Clubroot Steering Committee
Clubroot Steering Committee meeting (International Research Update)
April 30, 2020 (Virtual meeting)

Host and moderator: Dan Orchard (Canola Council of Canada)
Question moderator: Keith Gabert (Canola Council of Canada)
Notes: Autumn Barnes (Canola Council of Canada)

Sweden clubroot update: *Dr. Ann-Charlotte Wallenhammar*, Swedish University of Agricultural Sciences

- Have been doing bioassays for clubroot in fields starting in the 1980s. Worse infestations with tighter rotations: worst infestation was 72%.
- Total 103,350 ha of OSR in Sweden. Mostly winter cultivars (99,650 ha). Acreage is shrinking because of ban on neonicotinoid seed treatments leading to problems with flea beetles.
- Long term fertility experiments with OSR every 4 and 6 years started in 1950s/1960s. Found *P. brassicae* in soil after 13 rotations (2007).
    - Most fields with 4 year OSR rotation will have clubroot present. 6 year rotations are helping.
    - Some liming involved in fertility experiments.
- Started using clubroot resistant spring OSR cultivars in 1999. Have been testing winter OSR resistance since 2013. 10 cultivars currently tested in 2020. 2014 clubroot resistant winter OSR varieties introduced to the market.
- Ongoing project: Integrated mgmt. of clubroot in Winter OSR (WOSR) 2017-2019.
    - Characterizing R varieties & new guidelines for interpreting soil assays.
    - 16 different plots: susceptible and 3 R cultivars.
    - Results: clubroot in all plots at various spore concentrations in 2017. Only traces of clubroot in 2018 and 2019.
- Growing OSR every 4 years leads to clubroot and other problems including Verticillium longisporum. Have been having issues with blackleg as well. Don't think that they have BL resistance in Sweden, important that they also get other disease resistance in their genetics.

Questions & answers (Sweden clubroot update):

Q: Can you ask how they did actually detect the DNA? What equipment and methods did they use?

- **A:** Discussed online, following published protocols from 2011. Protocol is described in Wallenhammar et al., 2012. In field distribution of *Plasmodiophora brassicae* measured using quantitative real-time PCR. Plant Pathol 202, 61,16-28.

Q: How much clubroot is 'normal' in a resistant variety? i.e. seed is only 95% pure and hybrids will carry some non-resistant plants. Important when interpreting bioassay or field plot results and deciding whether varietal resistance is being eroded.

- **A:** Many cultivars have about 10% diseased plants. It is related to disease pressure in soil. In November2018 all of the resistant cultivars of WOSR and the susceptible control mixture showed no symptoms due to dry conditions in the soil at two field experimental sites in south Sweden. Assessment was performed after harvest with similar results.

Q: Could Anne please provide a bit more information on the role of liming for clubroot management in Sweden?

- **A:** Liming in Sweden has been an important issue for many years, it's recommended for sugar beets – but it's difficult to show that a yield increase occurs, but it's considered a good practice. Liming was introduced as an amendment in Swedish agriculture prior to artificial fertilizers, so there is a long tradition to bring out lime. Barley responds to lime and I know that in Norway recommendations are to keep a pH at 6.7 for barley. OSR itself grows well on soils with lower pH along with *P. brassicae*. The objectives of the study I was referring to was to find recommendations for liming soils with already high pH >7.0 to increase yield of sugar beets. Publication in English is underway.

Q: Is a 6 year break the recommended break in Sweden?

- **A:** It was typically every 4th year, but with clubroot it has been extended to every 6yrs (5 year break). In south Sweden a crop rotation is normally based on; malting barley-WOSR- winter wheat – sugar beets. Canning peas was cropped for many years, making OSR coming every 8th year, but some growers ran into problems with pea root rot, and finally the canning plant closed about 5 years ago. I have been to meetings where farmers have told med "without peas I will go for OSR every 4th year. This rotation also increases other soil-borne disease as Verticillium wilt, and in certain areas there are problems. Sugar beets are also infected with soil-borne diseases and the beet cyst nematode but here breeders are providing tolerant cultivars continuously.

