RESEARCH/INVESTIGACIÓN

A METHOD FOR SCREENING CANDIDATE NEMATICIDES AGAINST THE PACIFIC SHOOT-GALL NEMATODE, *ANGUINA PACIFICAE*

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ABSTRACT

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A growth chamber bioassay was developed for testing the efficacy of potential nematicides against the Pacific shoot-gall nematode, *Anguina pacificae*. Twenty nine products were assayed, eight of which showed some degree of control. Agroneem Plus, Avid, Cleary 3336 Plus, Levimisole, Neemix +Trilogy, Nemacur, Aldicarb and Vydate penetrated young galls on Poa annua, 10 days after inoculation, and interrupted the nematode's life cycle, either by preventing the development of second generation infective juveniles or by causing the formation of "empty" galls that contained no nematodes. A modification of the assay was used to demonstrate the acropetal systemic activity of Cleary 3336 Plus.

Key words: Anguina pacificae, chemical control, growth chamber assay, nematicides, Pacific shoot-gall nematode, *Poa annua,* systemic

RESUMEN

McClure, M. A., and M. E. Schmitt. 2012. Un método para evaluar candidatos de nematicidas contra el nemátodo, *Anguina pacificae*. Nematropica 42:146-152.

Se desarrolló un bioensayo en cámaras de crecimiento para evaluar la eficacia de varios nematicidas en contra del nematodo, *Anguina pacificae*. De los 29 nematicidas evaluados, 9 mostraron cierto grado de control. Agroneem Plus, Avid, Cleary 3336 Plus, Levimisole, Neemix +Trilogy, Nemacur, Aldicarb y Vydate fueron capaces de penetrar en las agallas jóvenes de Poa annua, hasta 10 días después de la inoculación, e interrumpieron el ciclo de vida del nematodo, ya sea al evitar el desarrollo de la segunda generación de juveniles infectivos o al ocasionar la formación de agallas "vacías" sin nematodos. Se utilizó una modificación en el bioensayo para demostrar la acción sistémica acropétalo de Clearly 3336.

Palabras clave: Anguina pacificae, control químico, ensayo en cámaras de crecimiento, nematicidas, nematodo 'Pacific shoot-gall', *Poa annua*, sistémico

INTRODUCTION

The Pacific shoot-gall nematode is a major pest of *Poa annua* (*Poa*) golf course greens in Northern California (McClure *et al.*, 2008). Since discovery of the nematode in 1978, golf course superintendents have relied exclusively on Nemacur (Fenamiphos) to minimize damage on infested greens. When Nemacur was voluntarily withdrawn from the market in 2008, superintendents and agronomists were left with few options for managing this pest and, in the past 10 years, numerous field trials have been conducted to find a replacement. Westerdahl *et al.* (2005) tested six different products, known or reputed to be nematicidal. Only one, fosthiazate, was found to reduce galling as well as, or better than, Nemacur. But, fosthiazate is not registered for use on turf grasses. Subsequent trials, conducted under the auspices of the Northern California Golf Association have examined the effect of 35 different products on *A. pacificae* and turf quality at a cost of more than $400,000 (M. McCullough, Personal Communication). Not only are field trials expensive, but manpower and other resources are wasted on field-testing chemicals that are not efficacious. Consequently, a biological assay to identify nematicidal products, prior to field testing, was developed and used herein to evaluate the efficacy of 29 products. In addition, we have utilized the technique to confirm the purported acropetal systemic activity of Cleary 3336 Plus (Cleary Chemicals, LLC, Dayton, NJ), and its effect on *A. pacificae*. Absorption of this material by the roots and its translocation to the infection site could enhance the product's efficacy and increase the options for application parameters.

