafer populations in group A, B and C lawn types after treatment with *H. bacteriophora***

	Percent reduction in chafer populations		
	Group A Lawns	Group B lawns	Group C lawns
1 <sup>st</sup> instar treatment	16 %	98 %	49 %
2 <sup>nd</sup> instar treatment	3 %	95 %	12 %

### Effect of Lawn Care Practices

The Greater Vancouver Regional District's 6 Steps to Natural Lawn Care outlines recommendations to lawn owners on how to keep their lawns healthy and vigorous. These recommendations include:

1. Mowing the lawn weekly to a grass height of 5-6 cm, and leaving grass clippings on the lawn
2. Fertilizing in May and September with slow release or natural organic fertilizer
3. Watering thoroughly but infrequently (2.5cm water per week in July and August) to wet the whole root zone but allow it to partially dry out between waterings

4. Aerating compacted soil and overseeding with a perennial rye/fine fescue mix
5. Avoiding the use of pesticides
6. Considering alternatives to lawn cover on slopes, in shady areas, and near watercourses

The lawn care practices undertaken by lawn owners participating in the chafer research in 2004 are identified in Table 3. A prerequisite for lawn owners participating in this study was that lawns were not to have any insecticides applied to them over the course of the spring, summer or fall. Additional lawn care practices not included in the GVRD's 6 Steps to Natural Lawn Care, but identified by lawn owners, included liming and power raking. One lawn owner suggested frequent mowing in May and June to suck or "vacuum" chafer adults into the lawn mower bag from the grass surface. This lawn had a very low infestation of chafer larvae.

**Table 3. Lawn care practices as identified by the lawn owners in the fall of 2004**

#	Class	Watering	Mowing	Fertilizer	Aerating	Seeding/Top dressing	Liming	Power raking	Soil Condition
1	A	2 hrs at a time, twice weekly on watering days	Every 4 days	Nov, June, Aug- slow release	No	No	March	March	Sandy soil, dense grass
2	A	2 hrs at a time, twice weekly on watering days	Once per week	June – slow release	April	Top dressing in April	April	No	Loose loamy soil, grass not dense
3	A	1-3 hrs at a time twice weekly on watering days	Every 2 weeks	April – slow release	No	Re-seeded in April	April October	No	Sandy, rocky soil, grass not dense
4	B	2 hrs at a time, twice weekly on watering days	Every 7-10 days	Every 6 weeks – slow release	No	Top dressing April, Sept	February, March, November	March	Clayey soil, dense grass
5	B	Daily at 2 hour intervals (underground sprinklers)	Once per week	No	No	No	No	No	Loose soil, dense grass
6	C	1-2 hrs at a time, twice weekly on watering days	Once per week	No	No	No	No	No	Loose soil, mossy grass
7	C	1-2 hrs at a time twice weekly on watering days	Once per week	April	No	No	April, November	No	Soil compacted, sparse grass
8	C	1-2 hrs at a time twice weekly on watering days	Once per 2 weeks	No	No	Seeding in late spring	No	No	Loose, sandy soil, sparse grass
9	C	2-3 hrs at a time twice weekly on watering days	Once every 5 weeks	No	No	No	No	No	Loose soil, grass not very dense

Group A lawns (#1, #2, and #3), which contained few chafer larvae and were in excellent condition throughout the summer and fall, shared similar lawn care practices. Lawns #1 and #2 were mowed weekly, while lawn #3 was mowed every 2 weeks. All three lawns were watered thoroughly for 2 hours on watering days, fertilized with slow release fertilizer and limed in the spring and/or fall. Lawn #1 was power raked in the spring, while lawn #2 was aerated. Top dressing was carried out on lawn #2, while reseeding was carried out in lawn #3.

Group B lawns (#4 and #5) were also in excellent condition throughout the summer, but contained larvae in their untreated sections when sampled in October. The lawn care practices of lawn #4 included mowing every 7-10 days, watering thoroughly for 2 hours on watering days, applying slow release fertilizer every 6 weeks, top dressing and liming in the spring and fall, and power raking in the spring. Lawn #4 survived the fall without visible signs of chafer damage. Lawn #5, however, suffered from isolated chafer damage in the control section of the lawn in the fall. The lawn care practices undertaken by lawn #5 were less thorough. The lawn was watered daily at two hour intervals by an underground sprinkling system, and mowed weekly. However, the lawn was not fertilized, limed, top dressed, aerated or power raked. Lawn #5 may have been more susceptible to chafer damage due to less vigorous turf growth.

