Progress Report – *Anguina* Project

Submitted by:

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Accomplishments and Findings

Nematicide Assays

Eartheart, ABC-1, ABC-2, and MeloCon were screened for nematicidal activity against *Anguina*. **ABC-1 and -2** are code names for materials that were tested in the field trial at Spanish Bay by Mark Mahady. The field trial also included the **Eartheart Program**, a mixture of three separate components: All purpose insect spray (0.5% Permethrin), EartheArt Fertilizer (10.83% ammoniacal nitrogen, 1.09% nitrate nitrogen, 1.32% Water insoluble nitrogen, 4.67% other water soluble nitrogen, and 9% potash) and Eartheart Elixir, a liquid material of unknown composition. **MeloCon WG** is a biological material from Certis USA. Its active ingredient is the naturally-occurring soil fungus, *Paecilomyces lilacinus,* that is purported to control a wide range of plant-parasitic nematodes.

None of these materials controlled *Anguina pacificae*. Treatments were no better than the untreated (distilled water only) controls (Appendix 1). There were more "empty" galls on plants treated with ABC-2 but this material was phytotoxic at the rate used and it could not be determined if the empty galls were due to an effect on *Anguina* or to the poor growth/death of the plants.

Field Tests of *Anguina* on Bentgrass

Samples from the field trial at Lake Merced GC (See plot diagram in Appendix) were examined on November 18, 2009, 5 months after inoculation with *Anguina* and *Rathayibacter*. No Anguina galls or bacterial galls were detected and *Anguina* juveniles were not recovered from the soil. It appears that, under the conditions of this test, Anguina was unable to reproduce on any of the three bentgrass cultivars in the trial: T-1, Tyee, and 007. The plot was re-inoculated on March 18, 2010 and sampling will continue at 5 to 6-month intervals.

A second bentgrass field trial was initiated at Cypress Point Club on March 17, 2010 on a separate bentgrass nursery that contains seven bentgrass varieties: T1, G2-G6 Mackenzie (40-40-20% mixture), 007-Tyee (50-50%), and A1-A4 (50-50%). See plot diagram in Appendix I for details**.** Treatments included inoculation with *Anguina* infective juveniles at the rate of 45 per square inch and inoculation with the same number of *Anguina* plus approximately 10^8 *Rathayibacter* per square inch. The trial will be monitored and samples from the plots examined for the presence of nematode and/or bacterial galls after 3 months and at 6 month intervals thereafter.

Infection of older *Poa* seedlings

In all past and current experiments (life cycle, host-range testing, nematicide screening, etc.) *Poa* seedlings were inoculated two weeks after sowing. To determine if *Poa* can be infected at a later stage in its development, an experiment was conducted in which *Poa* seedlings were inoculated 60 days after sowing.

Two months after inoculation, none of the plants were infected with *Anguina*. This suggests that a critical stage of plant growth is required for infection to occur and, based on these data, that stage lies in the interval somewhere between emergence and 60 days after emergence. The interval between 14 and 60 days was not tested because we were interested primarily in the susceptibility of older plants. Anguina always infects *Poa* just below the apical meristem. One explanation for this critical interval is that, after 60 days (or perhaps less), growth of the shoot slows and the meristematic region is either no longer attractive to the nematode, or that it no longer responds to the enzymes released by the nematode that are required for the formation of the cavity in which nematodes develop. Alternatively, after 60 days, active resistance

mechanisms may develop in *Poa* that prevent infection. Consequently, it is primarily younger plants that are killed when infected with large numbers of Anguina, a phenomenon that we have observed repeatedly in plugs and cup-cuts from infested golf course greens that are sent to us for nematode assays.

Samples Processed for the Spanish Bay nematicide trial: 40.

Plans for 2010

1. Monitor the field tests of *Anguina*/*Rathayibacter* at Lake Merced GC and Cypress Point Club.

2. Additional goals are pending, contingent upon submission of a new research plan that will emphasize the role of Root-knot nematodes, *Meloidogyne* spp., in turf decline.

Acknowledgments:

Thanks to Lou Tonelli at Lake Merced Golf Club and Jeff Markow at Cypress Point Club for providing the bentgrass greens and assistance in running the field trials.