MATERIALS AND METHODS

Methods for rearing *A. pacificae* in growth chambers have been described previously (McClure *et al.*, 2008). Briefly, *Poa* seedlings, were cultivated in 1.0 ml plastic pipette tips filled with quartz sand, sub-irrigated with nutrient solution. The pipette tips were placed in the plastic box in which they were supplied with their tips submerged in the nutrient and, with the lids closed, the plants growing in them were maintained for 60 to 90 days, during which the life cycle of *A. pacificae* was completed. A modification of this method was used for the present study. All tests were conducted in a growth chamber at 22ºC day/18ºC night, 50% relative humidity and 12 hours of daylight with an intensity of 215 µMol/m^2/s. Polycarbonate plastic boxes, 6X6X11 cm (Gary Plastic Packaging, Bronx, NY) were fitted with racks constructed from black plexiglas with holes bored to accept 8 pipette tips, containing 2 g of a 3:1 mixture of 30-mesh and 60-mesh quartz sand (Fig. 1B). The entire box, except for the top of the lid, was sprayed with flat black paint to inhibit growth of algae in the nutrient solution, and two holes, 2 mm diam., were bored in opposite sides of the lid to admit air. Support for 6 boxes was constructed from a polyethylene food-storage container by cutting square holes in the lid (Fig. 1A). When the boxes were filled with 75 ml of nutrient solution and the pipette tips inserted through the holes in the rack, the tips of the pipettes were submerged approximately 1 cm into the solution. Each pipette tip was sown with 2 to 3 seeds of *P. annua* cv. 'True Putt' and, after germination, the seedlings were thinned to one per pipette tip. Seedlings were inoculated with approximately 30-40 infective juveniles (J2) 14 days after sowing and the nutrient in the boxes removed for 4 days to prevent the J2 from migrating out of the sand and into the solution. After 4 days, the nutrient was replaced. Ten days after inoculation, the surface of the sand was drenched with the test chemicals, dissolved or suspended in 20 µl of distilled water which was sufficient to saturate the sand to a depth of 3 or 4 mm. This is the depth that includes the junction of the shoot and roots of *Poa*, which is the region of the plant where the J2 penetrate to initiate gall formation. Control plants were treated with 20 µl of distilled water. Granular materials, such as Nemacur 10G, that could not be dissolved or suspended in water, were mixed with 60-mesh sand and 10 mg of the mixture was applied directly to the surface of the sand surrounding the seedlings. Control plants were treated with 10 mg of sand only. A wetting