Group C lawns (#6, #7, #8, #9), which were in poor condition at the time of nematode application and extensively damaged in the fall, were less intensively managed throughout the spring, summer and fall. All lawns were watered for 1-3 hours on watering days, but with the exception of lawn #7, these lawns were not fertilized or limed, and none of these lawns were top dressed, aerated or power raked. Mowing was less frequent in lawn #9, which may have affected root growth and depth.

Treated lawns were visited on a monthly basis from October 2004 to January 2005, and photographed for chafer damage caused by chafer larvae feeding on the grass roots, and by secondary predators digging up the grass in search of chafer larvae. Damage to lawns was rated on a scale of 0-5, with 0 indicating no signs of chafer damage, and 5 representing extensive chafer and predator damage to the lawn. The results of the lawn assessments are shown in Table 4. Representative photos of the lawns are included in Appendix B.

**Table 4. Lawn damage assessed on a monthly basis following chafer treatments and sampling**

		Chafer damage to the lawn (scale of 0-5, 0 = no damage, 5 = extensive damage)		
Lawn #	Class	October	November	January
1	A	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar
2	A	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	0 – control, 0 – 1 <sup>st</sup> instar <b>1 – 2<sup>nd</sup> instar</b>	0 – control, 0 – 1 <sup>st</sup> instar <b>1 – 2<sup>nd</sup> instar</b>
3	A	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	<b>1 – control,</b> <b>1 – 1<sup>st</sup> instar</b> 0 – 2 <sup>nd</sup> instar	<b>1 – control,</b> 0 – 1 <sup>st</sup> instar <b>2 – 2<sup>nd</sup> instar</b>
4	B	<b>1 – control,</b> 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	<b>1 – control,</b> 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar
5	B	<b>1 – control,</b> 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	2 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	<b>3 – control,</b> 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar
6	C	0 – control, 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	<b>1 – control,</b> <b>1 – 1<sup>st</sup> instar</b> 3 – 2 <sup>nd</sup> instar	<b>1 – control,</b> 0 – 1 <sup>st</sup> instar <b>3 – 2<sup>nd</sup> instar</b>
7	C	0 – control, <b>1 – 1<sup>st</sup> instar</b> 0 – 2 <sup>nd</sup> instar	<b>1 – control,</b> <b>1 – 1<sup>st</sup> instar</b> <b>1 – 2<sup>nd</sup> instar</b>	<b>1 – control,</b> <b>2 – 1<sup>st</sup> instar</b> <b>3 – 2<sup>nd</sup> instar</b>
8	C	3 – control, <b>1 – 1<sup>st</sup> instar</b> <b>4 – 2<sup>nd</sup> instar</b>	<b>3 – control,</b> <b>1 – 1<sup>st</sup> instar</b> <b>5 – 2<sup>nd</sup> instar</b>	<b>3 – control</b> <b>1 – 1<sup>st</sup> instar</b> <b>5 – 2<sup>nd</sup> instar</b>
9	C	<b>1 – control,</b> 0 – 1 <sup>st</sup> instar 0 – 2 <sup>nd</sup> instar	<b>2 – control,</b> <b>3 – 1<sup>st</sup> instar</b> <b>2 – 2<sup>nd</sup> instar</b>	<b>3 – control,</b> <b>5 – 1<sup>st</sup> instar</b> <b>2 – 2<sup>nd</sup> instar</b>

## Discussion

*H. bacteriophora* provided significant control of European Chafer in all treatments. Preliminary research from 2003 showed *H. bacteriophora* to be more effective in controlling second instar larvae than third instar larvae and recommended that further research should look at the efficacy in controlling first instar larvae.