Appendix I

Anguina EartheArt & ABC Tests

Planted True Putt Poa: 3 November 2009
Inoculated with Anguina J2: 17 November 2009
Treated with EartheArt or ABC: 27 November 2009
Assayed: 12 January 2010

ABC Treatment & Reps	Number of Plants with Anguina Galls	Number of Plants with Empty Galls	Number of Plants without Galls
Controls 16 Reps	13	2	1 - dead plant
ABC 1 16 Reps	15	1	0
ABC 2** 16 Reps	7	7	2 - dead plants

EartheArt Treatment & Reps	Number of Plants with Anguina Galls	Number of Plants with Empty Galls	Number of Plants without Galls
Controls 16 Reps	16	0	0
EartheArt 32 Reps	30	2	0

**Note: All of the ABC treatments appeared to be somewhat phytotoxic, especially the ABC 2.

Anguina MeloCon Test March, 2010

Planted True Putt Poa: 14 January 2010
Inoculated with Anguina J2: 28 January 2010
Treated with MeloCon: 10 February 2010
Assayed: 23 March 2010

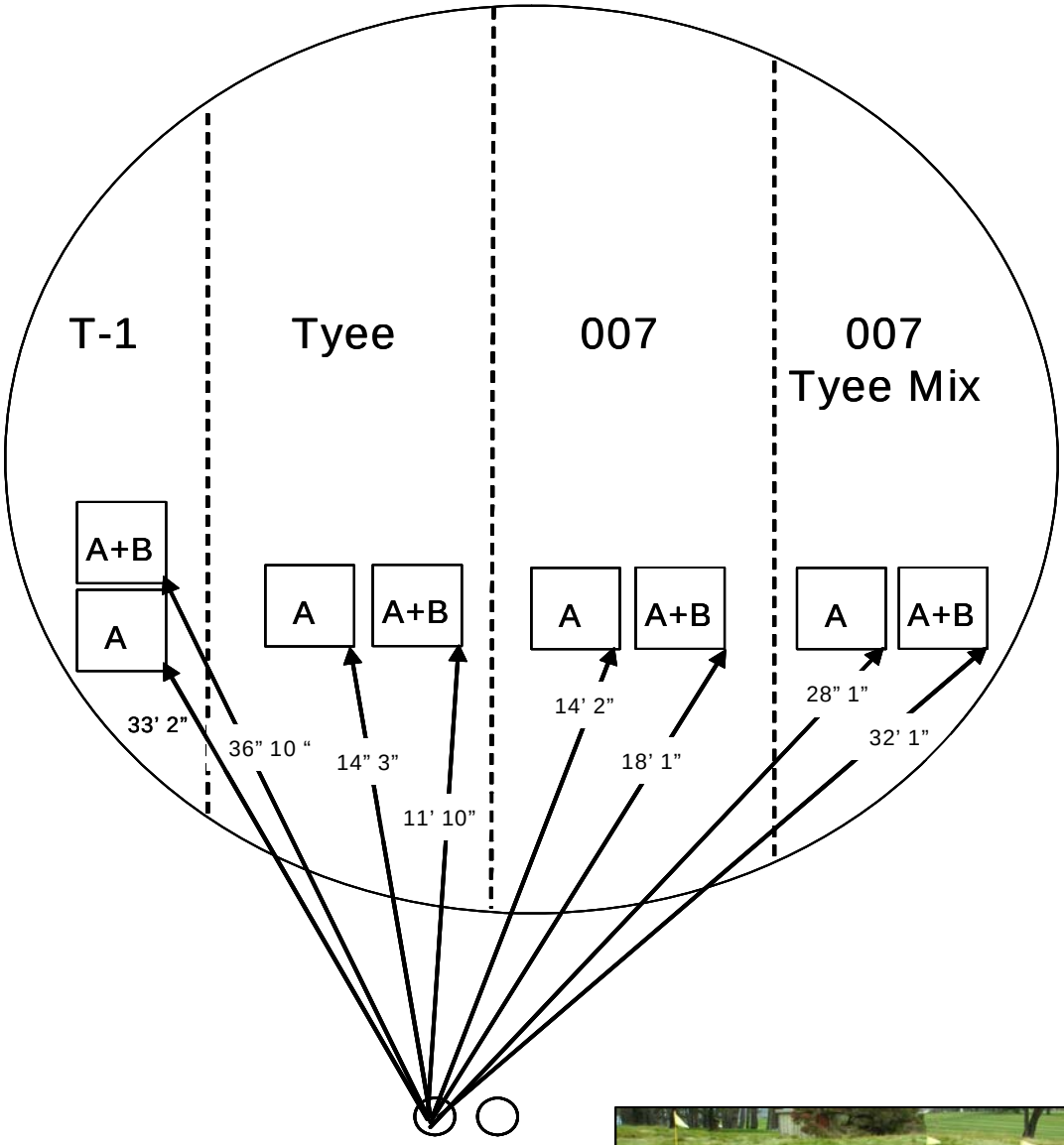
Treatment & Reps	**Number of Plants with Anguina Galls**	**Number of Plants without Galls**
Controls 16 Reps	16	0
MeloCon WP 3 oz. / 1000 sq. ft. 32 Reps	32	0

Individual Gall Analysis:

Controls 16 Reps	12 - Galls with 100's to 1000's of Anguina J2. 3 - Galls with about 2 dozen Anguina J2. 1 - Empty gall.
MeloCon WP 32 Reps	30 - Galls with 100's to 1000's of Anguina J2. 2 - Empty galls.