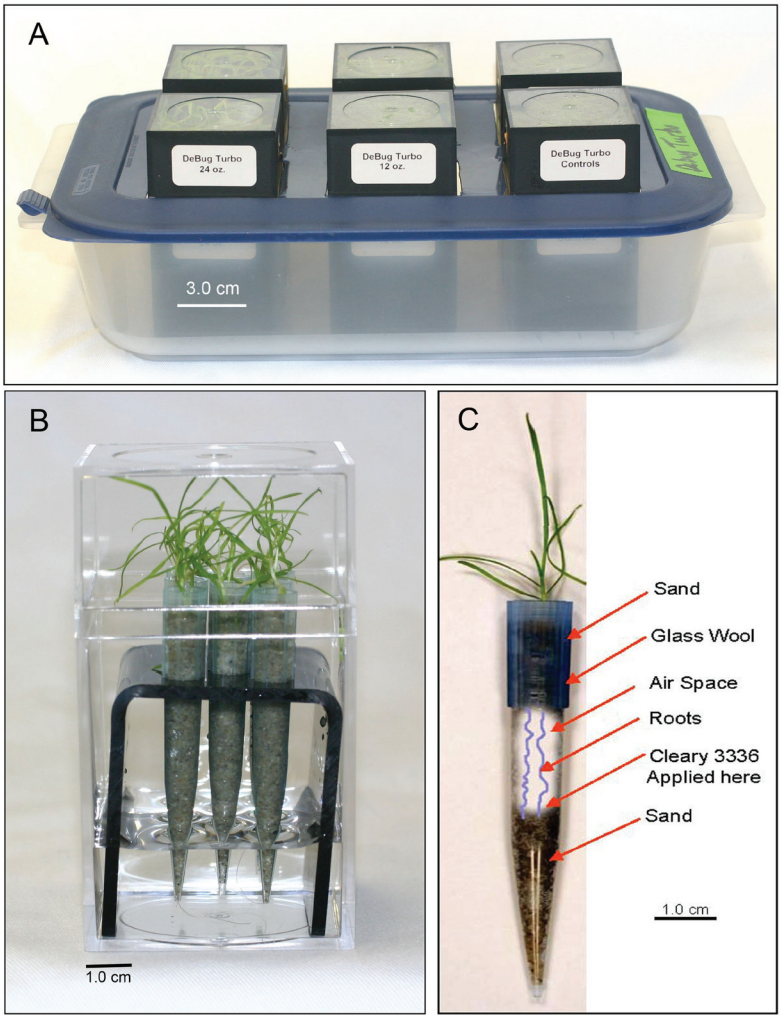

Fig. 1. Pipette tips used as "mini pots" for screening candidate nematicides against the Pacific shoot-gall nematode, *Anguina pacificae*. A rack, made from a refrigerator storage container (A) holds six polycarbonate boxes (B), each holding eight pipette tips filled with quartz sand and a single plant of *Poa annua*. By separating the roots from the shoot of the plant, injection of materials into the lower rhizosphere tested for acropetal systemic activity (C). Plants were irrigated by submerging the ends of the pipette tips into nutrient solution. To inhibit algal growth in the nutrient solution, the polycarbonate boxes were spray-painted black, except for the top surface. An unpainted box is shown in order to illustrate the method of suspending the pipette tips above the nutrient solution (B).

agent, Activator 90 (0.4 % v/v), was used to assist in the dispersal of Centric 40 WG. Plants were harvested 60 days after inoculation. The number of plants with galls and the developmental stages of the nematodes were determined microscopically.

To test the acropetal systemic activity of Cleary 3336 Plus, the pipette tip containers were divided into upper and lower portions, separated by a plug of glass wool with a layer of sand on top. This created an airspace through which the roots of the seedlings grew to reach another layer of sand at the bottom of the tube (Fig 1C). Seeds were sown in the top portion. The bottoms of the pipette tips were placed in the nutrient solution and the top portion of the sand was irrigated with only enough nutrient solution to support germination and growth of the roots until they passed through the air space and grew into the bottom layer of sand which was irrigated by capillarity from the nutrient solution in the bottom of the box. The plants were inoculated at the crown with approximately 30 *Anguina* J2. Two weeks after inoculation, Cleary 3336 Plus was applied in 20 µl of distilled water at the rate of either 8 or 16 ounces of formulated material per 1000 square feet (2.54 ml per m^2 and 5.09 ml per m^2, respectively). The material was introduced by puncturing the side of the tube with a 21-gauge (0.8 mm O.D.) hypodermic syringe needle attached to a 100-µl pipette (Rainin Instrument Company, Oakland, CA) and injecting it onto the bottom layer of sand containing the roots. Distilled water was used as controls. Plants were harvested 60 days after inoculation. Plants with galls were counted, and nematode development was determined by dissecting the galls at 40-60X magnification on a stereo microscope.

RESULTS

The methods described here enabled the screening of candidate nematicides against *A. pacificae* in laboratory growth chambers. Assays can be completed in 75 days, from sowing to harvest, and the number of products that can be tested is limited only by the capacity of the growth chambers. Twenty nine products were assayed (Table 1), eight of which showed some degree of control. Agroneem Plus, Avid, Cleary 3336 Plus, Levimisole, Neemix +Trilogy, Nemacur, Aldicarb and Vydate were able to penetrate young galls on *Poa*, 10 days after inoculation, and interrupt the nematode's life cycle, either by preventing the development of second generation infective juveniles or by causing the formation of "empty" galls that contained no nematodes (Table 1). Cleary 3336 Plus was systemic in *Poa* and equally effective when applied to the distal ends of the roots (Fig. 1C) or to the crown of the plant (Table 2). Juveniles in galls of plants treated with Cleary 3336 Plus were distorted, often devoid of contents, and obviously dead (Fig. 2). When juveniles did reach maturity, the eggs that the females produced were defective and did not contain mature embryos (Fig. 2, inset).

