The Development and evaluation of new system to control nematodes in golf course

Hanafy Fouly, Clemson University
Ken Watkin, University of Illinois

Turfgrass and Environmental Research Online
Volume 12, Number 2 | March–April 2013

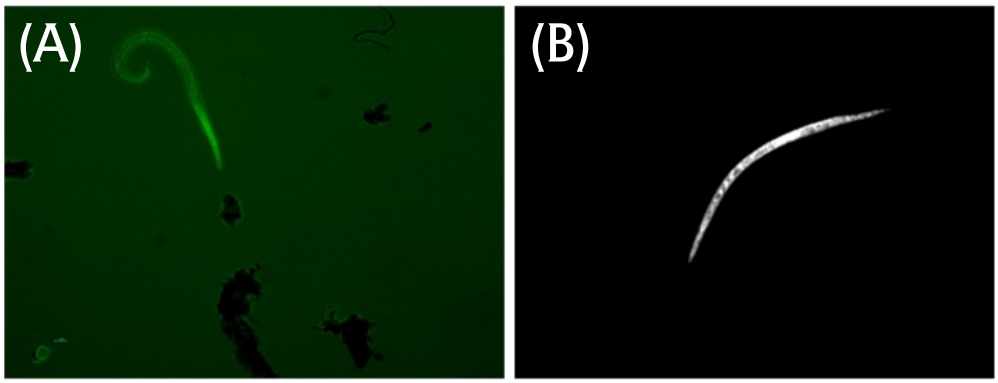
Objectives:

1. *Develop methods to visualize the ingestion of liposomes and determine their distribution within the body sting nematodes.*
2. *Develop liposomes–nematode biological control agent complexes and administration to sting nematodes in vitro and in vivo.*

Plant pathogenic nematodes are known to be serious pests of golf course turfgrasses. These microscopic worms feed on plant roots, thus weakening the plant and causing more damage from environmental stresses, such as heat, drought, nutrient deficiency and secondary infections by other pathogens. Nematode problems are most common and severe in sandy soils because they are more favorable for the growth and reproduction of most nematode species and also hold less water and nutrition to support plant growth.

There are some existing technologies and some under development for nematode control/suppression in turf systems. These include Curfew, and abamectin chemicals. Biological control products, which contain nematode–pathogenic or suppressive bacteria, are also being developed. Nortica is the recent trade name of a bacteria product (*Bacillus firmus*) being developed and sold by Bayer Environmental Science for suppression of nematodes in turf. The bacterium, *Pasteuria usgae*, is another biological agent that has been formulated for turf nematode control specific for sting nematodes, *Belonolaimus longicaudatus* and is sold under the trade name ´Econem´.

Figure 1. Nematode fluorescence after oral administration of fluorescent material. To monitor ingestion of liposomes containing chemical solutions, a water–soluble fluorescent compound was used. (A) A spiral nematode fed 25µl of liposmes containing fluorescent substance shows clear fluorescence along the esophagus digestive tract. (B) A Root Knot nematode fed 25µl of liposomes containing fluorescent substance shows clear fluorescence along the several organs of nematode body.



There is a need to develop, modify, or enhance existing technologies to control nematodes. In particular, development of a formulation that controls the rapid leaching of bacteria, and new potential nematicidal agents into soil would be very beneficial. A number of processes for coating or microencapsulating pesticides have been developed in the past. The objective of this proposal is to utilize liposomes as a new tool for designing slow release formulations of bacteria, abamectin and other nematicides to maximize their efficiency in controlling nematodes. Emphasis will be

©2013 by United States Golf Association. All rights reserved.
Please see Policies for the Reuse of USGA Green Section Publications.

TERO Vol. 12(2):20–21 | March–April 2013
USGA ID#: 2012–08–442
TGIF Number: 219650

given to control the virulent sting nematodes in golf course turf in the southeastern United States.

Liposomes are artificially–constructed spherical lipid vesicles with small and controllable size, diameter from tens to thousands of nm, whcih encapsulate and store various cargoes, such as proteins, DNA and various drug molecules. Liposome fluorescent materials were developed and different nematodes species ingested these materials. As a result, fluorescent material released in different parts of nematode body. Our data demonstrate that ingestion of liposomes loaded with fluorescent dye resulted in successful oral delivery of chemicals into the intestines of Root knot and Spiral nematodes (see Figure 1 . A and B).

Several sizes of liposomes (microns to sub microns) were developed and successfully ingested by nematodes. Dry preparations of liposomes were successfully developed and retained their structures.

