AGR1115

Determination of the Host Status of Field Pea and its Associated Rotations and Weeds to the Stem and Bulb Nematode in the Canadian Prairies

INVESTIGATORS

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STUDY SPONSORS

Alberta Crop Innovation and Development Fund, Saskatchewan Pulse Growers Association, Alberta Pulse Growers Association and Manitoba Pulse Growers Association

TYPE OF STUDY

AGRONOMY

OBJECTIVES

1. Conduct a field survey to confirm the infestation of weeds in pea fields with the stem and bulb nematode

2. Determine the species identity of the stem and bulb nematode from Prairie Canada

3. Conduct a greenhouse study to confirm what weed species are a source of contamination of harvest samples and that yellow field pea is not the primary host for the stem and bulb nematode from Prairie Canada

4. Conduct a greenhouse study to determine if rotation crops of yellow field pea in Western Canada are or are not a host for the stem and bulb nematode from Prairie Canada

5. Convey findings to the Canadian Food Inspection Agency that their analysis protocols for export pea shipments may be updated to differentiate *Ditylenchus weischeri* and *D. dipsaci* and reanalyze past positive samples

WHY STUDY NEEDED

This research will help to maintain market access of Canadian peas on the world market. With field pea exports being about $600,000,000 and the exports coming from AB, SK, and MB accounting for nearly all of the exports, the pulse industry of the these provinces benefit greatly. The results provide the pea export industry and its representative organizations with the knowledge to initiate discussions with Indian authorities and establish limiting export market access problems. Development of molecular identification of existing races of pest nematode will help in rapid identification of pest and a tool for shipment screening.

HYPOTHESIS

STUDY DESIGN

Objective 1: In the summer of 2011 a total of 14, 5, and 4 fields were surveyed in Manitoba, Saskatchewan, and Alberta, respectively. Mainly creeping thistle weed plant samples were collected from the different pea and other crop fields for the presence of stem and bulb nematode. Nematode extraction and microscopic evaluation using same protocol as Canadian Food Inspection Agency (CFIA) for screening the stem and bulb nematode in export shipments.

Objective 2: DNA sequences of the stem and bulb nematode from several regions of Prairie Canada were compared to that of *Ditylenchus dipsaci*. The Prairie Canada stem and bulb nematodes were obtained during survey of weeds fields in 2010 (MB only) and 2011 (AB, SK, and MB), and from pea harvest samples collected in 2009 (AB, SK, MB). DNA sequences of the nematodes were compared to the already published sequences of stem and bulb nematode species including *D. weischeri* and *D. dipsaci*. This work was done in collaboration with Dr. Sergei Subbotin, Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, California, USA.

Objective 3 and 4: Green-house trials were conducted for screening of creeping thistle, yellow pea, hard red spring wheat, canola, lentil, and chickpea as hosts for *D. weischeri* and *D. dipsaci*. *D. dipsaci* had been used to possibly contrast the host preference profile to D. weischeri. Garlic was included as a test plant because *D. dipsaci* was obtained from infested bulbs. The reproduction factor (Rf) of the nematodes was determined by dividing the recovered population by the added population with a Rf > 1 showing the plant was capable of being a host and Rf < 1 not being a host.

Another five trials were conducted with the following treatments:
Creeping thistle and yellow field pea challenged with *Ditylenchus weischeri*
Creeping thistle and yellow field pea challenged with *Ditylenchus dipsaci*
Garlic, spring wheat, and canola challenged with *Ditylenchus weischeri*
Garlic, spring wheat, and canola challenged with *Ditylenchus dipsaci*
Chickpea and lentil challenged with *Ditylenchus weischeri*
In addition to a control (no nematode challenge), a secondary control of creeping thistle and garlic were included when the trial included challenge with *D. weischeri* and *D. dipsaci*, respectively. Either nematode was not recovered from the control treatments.

Objective 5: Once the project results were completed, meetings were held with CFIA.