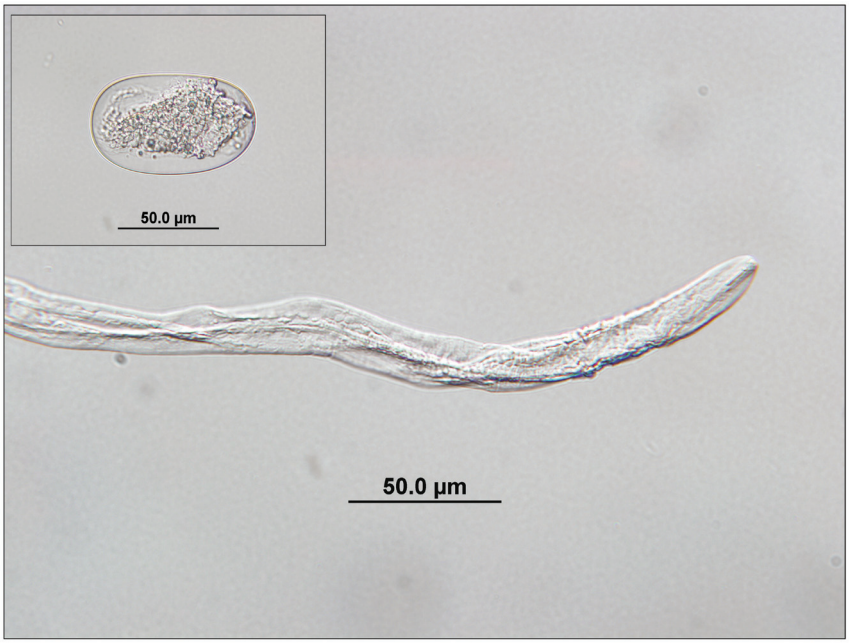

Fig. 2. Effect of Cleary 3336 on the development of the Pacific shoot-gall nematode, *Anguina pacificae*. Infective juveniles which had penetrated the crown of the plant failed to develop and were shrunken and distorted, often with ill-defined internal contents. Eggs from females that completed the molting process lacked definition and did not produce embryos (inset)

DISCUSSION

Under ideal conditions, the life cycle of *A. pacificae* can be completed in 60 days. Infective J2, liberated from mature galls, may be found free in the soil and are able to infect new plants in 48 hours or less. (McClure *et al.*, 2008). Once the J2 have penetrated host tissues and have begun to feed, they are less accessible to nematicides applied to infested sod. Consequently, products that are able to penetrate the tissues at the crown of the plant, either by translaminar movement or systemically via the roots or shoots are better suited for managing this damaging pest. Several of the products we tested have these properties, but only four, Agroneem Plus, Avid, Cleary 3336 Plus, and Neemix are registered for

Table 1. Products tested for their ability to control *Anguina pacificae* on *Poa annua*. Products in red typeface inhibited development of infective juveniles that had penetrated the plant. Rates are for formulated materials. The test chambers held 8 plants per box, so either 8 (one box) or 16 (two boxes) plants were used per treatment. Plants that died from causes unrelated to the treatment were removed, resulting in some treatments with fewer plants. a = second generation J2. b = adults and eggs. c = dead juvenile stages.