In 2003, a rate of 3 billion *H. bacteriophora* per acre reduced the population of second instar chafer larvae in constructed soil based plots by 76%. In 2004, the same application rate reduced second instar chafer populations in the constructed plots by 43%, and reduced first and second instar survival in lawn plots by 57%, and 29% respectively. In addition, a lower rate of 1.5 billion *H. bacteriophora* per acre applied to the constructed grass plots reduced first instar survival by 82%, and second instar survival by 43%.

The results from this year's *H. bacteriophora* treatments confirmed last years' findings that 2<sup>nd</sup> instar larvae can be controlled with *H. bacteriophora* in late August. Results were clear however, that younger (first instar) larvae can be controlled more successfully with *H. bacteriophora*. Although the level of control of first instar chafer by *H. bacteriophora* was significant, the results from the first instar grass plot treatments were mixed. Figure 1 shows a survivorship of only 40% of larvae in the control plots. The number of larvae found in control plots was quite variable and no plot had a survivorship of over 60%. This low percentage of survival is most likely due to the small size of the chafer larvae at this stage, and their susceptibility to damage through handling and drying out when stored overnight. Although the survivorship was low for all first instars in plot treatments, *H. bacteriophora* still provided significant control for this stage of chafer larvae. This outcome is supported by results in the lawn treatments where the higher rate of *H. bacteriophora* was shown to control on first instar more effectively than second instar.

The results obtained are important because they demonstrate that *H. bacteriophora* is readily able to enter and infect first instar chafer larvae. In fact the most effective way to treat chafer larvae appears to be at the first instar stage with *H. bacteriophora*.

*S. carpocapsae* was only used in the grass plot trial and was effective in controlling second instar chafer larvae at both low and high rates though the most effective rate was 3 billion nematodes per acre. The extra-high rate treatment was ineffective in controlling chafer larvae; this could be due to density-dependent factors that affect entomopathogenic nematodes. High densities of nematodes within a host can reduce nematode survival and fecundity due to competition for nutrients (Selvan 1993). The infected hosts would die but the nematodes ability to regenerate and attack more chafer would be affected. Our study was short term and therefore the mortality of chafer due to first generation nematodes was likely all that was observed. In this case competition within the host would not affect results. Competition may also occur in the soil or around the host. It is possible that the chafers behaviour could be affected by the presence of large groups of nematodes. *S. carpocapsae* is not a searcher therefore could be present in large concentrations at certain points in the soil. If the chafer larva can detect large concentrations of nematodes it may move away from those areas.

*S. carpocapsae* appeared to be more effective at controlling 2<sup>nd</sup> instar larvae than *H. bacteriophora*, but was ineffective in controlling first instar larvae. These somewhat conflicting results for chafer mortality with this nematode only represents one year of data and more research would need to be done to make conclusions about its efficacy in controlling chafer. In addition, grass plots are unlike real lawns, and if such inconsistencies are present in this very highly controlled environment, we would not expect better, more consistent results on lawns. It could be that this species will only provide inconsistent control of chafer.

*S. kraussei* was only applied to second instar larvae and it showed some control at the low rate but was ineffective at the high rate. It is unknown why a lower rate would provide better control but the difference between the two is small, 62% of chafer survived at the low rate and 70% survived at the higher rate. *S. kraussei* was the nematode with the least effective control. It is not a species we could recommend for control of European chafer at the second instar stage.

### Lawn Care

Lawns included in the chafer research were chosen based on their chafer infestation and damage in previous years, but were not dug up for chafer assessment purposes prior to treatment in 2004. Because of the uneven distribution of chafer larvae throughout any given lawn, some treated lawn



plots may initially have contained more chafer larvae than others. To help address this variation 9 lawns were included, and divided into the three groups.

Much of the lawn damage attributed to chafer larvae is done by crows, skunks and other predators that feed on larvae over the winter. These predators have learned that damaged grass can contain many appetizing larvae and will completely destroy lawns in search of chafer larvae. It appears that the predators do not attack lawns appearing to be healthy - with little or no dead grass even though larvae may be present.