The effect of Oxamyl (Vydate) and Thiodicarb (Larvin) within a liposome was determined on Root–Knot nematodes in vitro. First, efficient concentrations of nematicides (Oxamyl and Thiodicarb) that kill or suppress root knot larvae were determined. Root knot nematodes (*M. incognita*) eggs collected from tomato cultures by NaOC1 extraction. Egg suspension was incubated at room temperature until larvae were hatched (4–5 days). The Juveniles (J2) were counted and evaluated for activity/mobility for the duration of the study. Four different concentrations of both nematicides (Oxamyl and Thiodicarb) were used to assess their efficacy in killing the nematodes. These were untreated control, 200 ug, 1mg, 2 mg, and 10 mg. Three replicates of the each concentration were mixed with J2 suspensions and incubated at room temperature for 2 days (Table 1). Based on the results from this test, we eliminated Thiodicarb (Larvin) because it required higher concentration to kill root knot (J2).

Table 1. Effect of of two nematicides on root–knot nematode larvae.

Treatments	Root Knot larvae (J2)	
	Alive	Dead
Control	96.7	3.3
Larvin (Thiodicarb)		
200 ug	83.7	16.0
1mg	73.3	26.7
2mg	73.3	26.7
10 mg	73.3	26.7
Oxamyl (Vydate)		
200 ug	56.0	44.0
1mg	----	100
2mg	----	100
10 mg	----	100

We studied the efficiency of Oxamyl at 200 μg/ml and 100 μg/ml for suppression of root knot (J2) nematode larvae. We found that both concentrations lead to 100 % mortality of J2 larvae (Figure 2). We used 100μg of Oxamyl in subsequent greenhouse studies with liposomes.

Preliminary studies with nano–gram preparations of Avid inhibited root knot J2 larvae after brief exposure to the preparation. In a greenhouse experiment, we tested different concentrations of both Oxamyl and Avid in reducing root knot galls on tomato plants (in progress).

Figure 2. The effect of Vydate–liposome formulation on Root knot nematode larvae (A) No Vydate added – free and active larvae, (B) 100 μg of Vydate–liposomes was added–paralyzed larvae or lost

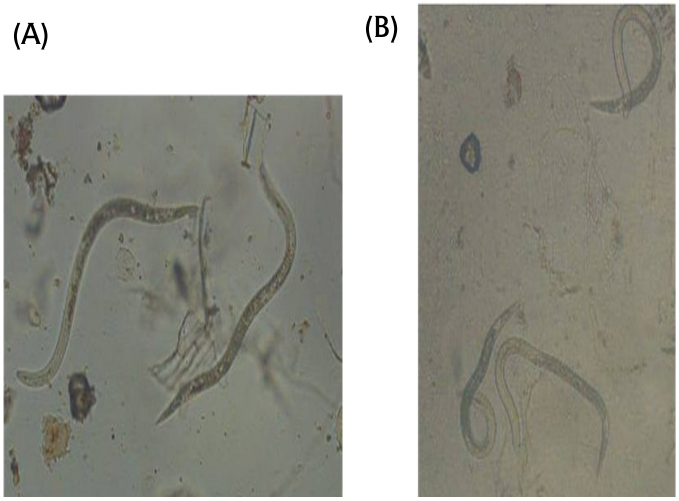
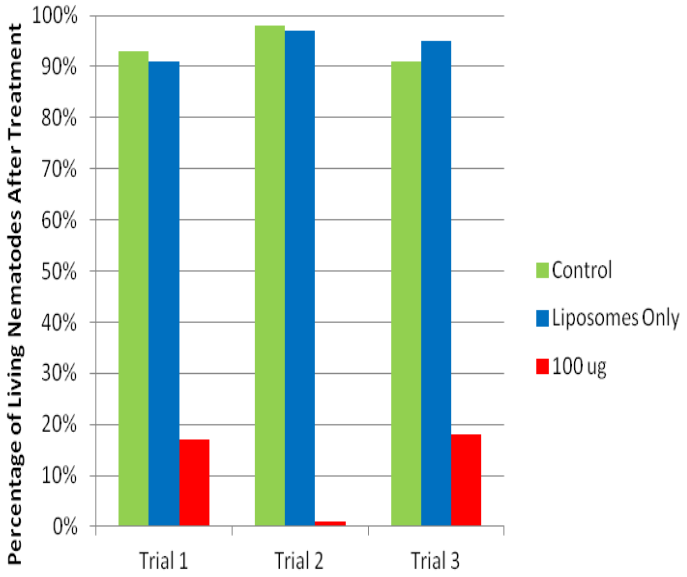


Figure 3. Nematicidal activity of 100 mg oxamyl–liposome formulation on root–knot nematodes (% living after treatment)



©2013 by United States Golf Association. All rights reserved.
Please see Policies for the Reuse of USGA Green Section Publications. .

TERO Vol. 12(2):20–21 | March–April 2013
USGA ID#: 2012–08–442
TGIF Number: 219650