Product	Active Ingredient	Formulation	Rate: USA Units	Rate: Metric Equivalent	Total Number of Plants Tested	Number of Plants with Anguina in Galls	Number of Plants with Empty Galls	Number of Plants without Galls
Agroneem Plus	Azadirachtin	0.15% EC	32 oz/1000 ft^2	10 ml/ m^2	8	1 b	1	6
			Controls	-----	8	7 a, b	0	1
Avid	Abamectin	2.0% EC	20 oz/acre	146 µl/m^2	16	0	15	1
			Controls	-----	16	14 a, b	2	0
BioFence	Brassica seed extract	Water dispersible granule	328 oz/1000 ft^2	100 g/m^2	16	15 a	1	0
			656 oz/1000 ft^2	200 g/m^2	15	14 a	1	0
			Controls	-----	15	12	3	0
Crop Guard	Furfural	90% EC	8.6 gal/acre	80 l/ha	8	7 a, b	1	0
			17.2 gal/acre	160 l/ha	8	6 a,b	1	1
			Controls	-----	8	7 a, b	1	0
Carbine 50 WG	Flonicamid	50% water dispersible granule	2 oz/acre	14 mg/m^2	15	14 a, b	1	
			8 oz/acre	56 mg/m^2	16	16 a	0	0
			Controls	-----	16	16 a	0	0
Carzol	Formetanate HCl	92% water soluble powder	8 oz/1000 ft^2	2.54 ml/ m^2	14	12 a,b	2	2
			16 oz/1000 ft^2	5.03 ml/ m^2	15	8 a,b	2	5
			Controls	-----	16	10 a,b	1	5
Centric	Thiamethoxam	40% water dispersible granule	2 oz/acre	14 mg/ m^2	16	11 a,b	2	3
			4 oz/acre	28 mg/ m^2	16	14 a,b	2	0
			Controls	-----	16	15 a	1	0
Cleary 3336 Plus	Thiophanate methyl	19.4% water dispersible suspension	1 oz/1000 ft^2	318 µl/ m^2	15	6 c	1	8
			2 oz/1000 ft^2	636 µl/ m^2	15	7 c	0	8
			4 oz/1000 ft^2	1272 µl/ m^2	16	7 c	0	9
			Controls	-----	8	8 a	0	0
Coragen	Chlorantraniliprole (Rynaxypyr)	20% water dispersible suspension	4 oz/acre	29 µl/ m^2	16	15	1	0
			8 oz/acre	58 µl/ m^2	16	16 a,b	0	0
			16 oz/acre	116 µl/ m^2	16	15 a	0	1
			Controls	-----		16 a,b	0	0
Denim	Emamectin benzoate	2.15% EC	8 oz/acre	56 mg/m^2	15	15 a, b	0	0
			Controls	-----	16	14 a, b	2	0
Fulfil	Pymetrozine	50% water dispersible granule	6 oz/acre	44 µl/m^2	16	8	8	0
			Controls	-----	16	12a	2	2
KMnO4	Potassium permanganate	99% Reagent grade crystals	4 oz/1000 ft^2	1.2 g/m^2	16	14 a,b	1	1
			8 oz/1000 ft^2	2.4 g/m^2	16	16 a	0	0
			Controls	-----	16	15 a, b	0	1
Knack	Pyriproxyfen	11.23% EC	16 oz/acre	117 µl/m^2	15	12 a,b	2	1
			Controls	-----	16	12 -3a	2	2
Levimisole	Levimisole HCl	90% water soluble powder	8 oz/1000 ft^2	2.5 ml/m^2	16	4 b	1	11
			16 oz/1000 ft^2	5 ml/m^2	16	3 b	1	12
			Controls	-----	16	13 a,b	1	2

