

Recommendations for Managing Clubroot Risks associated with Field Research February 2013

Background

The Saskatchewan Clubroot Initiative (SCI) was established in 2009 to promote awareness and identify priorities for clubroot prevention and management. One of the first priorities of this group was to develop a Clubroot Management Plan to promote awareness and minimize the risk of clubroot in Saskatchewan. The Saskatchewan Clubroot Management Plan is available on the Saskatchewan Ministry of Agriculture website (www.agriculture.gov.sk.ca/Default.aspx?DN=1c2e2764-6ad2-4b11-bfe0-207b1a60b931).

In 2010 a research sub-committee was formed by SCI to consider recommendations for managing risks associated with clubroot research in Saskatchewan, given the currently low presence of this pathogen in the province. This document is a result of the input of this sub-committee: Faye Dokken-Bouchard, Venkata Vakulabharanam, Sherrilyn Phelps, and Philip Northover (Saskatchewan Ministry of Agriculture), Pat Flaten (SaskCanola), Clint Jurke (Canola Council of Canada), Gary Peng and Bruce Gossen (Agriculture and Agri-Food Canada).

We recommend that researchers in Saskatchewan familiarize themselves with these guidelines and use them to develop suitable measures for their unique research situation. Funding agencies should also be aware of these recommendations and may wish to consider the importance of containment protocols in research proposals when considering supporting clubroot projects in Saskatchewan.

The following recommendations relate to:

- Risk assessment
- Clubroot research within a contained facility
- Clubroot field research
- Field research of all kinds

Risk assessment

Resting spores of the clubroot pathogen (*Plasmodiophora brassicae*) are extremely persistent in soils. However, clubroot is not regulated by the Canadian Food Inspection Agency (CFIA) due largely to already extensive presence of the pathogen in significant areas of Canada. *Plasmodiophora brassicae* is ubiquitous, especially in Europe and much of North America (Agrios 1988). Saskatchewan remains relatively free of the pathogen. Clubroot is a typical soil-borne disease and the pathogen inoculum (resting spore) is dispersed with soil particles or plant debris. Several preventative and mitigation measures can be implemented to reduce the risk of accidental release of the pathogen. Using the risk factors set by CFIA for regulated plant diseases (CFIA, 2007), a risk analysis was carried out by Agriculture and Agri-Food Canada (AAFC) to identify primary risk factors for accidental release of *P. brassicae* from a research facility (Peng and Gossen, 2010; Appendix 1). A list of preventative or mitigation measures were recommended to minimize the risk. Based on this analysis, it was concluded that the risk of accidental release of *P. brassicae* from a research facility is low when recommended operational guidelines are implemented.

Operational Guidelines for Working with P. brassicae in a Contained Facility (Greenhouse/Growth Cabinets/Laboratory/Containment)

To minimize the risk of accidental release of *P. brassicae*, the following guidelines¹ should be followed when conducting research involving *P. brassicae*:

1. Controlled access to trial area

Experiments should be carried out in an area designated for clubroot trials only. If applicable, the designated area should be approved by the Greenhouse and/or Biosafety

¹ adapted from those developed by and followed at the AAFC Saskatoon Research Centre (Peng and Gossen, 2010)

committee of the workplace where the research is taking place. Access to the clubroot work area should be restricted to authorized personnel only, including research staff working on approved projects and, if applicable, the Greenhouse Manager where the research is taking place. The facility should be locked at all times, and the signs “Clubroot trials in progress, do not enter!” should be posted on every entrance door of the clubroot work area. Unauthorized personnel such as visitors, maintenance and service staff should be discouraged from entering, but it is suggested they enter the areas only in the presence of an authorized member of project and under the same conditions.

All personnel working on clubroot projects should be trained to follow these clubroot operational guidelines when working in the designated area.

2. Entering and exiting trial area

Before entering the designated clubroot work area, all unnecessary articles of personal belongings, including sweaters, cell phones, MP3 players, etc, should be left outside the work area. Upon entering the restricted area, put on shoe coverings and lab coat immediately.

Before leaving the growth room or workroom, hands should be washed or sprayed with an alcohol-based sanitizer, lab coats and shoe coverings removed while standing on a pad soaked with 10% bleach or Javex (0.6% Sodium Hypochlorite). The disinfestation pad is placed by the inside of entrance door. The lab coats and shoe coverings are placed in separate containers and autoclaved prior to washing or disposal. Lab coats should be washed weekly.

3. *P. brassicae* inoculum preparation and application

Researchers conducting projects involving use of living propagules of *P. brassicae* should consult their workplace Greenhouse and/or Biosafety committees regarding measures to mitigate risks to other canola or related projects and minimize the possibility of releasing *P. brassicae* into the natural environment.

Autoclave growth media contaminated with *P. brassicae* inoculum to minimize the chance of selecting for new races.

