Alfalfa Disease Survey Of Ontario

Purpose:

Surveys are valuable tools to quantify disease problems, increase awareness and initiate appropriate management strategies or take proactive measures in order to minimize or reduce producer's losses. The information collected and reported from a project can alert extension personnel, grower organizations, industry and researchers to any new emerging pest issues in a crop such as Brown Root Rot and new races of Aphanomyces in alfalfa to name a few. This provides a valuable "heads-up" to potential disease problems and allows stakeholders the necessary time to develop management strategies hopefully before they become a problem.

During the course of this project we were able to survey forage fields in order to determine the incidence, distribution and severity of many common diseases of commercial alfalfa fields in Ontario. We used traditional field sampling, processing and mycological culturing to determine the fungal distribution in fields with typical alfalfa yellowing symptoms as well as evaluated new generation molecular techniques to determine applicability.

Methods:

Eighteen alfalfa fields were surveyed for disease severity and incidence. The numbers of healthy and diseased seedlings were counted in five random spots in each field as well as number of plants/ sq meter. Each disease seedlings was rated on a scale of 1-5 where 1= no necrosis of roots and hypocotyls, healthy; 2 = Slight necrosis of roots and hypocotyls; 3= necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stems; 4= extensive necrosis of roots, hypocotyls and cotyledons, and server stunting of stems; 5= dead seedlings/plants. Representative plants were brought to the laboratory where roots were cleaned and bleached with 15% sodium hypochloride solution prior to plating on Potato dextrose agar media. The resulting cultures were purified, sub cultured and allowed to grow on the selective media and pathogens were identified on morphological characteristics. This culture collection is being maintained and will be available to other researchers such as AAFC Ottawa.

Results:

During the course of this project we were able to survey fields in order to determine the incidence, distribution and severity of many common diseases of commercial alfalfa fields in Ontario. We used traditional field sampling, processing and mycological culturing to determine the fungal distribution in fields with typical alfalfa yellowing symptoms. We not only found new fungal pathogens of interest such as *Aphanomyces* and *Phoma* but traditional endemic soil fungal pathogens such as lower fungi (*Phytophthora*, *Pythium*), *Rhizoctonia* and to our surprise a large number of various *Fusarium* species which were very predominant (>50%) in our isolates. We will continue to identify these based on morphological characteristics and some such as *F. oxysporum* f. sp. *medicaginis* the causal agent of *Fusarium* wilt in alfalfa are very problematic and could indicate changes in *Fusarium* diversity in the province.

1) Disease Survey

Table 1: Distribution of alfalfa fields, average number of seedling per m^2, alfalfa yellowing severity and incidence in southern Ontario.

Field	Average number of seedling per m^2	Disease	
		Severity[x]	Incidence
1	28	1.2	10.7
2	32	1.1	5.6
3	18	1.5	30.8
4	28	1.5	27.5
5	16	1.0	2.5
6	29	1.3	25.9
7	21	1.0	4.9
8	25	1.3	25.8
9	20	1.7	32.7
10	13	1.3	31.7
11	42	1.3	14.3
12	34	1.8	23.5
13	23	1.2	8.7
14	20	1.2	17.8
15	18	1.3	18.2
16	26	1.4	26.4
17	30	1.2	14.1
18	20	1.2	24.2
Average	**25**	**1.3**	**19.2**
Range	**13 - 42**	**(1.0-1.8)**	**(2.5 – 32.7)**

[x] = Based on the 1-5 rating scale where; 1= no necrosis of roots and hypocotyls, healthy; 2 = Slight necrosis of roots and hypocotyls; 3= necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stems; 4= extensive necrosis of roots, hypocotyls and cotyledons, and server stunting of stems; 5= dead seedlings

2) *Aphanomyces* Race determination:

Another outcome of this project was the confirmation of *Apahnomyces* Race 2 in the province through the use of an *Aphanomyces* greenhouse differential test which was developed previously with Ontario Forage Council support. We partnered with AAFC Ottawa (Dr. Andre Levesque) to evaluate traditional and new state of the art molecular technologies developed through another Ontario Forage Council Project and the success of these technologies and the sensitivities of these tests and its potential

application in a diagnostic capacity will allow for not only the detection and quantification of *Aphanomyces* but basically any other alfalfa pathogens regardless of source (soil, plants, air, rain, etc.). Although alfalfa was used as the first commodity based study of this new technology it could be applied to other crops as well

Table 2: Percentage of different pathogens causing alfalfa yellows isolated from symptomatic roots in various fields in Ontario.