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Product	Active Ingredient	Formulation	Rate: USA Units	Rate: Metric Equivalent	Total Number of Plants Tested	Number of Plants with Anguina in Galls	Number of Plants with Empty Galls	Number of Plants without Galls
MeloCon WG	Paecilomyces lilacinous	6% water dispersible conidia of the fungus, P. lilacinous	3 oz/1000 ft^2	915 mg/m^2	32	30 a	2	0
			Controls	-----	16	15 a	1	0
Movento	Spirotetramat	22.4% water miscible liquid	10 oz/acre	73 µl/m^2	16	10 a	6	0
			Controls	-----	16	12 a	2	2
Neemix +Trilogy (volume of each)	Azadirachtin	4.5% water dispersable extract from Azadirachta indica seed	9 oz/1000 ft^2	2.9 ml/m^2	24	0	24	0
			18 oz/1000ft^2	5.7 ml/m^2	24	0	24	0
			36 oz/1000 ft^2	11.5 ml/m^2	17	0	17	0
			Controls	-----	24	23 a	1	0
Nemacur 10G	Fenamiphos	15% granule	2.3 lbs/1000 ft^2	11.2 g/m^2	36		1	35
			Controls	-----	12	12 a,b	0	0
Orthene	Acephate	75% Soluble Powder	2.5 oz/1000 ft^2	763 mg/m^2	15	13 a	3	0
			Control	-----	16	12a	2	2
Phosphite	Phosphite	Prepared from 99% Phosphorus acid	93 lbs/acre	10.4 g/m^2	7	7 a,b	0	0
			186 lbs/acre	20.8 g/m^2	6	4 a b	1	1
			372 lbs/acre	41.6 g/m^2	6	2 a,b	0	4
			Controls	-----	8	8 a,b	0	0
Piperazine	Piperazine	98% piperazine dihydrochloride hydrate Water soluble crystals	8 oz/1000 ft^2	2.4 g/m^2	16	16 a	0	0
			16 oz/1000 ft^2	4.8 g/m^2	16	16 a,b	0	0
			Controls	-----	16	16 a	0	0
Platinum 75 SG	Thiamethoxam	75% water soluble granules	8 oz/acre	58 µl/m^2	16	16 a,b	0	0
			16 oz/acre	116 µl/m^2	16	15 a,b	0	1
			Controls	-----	16	15 a,b	0	1
Rezist	Not registered as a pesticide	1.75% chelated copper, manganese and zinc	6 oz/acre	44 µl/m^2	16	13 a	3	0
			12 oz/acre	88 µl/m^2	16	15 a	1	0
			Controls	-----	16	14 a	2	0
Rugby 100ME	Cadusafos	100g/l Water dispersible liquid	6.3 oz/1000 ft^2	2.0 ml/m^2	8 * All Dead			
			12.6 oz/1000 ft^2	4.0 ml/m^2	8 * All Dead			
			Controls	-----	8	8 a,b	0	0
Sinocin	Plant extract	Water soluble liquid	2 oz/1000 ft^2	636 µl/m^2	7	7 a,b	0	0
			Controls	-----	8	7a,b	0	1
Success 480 SC	Spinosad	480 g/l Water dispersible liquid	10.9 oz/acre	80 µl/m^2	16	13 a,b	3	0
			Controls	-----	16	12 a	2	2
Temik 10G	Aldicarb	10% granule	15 lbs/acre	1.7 g/m^2	8	0	0	8
			Controls	-----	8	7 a, b	0	1

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Product	Active Ingredient	Formulation	Rate: USA Units	Rate: Metric Equivalent	Total Number of Plants Tested	Number of Plants with Anguina in Galls	Number of Plants with Empty Galls	Number of Plants without Galls
Vydate	Oxymyl	42% Water soluble liquid	4 pints/acre	468 µl/m^2	16	4 a, c	0	12
			8 pints/acre	936 µl/m^2	16	3 a, c	0	13
			Controls	-----	16	12 a, b	0	0
X-tra Power	Not registered as a pesticide	0.8% chelated copper, manganese, and magnesium. 3.2% chelated zinc.	12 oz/acre	88 µl/m^2	16	15 a	1	0
			24 oz/acre	176 µl/m^2	16	12 a	4	0
			Controls	-----	16	14 a	2	0

*Rugby 100ME was phytotoxic at the rates used.

Table 2. Effect of Cleary 3336 on the development of *Anguina pacificae* when applied to the roots of *Poa annua* two weeks after inoculation.

Treatment	Number of plants with mature galls containing second generation J2	Number of plants containing dead or undeveloped juveniles	Number of plants without galls
Cleary 3336 @ 8 oz per 1000 ft^2	0	3	5
Cleary 3336 @ 16 oz per 1000 ft^2	0	0	6
Controls	7	0	0