Lake Merced Bent Grass Test Green

June 17, 2009

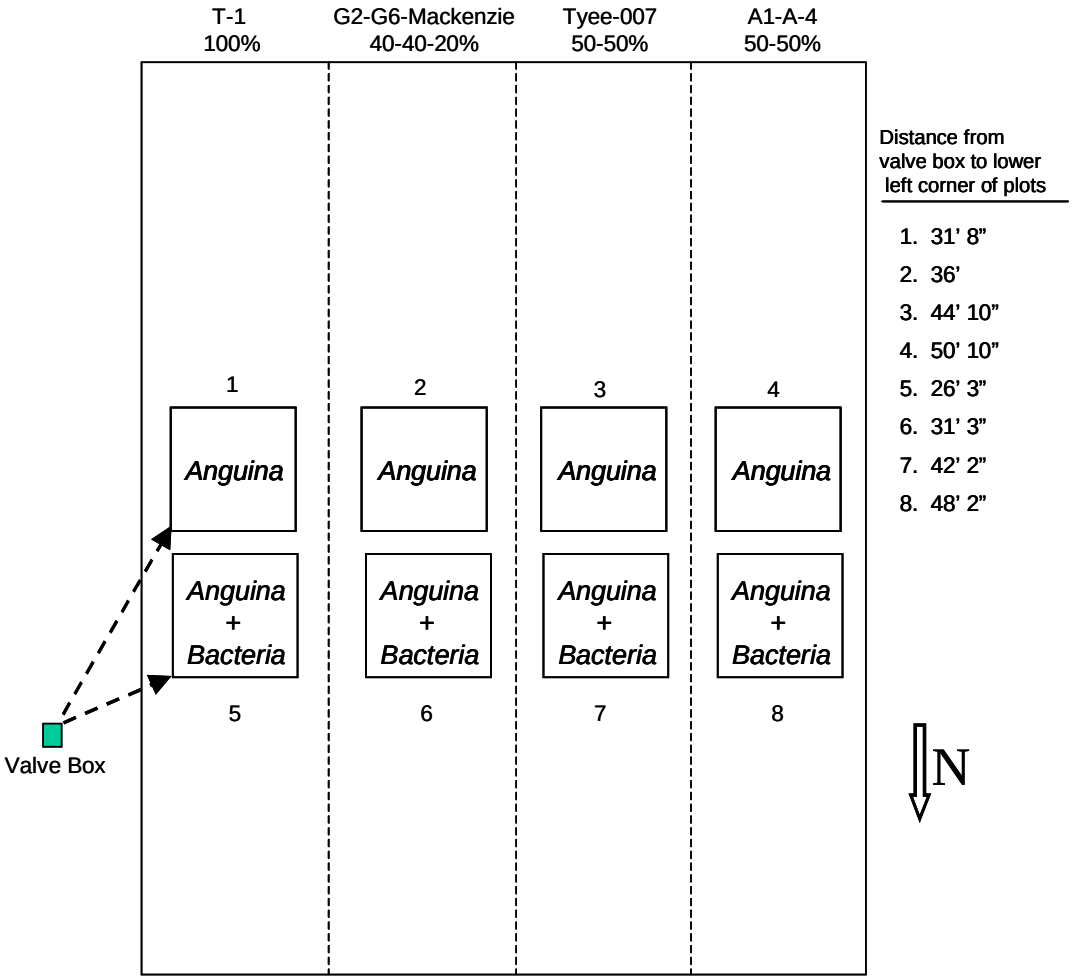


A = *Anguina*

A+B = *Anguina* + Bacteria

Treated areas are 1.0 meter square

Anguina-Bentgrass trial. Cypress Point Club. March 17, 2010



Plots are one meter square. *Anguina* plots were inoculated with 45 infective juveniles per square inch. *Anguina* + bacteria plots were inoculated with 45 infective juveniles per square inch plus approximately 10^8 *Rathayibacter* per square inch.

Anguina -- 2-Month-Old Poa Inoculation Test February, 2010

Planted True Putt Poa: 3 November 2009
Inoculated Controls: 17 November 2009
Inoculated 2-Month-Old Poa: 29 December 2009
Assayed Controls: 13 January 2010
Assayed 2-Month-Old Inoc. Poa: 23 February 2010

Treatment & Reps	**Number of Plants with Anguina Galls**	**Number of Plants with Immature Galls**	**Number of Plants with Empty Galls**	**Number of Plants with No Galls**
Controls (Inoc. 2 weeks old.) 18 Reps	16	2	0	0
Inoculated when 2 Months old 39 Reps	0	0	3	36

Individual Gall Analysis:

Controls 2 Week Old Poa
18 Reps

16 - Galls with 100's to 1000's of Anguina J2.
2 - Galls with Anguina Adults.

2 Month Old Poa
39 Rep

3* - Empty galls.
36 - No galls.

* These 3 empty galls were slightly swollen stems and hard to tell if they were originally started by Anguina J2 or due to something else.