FUSARIUM AS A POTENTIAL MYCOHERBICIDE FOR MANAGEMENT OF YELLOW LOOSESTRIFE

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Funding provided by:

Canada

Investment Agriculture Foundation of British Columbia

British Columbia

Funding for this project has been provided by Agriculture and Agri-Food Canada through the Canadian Adaption Program (CAAP). In British Columbia, this program is delivered by the Investment Agriculture Foundation of BC.

Project title: *Fusarium* as a potential mycoherbicide for management of yellow loosestrife

Commission Research Priority Addressed: High/Perennial Weeds/Yellow Loosestrife

Objectives: (1) Conduct additional pathogenicity assays in order to determine the most virulent isolates of *Fusarium* as a causal agent; (2) Determine when yellow loosestrife is most susceptible to infection by *Fusarium*, and (3) Perform a second year of field inoculations to see if *Fusarium* can be introduced and cause disease in a bed lacking the disease.

Project Summary: A naturally-occurring dieback disease of yellow loosestrife (YLS), one of the worst weed pests infesting cranberry beds in several producing areas, was initially found in four Massachusetts beds in 2010. *Fusarium oxysporum*, a known root pathogen, was cultured from symptomatic plants in all locations, as well as another *Fusarium* species. The disease was found in four additional beds in 2011. Pathogenicity studies conducted in 2011 in four field sites found at least four isolates of both *Fusarium* species were pathogenic, proving that this fungus was responsible for the dieback. Further inoculations will determine which *Fusarium* isolate(s) are most virulent and which YLS developmental stage is most susceptible to the fungus. Further field inoculations will be conducted in several Massachusetts cranberry beds with an infestation of YLS lacking the disease, and in Washington, if time allows it. This will be the first step towards the incorporation of this possible mycoherbicide into a management scheme for this important weed pest. Findings will be presented at the Cranberry Station Annual Research and Extension Update in January 2013 and in the Cranberry Station newsletter and website.

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Project Duration: Start 3/1/12; End 2/28/13

Location: Most of the research will be conducted in Massachusetts, although field plots may possibly be conducted on a limited basis in Washington

Literature Review (if applicable): In September 2009, an organically managed cranberry bed in Carver, MA with a significant infestation of yellow loosestrife (YLS, *Lysimachia terrestris*), commonly called mudweed or yellow weed by cranberry growers, was found to have at least 50% of the plants with severe dieback. Because it was late in the growing season and plants had been dying for two months, no attempts were made at isolating causal pathogens because there was a high probability of getting largely saprophytic microorganisms from the affected tissue. YLS plants in this same bed began dying in early July 2010 and plants were sampled for examination and culture. At this point, approximately 30% of the plants were showing dieback. Upon closer observation, significant necrosis of the crown and roots of the plants were noted. Of particular note, several of the necrotic areas of individual roots had visible white fungal mycelium on the surface. This mycelium was carefully teased from some plants and plated directly on to acidified potato dextrose agar (APDA) plates. The necrotic tissue of crowns and roots of other plants was sliced into thin 1 mm sections and surface sterilized in 10% Clorox for five minutes, after which it was blotted dry on sterile paper towels and plated on APDA. After seven days, individual fungal colonies were subcultured on to PDA plates for identification. The predominant fungus cultured was *Fusarium oxysporum*, a fungus known as a root rot pathogen in hundreds of crop plants but not infectious in either cranberry or blueberry (Farr *et al*.). There were other representative fungi cultured that were also noted, including another (as yet unidentified) species of *Fusarium*. Subsequently, YLS plants with dieback were also found in cranberry beds in Kingston, South Middleboro and Marion, MA. Symptomatic plants were