use on golf course greens. Two products, Agroneem Plus and Neemix/Trilogy are extracts from seeds of the neem tree, *Azadirachta indica*. The principal active ingredient is azadirachtin, although other triterpenoids also occur in neem seed extracts. Its mode of action on *A. pacificae* is not known but, in insects, azadirachtin interferes with the insect's chemoreceptors, producing anti-feeding effects. It also blocks peptide hormone release, resulting in growth and molting abnormalities (Aerts and Mordue [Luntz],1997). When our experiments were conducted, Trilogy was promoted as an adjuvant for Neemix. Trilogy is a botanical fungicide/miticide consisting of a clarified hydrophobic extract of neem oil. In subsequent tests, Trilogy alone had no effect on growth and reproduction of *A. pacificae* (data not shown). The product currently available, Neemix 4.5, contains no Trilogy and the use of Trilogy is not included in the specimen label. Galls on plants inoculated with *A. pacificae* and treated with products, such as Neemix, that contain azadirachtin generally do not contain any nematodes 60 days after inoculation. Either the developing juveniles stop feeding and migrate from the galls or their development is arrested and they are degraded enzymatically without leaving a trace. We have dissected hundreds of these galls without finding a single nematode. Empty galls, incited by *Meloidogyne incognita* and other nematodes, are also known to occur. (McClure *et al.*,1974).

Cleary 3336 Plus is a fungicide, registered for controlling a variety of fungi on turf grasses. Its active ingredient, thiophanate methyl, is a precursor of benzamidizole, which is well-known for its anthelmintic-ovicidal properties (Coles and McNeillie, 1977). Benzamidizole's mode of action in fungi appears to be related to its ability to interfere with the normal functioning of microtubules and, hence, nuclear division (Davidse, 1986). To our knowledge, this is the first report of its efficacy on plant-parasitic nematodes, where its activity in *Poa* infected with *A. pacificae* inhibits gall formation or results in an increased number of empty galls.

Avid is a pesticide containing the active ingredient, abamectin, isolated from the bacterium, *Streptomyces avermitilis*. Abamectin attacks the nervous system of insects and mites, causing irreversible paralysis. It has found use as a nematicide primarily as a seed treatment

for corn, soybeans and cotton. It is registered for use on turf, but not as a nematicide.

Because J2 in the soil are a fleeting target, we chose to treat infected plants 10 days after inoculation, when young galls contain developing and molting juveniles. Thus, we were not able to ascertain if the products we tested had any effect on second generation J2 *in planta* or in the soil. These J2 can enter a state of cryptobiosis (McClure *et al.*, 2008), and it seems likely that, in such a state, they may be more resistant to chemical treatments than are the active J2. In growth chamber assays, nematode development within individual galls may be relatively synchronized so that most eggs hatch to produce second generation J2 in a matter of days. However, in golf course greens, developmental stages in different galls can be widely disparate with generation overlap. Consequently, throughout the growing season, chemicals should be applied on a bi-weekly basis. In areas of California, where *Poa* greens are common, the growing season may extend from March to November.

It should be noted that the assay procedures described here do not lend themselves to statistical analyses of the results. The test chambers hold 8 plants per box, so either 8 (one box) or 16 (two boxes) plants were used per treatment, and untreated controls. But, individual plants cannot be considered "replications" because all plants in a box receive the same treatment and there is no way to randomize the pipette tips. However, we have had no difficulty in recognizing successful treatments, despite this shortcoming, and the method is adequate for preliminary screening. For more critical applications, each box could be considered a replication and 4 to 5 boxes utilized for each treatment.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of Arizona.

LITERATURE CITED

Aerts, R. J. and A. J. Mordue (Luntz). 1997. Feeding deterrence and toxicity of neem triterpenoids. Journal of Chemical. Ecology. 23:2117-2132.

Coles, G. C. and R. M. McNeillie. 1977. The response of nematodes in vivo and in vitro to some anthelmintics. Journal of Helminthology 51:323-326.

Davidse, L. C. 1986. Benzimidazole fungicides: mechanism of action and biological impact. Annual Review of Phytopathology 24:43-65.

McClure, M. A., K. C. Ellis, and E. L. Nigh. 1974. Post-infection development and histopathology of Meloidogyne incognita in resistant cotton. Journal of Nematology 6:21-26.

McClure, M. A., M. E. Schmitt, and M. D. McCullough. 2008. Distribution, biology, and pathology of Anguina pacificae. Journal of Nematology 40:226-239.

Westerdahl, B. B., M. A. Harivandi, and L. R. Costello. 2005. Biology and management of nematodes on turfgrass in Northern California. USGA Greens Section Record. September-October: 7-10.

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