Field	Percentage of Fungi Isolated			
	Lower fungi	*Fusarium* spp.	*Phoma* sp.	*Rhizoctonia* sp.
1	**11.1**	**44.4**	**37.0**	**7.4**
2	**11.5**	**46.2**	**34.6**	**7.7**
3	**3.1**	**40.6**	**53.1**	**3.1**
4	**0.0**	**50.0**	**50.0**	**0.0**
5	**7.1**	**67.9**	**25.0**	**0.0**
6	**6.9**	**58.6**	**34.5**	**0.0**
7	**15.2**	**45.7**	**23.9**	**15.2**
8	**21.6**	**35.1**	**18.9**	**24.3**
9	**13.5**	**40.5**	**18.9**	**27.0**
10	**10.3**	**53.8**	**5.1**	**30.8**
11	**20.4**	**30.6**	**20.4**	**28.6**
12	**15.4**	**42.3**	**19.2**	**23.1**
13	**28.9**	**42.1**	**15.8**	**13.2**
14	**23.7**	**39.5**	**31.6**	**5.3**
15	**12.2**	**55.1**	**28.6**	**4.1**
16	**7.7**	**84.6**	**7.7**	**0.0**
17	**25.0**	**75.0**	**0.0**	**0.0**
18	**7.1**	**57.1**	**25.0**	**10.7**

Summary:

Increased awareness amongst producers and the forage industry in the province of alfalfa issues including diseases would assist in limiting future losses and promoting better disease management strategies through best management practices as well as maintaining a sustainable and competitive forage industry well into the future. Project results remind us we must not forget traditional diseases especially *Fusarium* as well as they are still a major contributor to alfalfa yellowing and winterkill. New molecular identification tools will allow for earlier detection, quicker sampling of growers fields and plant samples as well as help breeders identify new lines with potential resistance further strengthening the sector in the future.

* *Aphanomyces* Race 2 – Soils A

Figure 1: In this soil A based on our differential test would indicate the presence of Aphanomyces Race 2. Additional work using the 454 DNA pyrosequencing analysis by AAFC Ottawas should the highest detectable (quantifiable) Aphanomyces levels also in this soil A. This is the first times these new molecular technologies have been used in a commodity based system.

(Note the yellow colour as well as severe stunting of seedlings and death. Additional symptoms included necrotic roots and hypocotyls, chlorosis and purpling of foliage.

Soil A – High overall rating score with DART and WAPH-1 highest suggesting Race 2 *Aphanomyces*.	Soil B – Low rating score (No *Aphanomyces* or *Phytophthora*)

Figure 2: comparing soil B with very low levels of disease pathogens (note lack of symptoms) in soil compared to soil A with the highest level of Aphanomyces as well as Race 2.

Figure 3: Showing typical *Aphanomyces* root rot symptoms of Race 2 on seedlings of susceptible line WAPH-1 in greenhouse differential test grown on soil A.

Next Steps:

Project results remind us that although we often highlight or focus on the new and interesting, we must not forget traditional or endemic diseases especially Fusarium as well since they are still a major contributor to alfalfa yellowing and winterkill. Efforts post project around communication of results will continue by OMAFRA, Ontario Forage Council, AAFC and other stake holders. As mentioned earlier new molecular identification tools will allow for earlier detection, quicker sampling of growers fields and plant samples as well as help breeders identify new lines with potential resistance further strengthening the sector in the future. This project has been successful but as with any project new questions arise and will require further post project activities by OMAFRA and AAFC.

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