Clubroot in Poland- What's new: pyramiding of resistance and mining the soil microbiome – *Dr. Małgorzata Jędryczka*, Polish Academy of Sciences

- 0.8 million ha of OSR in Poland. 95% is winter OSR, unless lots of winterkill (re-seed with spring type). Clubroot has been a problem since 2000.
- Sampling for clubroot (plants, soil, water) all over Poland, except for 2020 because of COVID-19. Most clubroot in north and south, in places where lots of OSR grown. Basically if OSR is present, clubroot is present.
- Incidence quite high. 2018: 36.3%. 2020: 43%
- Pathotypes P1-P8. Mostly P1 & P3, some P2, P4, P5.
- Severity of disease: very bad in 2017 rapeseed, was looking like radishes. Even weeds (like pennycress), so much galling that it was difficult to recognize.
- Testing & results:
    - qPCR soil testing, bio-testing and LAMP.
    - Also doing a lot of screening tests, about 300 lines from breeders each year and from gene banks. Symptoms from field are similar but smaller in glasshouse.
    - Physical rating on 1-4 scale.
    - Most of the lines are totally susceptible but still possible to see some resistance to some pathotypes.
- Might be possible to do pyramiding of resistance but not very precise: not based on gene to gene relationship. Segregation of resistance traits – plants growing in same pots can be galled or asymptomatic.
- IHAR – institute of plant breeding looking at HOLL lines (high oleic, low linoleic) in addition to clubroot resistance.
- Shotgun metagenomic (DNA cut to 100-300 bp fragments, sequenced and scaffolded into databases.
    - Microbiome present in soil under OSR heavily infected. Possible to see which fungi or bacteria are present in these soils. Not easy to study, over 6,000-7,000 microbes present in

soil sample. Possible to see which are different in different samples from infestations of *P. brassicae*. Trying to develop different informatics tools to study all this big data. Some rare microorganisms are present in some samples but not others.
    - Also studying enzymes present in soil samples: DHA, PAL, PAC, URE, PROT. No standards that determine what level of their activity should occur in a given class of the soil.
- Summary:
    - High incidence of clubroot in WOSR in Poland
    - High severity of CR on WOSR in Poland.
    - Numerous pathotypes 5 by <publication>, 9 by *ECD*
    - High incidence of P1+ (overestimation due to sampling)
    - Some pathotype specific resistance, infrequent in brassicas, possibilities of resistance pyramiding.
    - General resistance frequent in *Raphanus*, necessity for distant crossing and embryo rescue.
    - Differences in soil microbiome: difficult to fish out
    - Differences in soil enzymes: crude tool

Questions & answers (**Clubroot in Poland**):

Q: Any additional comments/experience with gene pyramiding improving CR resistance

- **A:** As resistance to clubroot is pathotype-specific, it is a reasonable tool to be used by the breeders. This work must be combined with recognition of the pathotypes in the area of interest (at local, regional, country or global scale).

Q: Any evidence that differences in microbiome are linked to how severely clubroot develops? Veg growers have historically reported soils where despite infection being introduced on transplants nothing develops.

- **A:** Just beginning to look at these topics, so by now we were only looking at results from severely infected soils.

<u>The changing clubroot threat and sustainable management options in the UK</u> – *Dr. Fiona Burnett*, Scotland's Rural College (SRUC)

- Finished project last year with SRUC, AHDB, ADAS: developing sustainable management methods for clubroot.
- Clubroot continues to be a major issue commercially. Warm autumns and tight rotations are making the issue worse. Reports of poor control with R varieties continue. Conflict between short term profit and long term sustainability.
- Current control largely based on use of R varieties.
- How widespread are R-breaking strains? 75 commercial fields sampled. WOSR varieties Mentor & Tolkin, a Chinese cabbage control. Soils tested for +/- of Mendel virulence.
    - Mendel resistance breaking strains. Even R varieties show some level of infection. They are building up virulent strains by using resistance. Probably around 10% resistance is normal in R varieties in UK. About 17% of infected sites had R breaking strains (30%+ infection). Major concern for long term sustainability of genetics to manage problem.
    - Survey doesn't cover all of UK but they are finding R breaking strains across the country.
    - Looked at diversity clubroot population.