sampled from these other locations, and the plants showed identical symptoms including the visible fungal presence. Isolations performed from these other beds yielded the same fungal isolates as the initial bed. Thirty four fungal isolates were subcultured from these four beds with the disease and set aside in a refrigerator for subsequent use in pathogenicity studies. A set of these 34 cultures was sent to Dr. Wade Elmer, plant pathologist/mycologist at the Connecticut Agricultural Experiment Station in New Haven. Dr. Elmer is a world-renowned expert on the genus *Fusarium*, and he tentatively identified at least half of the isolates as *Fusarium oxysporum*. The primary goal of research conducted in 2011 was to prove that the fungus cultured from diseased plants caused the disease. Eighteen of the 34 isolates were selected for pathogenicity studies and grown on autoclaved white millet seed as described in LaMondia and Elmer (1989). After 10 days of growth, millet seeds colonized by fungal mycelium were harvested and air dried. Four cranberry beds with healthy yellow loosestrife were selected for field studies. Nine isolates were used for field trials in two of the four beds. One teaspoon of inoculum was placed at the base of 15-20 plants in each bed. Plots were briefly irrigated before and after inoculation. Growers could use any fungicide except Abound in these beds, as Abound is the only fungicide that might negatively affect *Fusarium*. YLS plants were monitored for symptom development, beginning at 4 weeks. Four of the *Fusarium* (both species) isolates caused loosestrife plants to develop similar symptoms as was seen in naturally-occurring diseased plants, and the fungus was successfully cultured from these dying plants. Now that the fungus has been conclusively shown to cause the disease, this project aims to explore further aspects of this host/pathogen combination, taking the necessary steps toward integrating this fungus as a mycoherbicide into a management scheme for YLS.

Procedures

Because the funding was half of what was requested from the BCCMC and because no funds were procured from the Cape Cod Cranberry Growers' Association for a second year of research, the work presented in the proposal had to be scaled back. All of the research was conducted in Massachusetts cranberry beds, as there were inadequate funds to expand the research to beds in Washington (where Kim Patten could perform and evaluate the trials).

It was decided to perform a second year of field trials in beds with yellow loosestrife populations had not had field trials in 2011 and that (to our knowledge) had not had any naturally-diseased plants in 2011. Six isolates of *Fusarium* which had shown pathogenicity in 2011 field trials were selected for further study. These isolates were LF-3, LF-4, LF-6, and LF-9 (*Fusarium oxysporum*) and LF-11 and LF-29 (*Fusarium* sp., still to be speciated). The fungal isolates were grown on white millet seed and inoculations were performed as described earlier. Symptom development was monitored and at the conclusion of the experiment, plants were harvested for isolation of the causal agent. Disease was rated using a numerical index wherein 0 = no symptoms and 4 = severe symptoms and death. Roots and crowns were washed, segments of plant tissue were selected and surface-sterilized in 10% Clorox, and segments were plated on APDA. Plates were evaluated for the recovery of *Fusarium* at 14 days. These inoculations were performed at five different cranberry beds located in Falmouth (June 7), Middleboro (2, both on June 5), Rochester (June 6) and Wareham (June 6).

In a separate field trial, 32 isolates of the fungus in our collection (14 isolates of *Fusarium oxysporum* and 18 isolates of *Fusarium* sp.) were evaluated for pathogenicity in a cranberry bed in Middleboro. A flask with 125 ml of potato dextrose broth was seeded with a disc of fungal

mycelium and the fungal isolates were grown for 10 days. Fungal mycelium was harvested for each isolate by running the liquid culture through sterile cheesecloth, the mycelium was macerated in a blendor and a spoonful of the fungal mycelium was deposited at the base of 10 plants as described earlier on June 19. Symptoms were monitored as they were in the other field plots.

In all field experiments, symptomatic plants were dug up and the causal fungus was isolated as described earlier.

Results

The disease ratings of the six isolates evaluated in five cranberry beds can be seen in Table 1.