Clubroot galls should be stored in double-sealed plastic bags in a freezer in the clubroot work area, with inoculum preparation and application to be carried out in the designated area. For inoculation, pipette about 1-5 ml of resting spore suspensions at 10^7 - 10^8 spores/ml into the growth medium in a conetainer (1.5-in in diameter and 8-in deep) on a rack which is held in a 6-in deep plastic tray to retain spill.

The work area should be cleaned after inoculation is completed, initially by sweeping off debris into a biohazard bag, and then wiping or mopping benches, tools and floor with 10% bleach. Inoculated plants can be transferred using a designated cart to growth rooms that are locked when not in use. The cart wheels will be disinfested on the 10% bleach mat before leaving the workroom or growth room.

To prevent the escape of resting spores attached to dust particles from growth rooms or the workroom, a HEPA air filter (>99% efficiency) will be installed for air outlets.

4. Disposal of trial materials

All experimental materials, including potting mix, pots, and plants, should be autoclaved or disinfested prior to disposal or reuse. Plants and potting mix should be autoclaved at 121°C for at least 20 minutes, and plastic pots disinfested with 10% bleach for over 12 h. Aliquots of plant samples to be used for genetic/molecular characterization, or root samples for microscopic observations may be removed from the trial area, but these samples have to be placed in sealed containers that are then surface sterilized with 10% bleach. These samples should be autoclaved after use. Carts, tools, benches, and other material that cannot be autoclaved must be thoroughly wiped with 10% bleach.

5. Preventing *P. brassicae* inoculum from entering sewage system

All experimental plants should be placed in self-containing trays that hold excess water. Any waste water resulting from root or pot washing should be treated with 10% bleach

overnight prior to being discharged into the city sewage system. A berm should be installed at the entrance of the designated clubroot work area(s) to prevent major spills going directly into the drain.

6. Disinfestation and decontamination

All spills of liquids containing clubroot resting spores should be contained with an absorbent pad which is then put directly in an autoclave bag for autoclaving and disposal. The spill area should then be immediately sprayed with 10% bleach. The project supervisor should be notified of any spill and mitigation measures applied.

At the end of each work period, the counters and other work surfaces should be cleared of experimental samples and wiped with 10% bleach. Floors in all work areas should be kept clean of debris and soil. When a research project is completed, project staff are responsible for decontaminating the trial area by wiping benches, and sweeping and washing the holding trays and floors with 10% bleach. The project supervisor will oversee disinfestation and decontamination procedures.

Operational Guidelines for Conducting Clubroot Field Research

Because clubroot is not widespread in Saskatchewan (Dokken *et al.* 2010), plot research may only be conducted in locations *outside of Saskatchewan* (for example established field nurseries in Alberta or Ontario/Quebec). Exceptions may be made for eradication projects, under consultation with SCI and the RM council for the municipality where the field site is located.

The following guidelines are recommended for canola field surveys in Saskatchewan.

Plant Sample Survey Procedure:

1. As clubroot may take six to eight weeks to develop, symptoms are most detectable later in the growing season (late July or August).
2. Records must be kept for all fields visited using detailed clubroot survey sheets that include surveyor name, landowner name along with his or her permission to sample, field location and history.
3. Do not drive into field or access, but park on the road whenever possible. Surveyors can walk into infested fields but must follow human sanitation procedures.
4. If survey personnel enter a field in any potentially infested regions, whether it is known to have clubroot or not, they are to follow these human sanitation procedures:
 - Wear disposable footwear that can be removed immediately after leaving the field. Another option is to use rubber boots or other footwear that can be sterilized (misted) with a disinfectant solution (10% bleach) upon leaving the field.
 - Dispose of the disposable footwear in a sterile fashion. Sealing in a garbage bag and burning is preferred. Do not reuse disposable footwear.
 - Clean and disinfect any tools that may have been in contact with soil in the field.
5. Collect 20 plants at each of five sites in the field, for a total of 100 plants and observe for disease symptoms. Each of these five sites need to be at least 20 metres from each other and at least 20 metres from the field edge.
6. If patches of premature ripening are observed, particularly in field entrances or corners, dig or pull up plants, shake off excess soil and inspect roots for the presence of galls. If clubroot is suspected, cut off stems and collect root samples.
7. Air-dry root samples in a double paper bags OR freeze the samples in a double Ziploc bag (samples must remain frozen if this option is chosen) and send them to the Ministry of Agriculture's Crop Protection Laboratory at 346 McDonald Street, Regina SK, telephone (306) 787-8130. You may mail, courier or drop off samples in person. There is a \$20 fee for visual inspection.
8. If the visual diagnosis is positive, root samples will be forwarded to an accredited laboratory on behalf of the municipality for DNA testing. Cost of the DNA testing will depend on the current fee set by the accredited laboratory (approximately \$100).