- Field mapping has potential to target treatments and help with decision making (project where a bunch of fields were mapped).
    - One field example: mapped 30 points, looked at in December, April, June. Can see how the disease developed in the crop. The C plot showed how using qPCR to map. Some link between qPCR result and what seeing in field but driven by other factors aside from just the amount of clubroot DNA in soil.
    - Pencombe field: looking for cheaper ways to map for clubroot. Tried many different approaches including green leaf area, hyper spectral. Aerial images of the field. Haven't found any cheap and accurate ways of mapping clubroot yet. Measuring green at 2 times in season, still not great.
- Novel control methods: Elicitors as foliar spray or drench.
    - Bion® drench & foliar spray gives some response but this research is still quite experimental.
- Clubroot summary for UK:
    - Clubroot is extremely variable across UK. Gives potential for rapid adaptation.
    - Mendel breaking strains are present throughout the UK (18% of infected sites had no symptoms on Mendel).
    - UK advice is that deploying R variety should not be the first/only recourse in an identified clubroot field or it will be eroded rapidly as a tool for that field.
    - Alternative non-susceptible crops are the most sustainable long term method of control.
- Clubroot key messages in the UK
    - Keep accurate records of clubroot occurrence, location and intensity. Note where varietal resistance has been deployed in fields to aid long term planning and help prevent spread.
    - Where R varieties are being used, monitor crop carefully. Assess levels of clubroot present. If infection increasing, change strategy.
    - Buying certified seed ensures that susceptible plant numbers are minimized in a resistant variety seed batch. Do not home save resistant varieties.
    - Manage volunteers and susceptible weeds within and between OSR crops. Manage weeds as early as possible to minimize spore multiplication.
    - Be mindful of other susceptible crops when planning rotations: spring OSR is susceptible, cover crop mixes often contain susceptible species.
    - Long term planning should be based on the long term profitability of a field and not on a single season's predicted margin.

Questions & answers (**The changing clubroot threat and sustainable management options in the UK**):

Q: What is the proportion of farmer saved seeds?

- **A:** Very low and <5%.

Q: What is the recommended rotation advice to protect the resistant varieties?

- **A:** We advise no closer than a 1 in 5 rotation with a susceptible crop and that's closer to practice now as growers have moved away from 1 in 3 being standard. But that has more to do with the price of rape being low and problems with flea beetles than it does with better clubroot practice.

Q: Are there any opportunities for rotating resistant sources?

- **A:** Not at the moment as all our resistant varieties, present and past, have been based on Mendel. We advise using resistant varieties in combination with other methods such as extended rotations rather than relying on it exclusively in affected fields.

Q: What kind of product is Bion®?

- **A:** It is an plant host defense elicitor (BTH benzo-(1,2,3)-thiadiazole-7 carbothioic acid S-methyl ester). It works well in pot experiments as a root drench but control in the field is variable. We have tried seed treatment use and it is erratic. It has potential but is not yet at a stage to use with growers.

Q: Did severity across the field match the soil moisture level better than qPCR spore levels?

- **A:** We didn't measure soil moisture so I can't answer that. But it was really evident that the level of spores alone was a poor indicator of ultimate severity in the plants so a combination of environmental drivers, of which soil moisture would be a key one, are certainly important. The other observation would be variability plant to plant in the field. So we would frequently sample neighbouring plants of which one would be severely affected and the other clear even although they were only 20 cm apart.