Table 1. Six isolates inoculated in five beds with yellow loosestrife

Isolate	Falm	Mid-1	Mid-2	Roch	Ware	Overall
LF-3	0.5	1.5	1.8	0.5	3.1	1.48
LF-4	0.4	1.9	0.9	0.9	1.6	1.14
LF-6	1.5	0.5	1.0	0.5	1.0	0.90
LF-9	0.6	0	0.7	0.6	2.8	0.94
LF-11	1.7	1.3	1.6	2.1	1.7	1.68
LF-29	1.1	1.4	0.6	0.9	2.4	1.28

There was great variability in the pathogenicity of the six isolates from location to location, indicating either site differences or differences in the individual loosestrife populations/genotype. LF-11 (*Fusarium* sp.), LF-3 (*Fusarium oxysporum*) and LF-29 (*Fusarium* sp.), respectively, were the most pathogenic, although they did not result in much mortality of the inoculated loosestrife plants. The highest mortality occurred in the Wareham bed, which interestingly, is an organic cranberry bed.

The disease ratings for the 32 isolates evaluated in one cranberry bed can be seen in Table 2.

Table 2. Thirty two isolates inoculated in one bed with yellow loosestrife

Isolate	Rating	Isolate	Rating	Isolate	Rating	Isolate	Rating
LF-1	**0.3**	**LF-10**	**0.6**	**LF-18**	**0.4**	**LF-27**	**1.2**
LF-2	**0.4**	**LF-11**	**1.6**	**LF-19**	**0.4**	**LF-28**	**0.4**
LF-3	**1.5**	**LF-12**	**0.4**	**LF-20**	**0.7**	**LF-29**	**0.4**
LF-4	**0.4**	**LF-13**	**1.5**	**LF-22**	**0.7**	**LF-30**	**1.1**
LF-5	**0.4**	**LF-14**	**0.9**	**LF-23**	**1.0**	**LF-31**	**1.2**
LF-6	**0.3**	**LF-15**	**0.4**	**LF-24**	**2.3**	**LF-32**	**0.4**
LF-7	**1.1**	**LF-16**	**0.5**	**LF-25**	**0.4**	**LF-33**	**0.4**
LF-9	**1.4**	**LF-17**	**0.4**	**LF-26**	**0.4**	**LF-34**	**2.0**

The most pathogenic isolates were LF-24 (*Fusarium* sp.), LF-34 (*Fusarium* sp.), LF-11 (*Fusarium* sp.), LF-3 (*Fusarium oxysporum*), LF-13 (*Fusarium oxysporum*), and LF-9 (*Fusarium oxysporum*). The unspeciated *Fusarium* was the most pathogenic in this trial. As in the other field trial, in most instances, the fungus did not kill most of the loosestrife plants. LF-4, LF-6, LF-11 and LF-29 (used in the other field trials) showed very low pathogenicity in this trial, again showing a lack of consistency from site to site.

Recovery of the *Fusarium* isolates from inoculated symptomatic plants was very low in frequency, due to extensive growth of the common soil fungus *Trichoderma*. Not only was this fungus present in great frequency on all roots, but it grew at a much faster rate than *Fusarium*. In retrospect, a highly selective medium should have been utilized to culture the pathogen for a better determinant of its presence in the inoculated plants.

References

Farr, D.F., Bills, G.F., Chamuris, G. and Rossman, A.Y. 1989. Fungi on Plants and Plant Products in the United States. APS Press. St. Paul, MN. 1252 p.

LaMondia, J.A. and Elmer, W.H. 1989. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus. Can. J. Bot. 67:2420-2424.

Probable Subsequent Research Work Required

It is apparent that the fungal pathogen *Fusarium* is not capable of consistently causing disease, and especially, mortality in inoculated yellow loosestrife plants in the field. A logical follow-up would be to use the fungus in conjunction with herbicides that are registered for management of loosestrife, such as Curio and Quinstar. Lower rates of these herbicides could be utilized to stress the loosestrife with follow-up inoculations of *Fusarium* inoculum to "finish the job." Down the road, a better formulated preparation of fungal inoculum would be necessary for the mycoherbicide to be grower friendly. Field trials should be conducted in several cranberry beds in British Columbia, Oregon and Washington to see if the populations of yellow weed behave any differently that the populations of yellow loosestrife in Massachusetts. A survey should be started to look for any dying loosestrife plants in the Pacific Northwest with subsequent isolations aimed at the determination of whether a fungus is involved in the symptoms, and if *Fusarium* is also causing disease in those beds.