The effect of lawn care practices on chafer damage was best exemplified by the results of neighbouring lawns that were included in this research study. Lawns #1 and #9 were neighbours, both south facing, level lawns. Lawn #1 had suffered some damage to the boulevard in 2003, but had recovered by the time of treatment in 2004. Lawn #9 was badly damaged in 2003, and the grass was still sparse at the time of treatment in 2004. Lawn care practices were intensive in lawn #1 throughout the spring, summer and fall, while in lawn #9 there was less intensive management because ownership of the property had just changed hands. At sampling in October, Lawn #1 contained few chafer larvae (Group A), while lawn #9 was thoroughly infested (Group C).

The results of the lawn trials suggest that lawn care practices such as weekly mowing, thorough watering, annual fertilizing, liming, top dressing, power raking and aerating, in combination with an application of the nematode *H. bacteriophora* in July, can effectively reduce the damage caused by chafer and chafer predators to lawns.

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## Appendix A

Figure 1. Diagram of the spatial arrangement of tulip box plot treatments. 1<sup>st</sup> = 1<sup>st</sup> instar, 2<sup>nd</sup> = 2<sup>nd</sup> instar, H.b = *Heterorhabditis bacteriophora*, S.c = *Steinernema carpocapsae*, S.k. = *Steinernema kraussei*

2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate
1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water
2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.
2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate
1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate
1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water
2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.
2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate
2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate
2 <sup>nd</sup> S.k. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> H.b. high rate	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate
2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> water	1 <sup>st</sup> S.c.	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.c. Xhigh rate	1 <sup>st</sup> water	1 <sup>st</sup> H.b.	2 <sup>nd</sup> S.k. high rate	2 <sup>nd</sup> H.b. low rate	2 <sup>nd</sup> S.c. low rate	2 <sup>nd</sup> S.k. low rate

Table1. Statistical results for first instar chafer in plot trials.

Treatment	Application Amount	Survival of Chafer larvae	Statistically Significant Control	df	F-value	p-value
Control	N/A	36%	N/A	N/A	N/A	N/A
<i>S.carpocapsae</i>	3 billion /acre	42%	Yes	1	0.4633867	0.5038509
<i>H.bacteriophora</i>	1.5 billion	7%	Yes	1	27.03829	4.35E-05

Table 2. Statistical results for second instar chafer in plot trials.

Treatment	Application Amount	Survival of Chafer larvae	Statistically Significant Control	df	F-value	p-value
Control	N/A	75%	N/A	N/A	N/A	N/A
<i>S.carpocapsae</i>	1.5 billion	42%	Yes	1	27.1139	4.278E-05
<i>S.carpocapsae</i>	3 billion /acre	38%	Yes	1	19.877137	0.0002411
<i>S.carpocapsae</i>	6 billion /acre	72%	No	1	0.1043406	0.7500345
<i>H.bacteriophora</i>	1.5 billion	42%	Yes	1	42.432024	2.383E-06
<i>H.bacteriophora</i>	3 billion /acre	50%	Yes	1	12.718601	0.0019338
<i>S.kraussei</i>	1.5 billion	62%	Yes	1	7.7368421	0.0115179
<i>S.kraussei</i>	3 billion /acre	70%	No	1	1.4414414	0.2439337

Table 3. Statistical results for treatment of chafer in lawn plots

Group	Lawn	Treatment	Statistically Significant Control	df	F-value	p-value
A	1	1st instar	No	1	1.301205	0.268944
		2nd instar	No	1	0.231797	0.635996
	2	1st instar	No	1	0.9	0.355346
		2nd instar	No	1	0.047619	0.829714
	3	1st instar	No	1	0.568421	0.460637
		2nd instar	No	1	1.359517	0.258839
B	4	1st instar	Yes	1	26.1712	7.2E-05
		2nd instar	Yes	1	27.249	5.8E-05
	5	1st instar	Yes	1	19.687	0.00032
		2nd instar	Yes	1	17.3632	0.00058
C	6	1st instar	Yes	1	11.8416	0.00291
		2nd instar	No	1	3.890822	0.064113
	7	1st instar	Yes	1	10.7041	0.00424
		2nd instar	No	1	3.340895	0.084203
	8	1st instar	Yes	1	18.4312	0.00044
		2nd instar	No	1	0.101251	0.753995
	9	1st instar	No	1	2.725437	0.1161
		2nd instar	Yes	1	40.9091	5.1E-06