Clubroot situation in Germany – *Dr. Elke Diederichsen*, Freie Universität Berlin

- 900,000 ha OSR in Germany. Was increasing until 2019 when it dropped due to neonicotinoid ban and drought in 2018. 8-10% OSR crops infested with clubroot. Major cropping areas have a higher proportion.
- Expecting that clubroot infested area will increase (NPZ breeding company projection)
- Market share of CR cultivars: varies in different states in Germany – the two most northern states with longer tradition of growing OSR have more than 40% of Clubroot resistant cultivars.
- Pathotypes in Germany:
- Mendel resistance has been used since 2001. Virulent isolates are present all over cropping area (39% of all tested isolates) but frequency not as high as expected. 12% of isolates showed some virulence on *B. rapa* ECD hosts.
- P1 pathotype prevailing in the north. Also found in other states in Germany. P2, P3, P5 present as well. Mendel is not part of any differential sets yet but on map (slide), boxes marked with 'x' show that the pathotype is virulent to Mendel resistance. Same slide, graph on left: Top in light grey part means that that proportion is virulent on Mendel resistance, happens on all different pathotypes found.
- Clubroot resistance in new breeding material (NPZ breeding company): no complete resistance, but significant improvement. CRE1 is the new type, will be more reliable for its clubroot resistance compared to Mendel.
- Update on nomenclature initiative for CR genes initiated by Gary Peng.
- Gene nomenclature rules help communicate and distinguish between CR genes. For gene pyramiding or resistance management, we need to know whether genes are really different. Want to find novel CR sources. Composition of differential sets based on known resistance genotypes.
- Current status: 95% of contacted authors have added their data to table. Manuscript in preparation to help share all that information. Once published, the table will be shared on the Brassica Info website. In respect to publication, there is an invitation to publish as open-access in European Journal Plant Pathology.

Questions & answers (Clubroot situation in Germany):

Q: Any comments on Tosca resistance.

- **A:** Tosca has different race specificity, but as a line cultivar it was not competitive in respect to yield.

Q: Why genes are only from A genome if both *B. oleracea* and *B. rapa* are resistant?
- **A:** This was an example only showing the A genome genetics.

Q: Will the proposed gene nomenclature indicate the source of the resistance gene? We would like to know how is it going to be addressed in the proposed nomenclature the R genes in different species, because I can see the names are defined by the chromosome where they are found but the specie is not addressed and it can cause confusion.
- **A:** The origin of the studied CR QTL/gene is part of the data collection, as this can be an important criterion to distinguish different sources. The species is not part of the gene name, but the table contains the information whether a certain QTL/gene has been mapped in i.e. *B. rapa* or *B. napus*. I prefer to keep it like this, as adding the species to the name would not be in accordance with the suggested rules by Östergaard and King (2008) and would further increase the length.

Survey/Management options in North Dakota, USA – *Dr. Venkat Chapara*, North Dakota State University

- Spring canola acres are increasing in North Dakota. First report of clubroot was made in 2013, the survey group found increased numbers over the years with highest numbers found in 2018, 31 fields (33% of surveyed). In 2019 saw drastic reduction (only 8% of surveyed) as most growers opted for Resistant Cultivars.
- Soil sampled infected fields and found that pH varied in infected fields: 4.8-6.4 in 2018, 4.7-6.8 in 2019.
- Besides visual survey, clubroot positives were also identified through molecular assays. Found low spore populations per gram of soil mostly in higher pH soils without visible symptoms. Three new Counties of North Dakota will be monitored closely from here onwards based on these results.
- Evaluation of soil amendments:
    - Different rates of beet lime, pellet lime and wood ash.
    - Tested a surfactant alone and in combination with the best treatments tested over the years.
- Lime research under field conditions: disease index observed in two years of field study. Base population of resting spores was 5.5 million spores/gram of soil in 2017 (high spore pressure). Many of the growers thought that 15 t/ac very high application rate so had to look at different (lower) application rates for control. Found that beet lime at 15 t/ha and 10 t/ha were good.
- Evaluation of ORO-RZ surfactant to manage clubroot under field conditions. On its own and with chemicals that were tested previously. Beet lime and lime showed good control and ORO-RZ showed less than 30% infection. The trials will be repeated this year to see if the control is consistent.
- Clubroot resistance in commercial cultivars of canola. All R varieties showing pretty good activity, seen some growers already using multiple gene or 2nd gen resistance in North Dakota.
- Pathotyping: in 2018 collected galls from 33 fields and sent to U of A Strelkov lab to pathotype:
    - *<publication>*: P2 and P3
    - *Williams*: 8, 2
    - *CCD*: 1N and 1A, the rest are all novel (will be named soon). Good news = whatever pathotypes prevalent in North Dakota, can be controlled by 1st generation Clubroot resistance (Mendel).