Soil Sample Survey Procedure:

1. Soil samples can be collected at any time but soil should be dried after collection.

2. Records must be kept for all fields visited.
3. Do not drive into field or access, but park on the road whenever possible. Surveyors can walk into infested fields but must follow human sanitation procedures.
4. If survey personnel enter a field in any potentially infested regions, whether it is known to have clubroot or not, they are to follow these procedures:
 - Wear disposable footwear that can be removed immediately after leaving the field. Another option is to use rubber boots or other footwear that can be sterilized (misted) with a disinfectant solution (five per cent bleach) upon leaving the field.
 - Dispose of the disposable footwear in a sterile fashion. Sealing in a garbage bag and burning is preferred. Do not reuse disposable footwear.
 - Clean and disinfect any tools that may have been in contact with soil in the field.
5. Soil samples should be comprised of a mixture of small scoops (approximately one cup each) of soil taken at each of 5 sites visited in one field. Because clubroot is most likely to arrive on soil attached to vehicles and field equipment, IF the entrance to the field is evident, these 5 sites should be located in the vicinity of this approach. Clear away residue from the soil surface, and scoop approximately 1 cup of the top 0 to 10 cm of soil at each site (total 1 litre from all 5 sites combined into one sample). Keep each of these five sites at least 20 metres from each other and at least 20 metres from the field edge.
6. Air-dry soil samples in paper boxes and send them to a laboratory for DNA testing. Cost of the DNA testing will depend on the current fee set by the credited laboratory (approximately \$100).
 - For a list of laboratories providing clubroot testing, please visit: www.clubroot.ca (click on Identify Clubroot) or contact the Crop Protection Laboratory in Regina.

Operational Guidelines for Conducting Field Research

Because of the risk that clubroot is already present in Saskatchewan soils or that it may be introduced through plot equipment and increased in disease nurseries where canola is grown more than once every four years, caution must be taken in canola field research, even when clubroot is not the disease of interest.

The following guidelines are recommended for canola field research and co-op tests conducted in Saskatchewan.

1. **Establishing Field Sites:** Industry personnel should enquire with the grower and municipality if clubroot is known or suspected to be present in the field or surrounding area.
2. Discuss with the grower the type of field practices (from rotations, custom field operators, oilfield activities, etc.), which potentially increase the chance of spreading clubroot, as a part of risk assessment, as well as past crops and weeds history, noting those in the Brassica family are susceptible to clubroot.
3. Inform the grower of the precautionary measures being taken to prevent clubroot spread. Enquire with the grower if he requires any additional measures and what those should be. Growers should feel encouraged to inspect industry equipment and protocols to be satisfied that there is no risk of contaminating their land. If tours are to be conducted, then establish clearly what precautions will be implemented.
4. Industry personnel should discuss with growers the implications of their privacy policy and corporate responsibility in regards to clubroot findings. Due diligence in Saskatchewan involves to informing the RM of the exact location of any clubroot findings, so they can fulfill their obligations under *The Pest Control Act*. Clubroot bylaws in the RM may require duty to inform. In Alberta, there is not a regulatory requirement to report clubroot to authorities.
5. The public will be informed of the general area of the province of clubroot findings in the interest of clubroot awareness, as per the communication strategy defined by SCI. In Alberta, the geographic detail for disclosure of confirmed infestations is determined by the local municipal authorities.
6. Fields selected for trials should be sampled prior to planting to determine if clubroot is present, as well as every year. If a susceptible crop is grown, watch for clubroot

symptoms. Note that consecutive or tight rotations of susceptible crops, creates a high risk environment for increasing clubroot if it is introduced to the soil. Land in proximity to the entrance way to the field and/or the plot area should be sampled in a W pattern providing 5 samples, which can be submitted as a composite. Sampling canola volunteers/Brassica weeds should ideally be done in the year prior to the trials, if the potential plot location is known.

- If detected positive by PCR test or by other identifiable means (infected crops or weeds), these fields will not be utilised for research. Inform the grower, the Ministry of Agriculture and the RM of any clubroot findings, so they can fulfill their obligations under *The Pest Control Act*.
 - If clubroot is discovered at the site while the site is in use (e.g. on plants in plots), use of field equipment needs to be minimized, and any such equipment must follow vehicle sanitation procedures. Inform the grower, the Ministry of Agriculture and the RM of any clubroot findings, so they can fulfill their obligations under *The Pest Control Act*.
7. Trucks, trailers, etc. should be parked or unloaded off-site. Any fields known to have clubroot infestation will be off-limits to any vehicle access and will be strongly avoided for foot-traffic as well.
 8. Records should be kept of all fields visited and sanitation procedures followed.