FINDINGS

From the fields surveyed in 2011, 13 fields were positive from Manitoba, three from Saskatchewan, and no field was positive from Alberta for the presence of stem and bulb nematode. Pigweed (*Amaranthus retroflexus*) collected from one field in Manitoba was also observed to be positive with nematode.

The results of multiple lines of molecular DNA evidence show the Prairie Canada stem and bulb nematode to definitively be *Ditylenchus weischeri* and not the quarantine stem and bulb nematode *Ditylenchus dipsaci*. Various research reports have shown that the *D. dipsaci* species was not monophyletic (one species) but really a cluster of different species, including *D. weischeri* (commonly found on creeping thistle plant parts) and D. gigas (stem and bulb

nematode that primarily infests faba or broad bean). The results of this study are further evidence validating species separation of *D. weischeri* and *D. dipsaci*. Therefore, establishing quarantine restrictions to trade for species across all taxa within *D. dipsaci* may not have been needed. Further it seems it is weed seeds and particularly, creeping thistle seeds, are the source of the very rare occurrence of *D. weischeri* in yellow pea export grain.

The greenhouse host screening trial results confirm *D. weischeri* to parasitize creeping thistle. The nematode had a relatively high Rf value which was not surprising to find considering it was often present on the weed in the field surveys given above and being reared on creeping thistle. Yellow pea needs to be considered a possible host of *D. weischeri* because the nematode was able to reproduce, though not to the extent as on creeping thistle. Confirmation with field experiments is needed for yellow pea including possible effects on yield and quality, based on these results. Large green seed lentil, Kabuli and Desi chickpea, garlic, spring wheat, and canola were not hosts for *D. weischeri*.

The greenhouse host screening trials also confirmed that *D. dipsaci* reproduced aggressively on garlic, which is not surprising since the nematode is causing large yield losses of garlic in northeastern North America. It survived on creeping thistle but spring wheat and canola were not hosts. *D. dipsaci* was also able to parasitize yellow pea though not with same preference as for garlic but more so than *D. weischeri*. Therefore, there is potential for concern for the nematode to move from garlic fields to yellow pea. Fortunately most commercial garlic in Canada is not grown on the Prairies. Nevertheless growers should in future avoid the possibility of yellow pea and garlic being grown near each other.

The study results, developed methodologies, sequences, and creeping thistle material colonized with *D. weischeri* from this project were transferred to CFIA. Since the initial meeting, much progress by CFIA has been made to advance addressing the market access issue of yellow pea with India.

SIGNIFICANCE OF STUDY

These reports and the results of this project are important to Canadian growers because they indicate the *D. dipsaci* species was not monophyletic (one species) but really a cluster of species. In light of the results, phytosanitary protocols used by government agencies for screening of stem and bulb nematode species has been updated and past positive pea grain export shipments have been reanalyzed.

PUBLICATIONS, PRESENTATIONS, EDUCATIONAL MATERIALS PRODUCED

Tenuta, M., Madani, M., Briar, S., Molina, O., Gulden, R., and Subbotin, S.A. 2014. occurrence of Ditylenchus weischeri and not D. dipsaci in Field Pea Harvest Samples and Cirsium arvense in the Canadian Prairies. Journal of Nematology 46:376-384.

Madani, M., Tenuta, M., Chizhov, V.N., and Subbotin, S.A. 2015. Diagnostics of Stem and Bulb Nematodes, Ditylenchus weischeri and D. dipsaci (Nematoda: Anguinidae). using PCR with species-specific primers. Canadian Journal of Plant Pathology 37:212-220.

VALUE TO PRODUCERS

The results for this project demonstrate that the stem and bulb nematode present in yellow pea fields in the Prairie Provinces to be *Ditylenchus weischeri* and not the quarantine stem

and bulb nematode *Ditylenchus dipsaci*. Protocols have been provided to CFIA to update monitoring of yellow pea exports. It is expected the updating will remove the necessity for continued sampling and fumigation thus reducing costs to exporters. This should increase the competitiveness of Canadian exports.