- Summary:
    - Clubroot is spreading.
    - Visible symptoms were reported from acidic soils.
    - Beet lime, pellet lime can be used in clubroot patch management.
    - Surfactants need more study.
    - R varieties available to manage clubroot in combination with rotations (minimum 1/3 year rotation).
    - Pathotypes in North Dakota so far are manageable with the currently available CR resistant varieties.

Questions & answers (Survey/Management options in North Dakota, USA):

Q: What was the Disease Severity index in the untreated control trial with 3 amendments?

- **A:** The trial was designed in a Factorial RCBD. There were 3 levels of soil amendments and 4 levels of rates along with control. In the graph we presented DSI of Untreated Checks as: Woodash-0 (77.3%), Pelletlime-0 (65.7%) and beet lime-0 (80%)

Q: How and when is the ORO-RZ surfactant applied?

- **A:** It's a liquid formulation, applied in furrow, prior to planting. The furrows were closed immediately after planting (Pic attached).

Q: There are several studies showing that surfactants can reduce clubroot, but some are also phytotoxic and it can be difficult to get them registered. Did you see any phytotoxicity?

- **A:** No phytotoxicity observed. Only one year of research done so far; repeating the trial this year. Earlier in my 2016 and 17 study, noticed phytotoxicity in a surfactant treatment (AquaGro-Granular formulation).

Evaluation of various strategies for the integrated management of clubroot of canola – *Brittany Hennig*, University of Alberta

- 2019 Alberta clubroot survey: cumulative 3,353 fields with confirmed clubroot infestations (assumed to be much higher). Spreading to new counties (total of 42 counties in AB now positive). Some of the most infested fields were planted to R varieties.

- Clubroot was first found in AB in 2003, first commercially available CR variety was sold in 2009. 2013 had first case of resistance being overcome. 2019: more than 320 fields where resistance has been overcome.
- Pathotypes:
    - Currently 36 pathotypes in Canadian prairies. 19 of them can overcome 1st generation (Mendel) resistance
    - Predominant pathotypes: continue to be 3A, 3D and old pathotype 3H. Many of the new pathotypes confined to specific area.
- Trials
    - Weed/pathotype trial: are known clubroot susceptible weeds equally susceptible to all pathotypes? 6 plant species (susceptible canola, pepperweed, shepherds purse, stinkweed, flixweed, alsike clover). 3 pathotypes: 3A (most common across province), 3H (common in central AB), 5I (common in south AB). Bioassays, inoculate after germination, evaluations at 8 weeks, other tests TBD.
    - Rotational trial: is there a detriment to early clubroot resistance deployment? 4 crop rotation (canola-wheat-barley-canola) in 4 different rotations with 5 different concentrations of spores. Each crop grown for 8 weeks with a 4 week break between crops. Seeded in greenhouse tubs, fertilized all according to recommendations. Sanitation very important. 8 week canola evaluations. All root material removed, dried, reincorporated prior to next crop. Soil samples after every crop and lab analysis by qPCR. Preliminary results: trace or no symptoms at </= 10,000 spores/gram. Resistant varieties lowered spore concentration at first. After 4th crop, spore concentration has increased. qPCR & duplication in progress.
    - Field trial: what is the effect on clubroot resting spore load with the collective use of integrated strategies? Genetics (resistant and susceptible cultivars), weed management (hand weeded vs not weeded), lime application (hydrated lime to desired pH of 7.2). Results: weeds not significant for the incidence of disease in the canola growing season but the application of lime and use of R genetics were. Surprising how much the lime helped the susceptible variety (under 30% incidence) with the high concentration of clubroot spores in soil. Resistance and lime complement each other. Will soil sample and do qPCR this spring.
- Goal: quantify the consequences of NOT implementing an integrated clubroot management plan and determine the most effective clubroot management recipe for growers.