Vehicle Sanitation Procedures:

1. The largest clearly established factor contributing to clubroot spread in Alberta is contaminated soil on agricultural equipment. Do not drive into field or access, but park on the road whenever possible.
 - Exceptions can be made for field-trials with permission of the grower. In these cases vehicle sanitation procedures will apply. Industry personnel can walk into fields but must follow human sanitation procedures.
2. Vehicles should especially stay out any of these fields following a rain – wet soil is much more difficult to remove than dry.
3. If a vehicle enters a field in the infested municipalities then it will follow these procedures:
 - Before entering any field, vehicles and equipment must be clean. Growers should be encouraged to inspect any vehicles/equipment as well. This will reduce concern that soil (infested or not) is being transported.
 - When leaving the field, knock off all clumps of soil in field before leaving field – preferably not in the field's approach, but off to one side.
 - If a pressure washer is available, pressure wash any visible soil, focus on tires, undercarriage, and any other parts that may have contact with soil. If this is not available, drive directly to a car-wash and clean vehicle and equipment as best as possible.
 - Mist down tires and other points of contact with a disinfectant, such as 1-2% bleach solution (bleach can be corrosive), Rocal, or 1% Virkon. This disinfectant process should be the last step, since most disinfectants do not effectively penetrate soil. The disinfectant will need to be in contact for 15 to 20 minutes with the pathogen to be effective. Vehicles and equipment need to be clean and free of soil for the disinfectant process to be effective.

Human Sanitation Procedures:

1. If industry personnel enter a field in any potentially infested regions, whether it is known to have clubroot or not, they are to follow these human sanitation procedures:
 - Wear disposable footwear that can be removed immediately after leaving the field. Another option is to use rubber boots or other footwear that can be sterilized (misted) with a disinfectant solution (10% bleach) upon leaving the field.
 - Dispose of the disposable footwear in a sterile fashion. Sealing in a garbage bag and burning is preferred. Do not reuse disposable footwear.
 - Clean and disinfect any tools that may have been in contact with soil in the field.

References

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- 2009 Guidelines for Canola Field Research Activities, Alberta.

Appendix**Risk assessment of clubroot based on CFIA pest risk assessment factors**

Risk factor	Risk level	Risk mitigation options
Known presence/absence of the pathogen in Canada	Low - presence in several provinces including Saskatchewan	N/A
Host range/local presence of potential hosts	Medium – infect canola and weeds in mustard family	Crop rotation out of canola crops
Potential for significant exotic biotypes or strains	Medium - 4 races exist on the prairies, potentially more races	Judicious use of R cv. to delay race-structure change
History of the organism in other new environments	Low - one of the old plant diseases described, ubiquitous, endemic	Clubroot is manageable by R cultivars, agronomic/cultural practices, and fungicides
Virulence/aggressiveness of the organism	High - aggressive under favorable soil conditions	Cultivar resistance, agronomic or cultural practices, and use of fungicides
The availability of pest risk information	Low - information abundant about clubroot, but most is on vegetable crops	Research on clubroot infection and yield impact is being studied
Nature of the proposed work (scale <i>in vivo</i>)	Low – indoor, small-scaled trials with < 100 plants, pathogen amount is much less than that in infested fields	Procedures designed to prevent pathogen escape from indoor trials will be implemented
The location, proximity of suitable hosts	Low – no canola fields within 5 km radius of the trial area	Procedures preventing/mitigating pathogen escape implemented
Mode of transmission or spread (airborne/soilborne)	Low – soilborne, no airborne spores	Measures to prevent/mitigate pathogen escape implemented
Potential rate of local and long-distance spread	Low – spread by wind/water erosion and field equipment	Equipment cleaning, use no-till /cover crops- reduce wind erosion
Presence of vectors in Canada	Low – no vector organism known	N/A
Presence of vectors in or near the trial facility	N/A	N/A
Persistence and potential for overwintering	High – resting spores persist in soil for up to 20 years	Grow non-crucifer crops, or R cv. of canola
Environ. requirements for establishment and spread	High – resting spores are tolerant to a wide range of environ. conditions	Long-term rotation or use of R canola cultivars
Capacity to control/eradicate if escapes;	Medium - eradication is impractical and unnecessary	Disease can be managed with R cultivars and crop rotation
Potential for economic or environmental losses	High – up to 50% yield reduction on canola under severe infection	Impact of the disease can be alleviated using R cultivar, crop rotation, and soil fungicides
Economic and environmental significance of pathogen and their host plants	High – the host canola is of significant economic value in western Canada, and can be affected substantially by the pathogen	Use of an integrated strategy for sustainable management of clubroot
Biosecurity-related risks (e.g. the potential for theft/misuse)	Low – trials are on secured AAFC property, with only employee access to the building	Trial area is locked at all times with restricted access by approved personnel