Questions & answers (Evaluation of various strategies for the integrated management of clubroot of canola):

Q: Brittany's rotational study was in the greenhouse, with short breaks between crops, would that affect spore viability or the drop in spore loads seen after a two year break in field studies?

- **A:** Unfortunately it doesn't go through all the seasons - but looking at the lifecycle, there is opportunity for spore loads to increase - therefore differentiating between treatments.

Q: Is your research showing pH of 7 and higher will be less impacted by clubroot?

- **A:** Yes, but other research is showing pH 7.2 is required to reduce clubroot. A pH of 7.2 is optimal for management, but it does not prevent clubroot. Clubroot symptoms have appeared in soils with a pH over 7.2 – but it is a gradient i.e.: a pH of 7 is better than 6.5, and a pH of 7.2 is better than 7.

Clubroot Pathotypes – *Keisha Hollman*, University of Alberta

- History of clubroot in Alberta:
  - Isolated cases in home & market gardens since 1970s.
  - First cases on canola identified in 2003 (12 fields near Edmonton). Rapid increase in confirmed infestations and spread further in the province in subsequent years: 3,353 confirmed by 2019.
  - Some of most severely infested fields were planted to R varieties.
- Annual surveys have found increasing numbers of fields where resistance has been overcome (occurring in provinces of AB and MB but not SK yet).
  - Samples from fields with resistance issues are evaluated in greenhouse for ability to overcome resistance and for pathotype designation.
  - 2 fields with resistance breakdown in 2013, up to 204 fields in 2018. 134 more potential cases in 2019 (results available in a couple of months)
- Challenge: pathotype ID
  - New strains that overcome resistance cannot be distinguished from old strains based on previous commonly used pathotypes. First of the new strains were classified as 5 on Williams, but that didn't reflect what we were seeing in the field (virulence on CR canola).
- Pathotyping
  - Long process (it takes months to get results).
  - High demand (agronomists, counties, etc.).
  - Important to determine spread of new pathotypes & pathotype diversity.
  - Helps determine management plans and genetics deployment schedules.
- Canadian Clubroot Differential set (CCD)
  - Populations from fields with resistance issues are tested for pathotype designation on the CCD. Results from 2018 are complete, currently working on 2019.
  - Inoculate all differential hosts (left side of CCD table). Then rate on 0-3 scale. +/- reactions (unique virulence pattern) through differential hosts gives pathotype designation. CCD table includes designations of *Williams* and <*publication*> as well as *CCD*.
- Pathotypes identified in 2014-2016
  - CCD set has good differentiating capacity
  - Enabled ID of multiple distinct virulence phenotypes among pathogen populations able to overcome resistance. <*publication*> breaks down into 2, *Williams* further, *CCD* is more precise.
  - In 2016, 17 pathotypes identified in Canada under *CCD*. If using *Williams*, would only ID 4 unique pathotypes. <*publication*> would only ID 2 unique pathotypes.
- Current pathotypes:
  - Number of new pathotypes continuing to increase. Several novel virulence patterns identified in 'new' clubroot regions and from single-spore isolates. 36 unique pathotypes to date.
  - Challenge: rapid ID of new pathotypes = running out of letters in CCD. Have modified pathotype nomenclature to streamline the CCD system (2019)
- Revised CCD nomenclature
  - Original CCD system: pathotypes assigned a letter in the order they were discovered. Each letter used once. Could also be assigned number under Williams' system but that was not officially part of CCD designation.

- Refined CCD system: pathotypes designations include Williams' number first, followed by a letter. Entire alphabet may be applied to distinguish multiple variants of a single Williams pathotype.
- Pathotype composition:
    - 19 of the known 36 pathotypes can overcome 1st generation resistance (Mendel).
    - 17 of the known 36 pathotypes do not overcome 1st generation resistance (Mendel). Other sources of genetic resistance may work on some of these pathotypes.
- 9 new pathotypes identified from collections in 2017 and 2018 (including some that are new *Williams* designations). Most new ones confined to specific area/county. CCD designations help further identify a pathotype's unique virulence pattern to more accurately focus breeding efforts.
- Despite the large number of pathotypes, only a few are common or widely distributed
    - Predominant pathotypes still 3A, 3D and 'old' pathotype 3H. Most of other 'new' pathotypes confined to specific areas/counties or identified only from single-spore isolates.
- Conclusions:
    - Clubroot continues to spread.
    - Biggest issue in established clubroot areas is emergence of new pathotypes
    - With so many new pathotypes, breeders have to make strategic decisions. Focus on dominating pathotypes. Genetics may not be an option for some farms with unique/rare pathotypes (will have to focus on cultural practices and soil amendments).

Questions & answers (Clubroot Pathotypes):

Q: Do you think the 'new' pathotypes have always been there as variants within your known strains?

- **A:** There is strong evidence to suggest that some of the new pathotypes existed before the introduction of genetic resistance. This evidence is both phenotypic (based on virulence of a couple of old populations) and genomic. It is likely that these strains that were already present increased greatly as a result of the selection pressure from CR canola. This doesn't rule out the emergency of completely new strains (e.g., via mutation), but it doesn't appear to be the main mechanism.

Q: Are you testing only field collections or also Single Spore Isolates?

- **A:** We do both, my project focuses specifically on field collections, whereas another colleague within Dr. Strelkov's lab works with single spore isolates.

Q: Given that spores are collected form galls, is it possible that some of the variation in new pathotypes isn't necessarily novel genotypes, but could also reflect different mixtures of pathotypes present in the starting gall? That is a mix of 3A and 3H could pathotype out differently than a mix of 3A, 5X and 3H coming from a single gall? Or different ratios of pathotypes?

- **A:** The system is that the spore suspension is a mix, and the CCD shows the only dominant pathotype. It is also possible that a mixture of pathotypes could differentially influence the host reaction (example: avirulent pathotypes triggering resistance to more virulent pathotypes in the same mixture). To specify in detail would require single spore isolates.

Q: Any pathotypes that have broken down all resistance varieties known in Canada?

- **A:** 19 overcome first generation resistance. Breeders focus on 3A and 3D. We know that pathotypes 3A and 3D (both of which overcome first generation resistance) dominate heavily from 2014-2018. Therefore, breeders have focused on breeding for resistance to these pathotypes given their widespread distribution. These appear to overcome the resistance nearly all CR canola with 'first generation' resistance.

Q: Have you tried pathotyping individual galls obtained from surveys?

- **A:** We generally pathotype multiple galls from a field (usually derived from the same plant). We need a good amount of spore suspension in order to inoculate the whole CCD with replicates. Nonetheless, in the past, we have also tried pathotyping spores from SINGLE galls… the reactions on the differential hosts were not very different. Single-spores remain the ideal.

<u>Development of harmonized clubroot maps</u> – *Dr. Yoann Aigu*, University of Alberta

- This project funded through CARP by Alberta Canola, SaskCanola, MCGA. 3 main objectives:
  - Examine feasibility of harmonized clubroot map.
  - Determine what map would look like.
  - Communicate results with stakeholders.
- Clubroot well established in Canada. Isolated reports from home/market gardens in AB & MB starting 1920s. Found in 1997 in canola in Quebec. 98% of Canadian canola is grown in the prairies:
  - Alberta first fields of clubroot identified in 2003, confirmed cases up to 3,353 in 2019 survey
  - Saskatchewan first fields clubroot identified 2008, 60 fields confirmed as of 2019
  - Manitoba first fields clubroot identified 2009, 35 fields confirmed as of 2019
- Currently, each province makes their own clubroot map and represents data differently. Comparison can become confusing.
  - AB = number of infested fields with clubroot symptoms
  - SK = number infested fields with symptoms and infested fields with no symptoms
  - MB = maximum quantity of spores per gram of soil
- Had to select which data to use for graphic representation: spore concentration? Collection of the data over a large number of fields should NOT be prohibitively expensive, labour intensive, time consuming.
  - Resting spore detection & quantification: not ideal because of cost, labour, time.
  - Confirmed symptoms: good basic data, checks can be completed without any special equipment (pulling roots at main entrance)
- How to create the maps? Project started with ArcGIS but moved to a more customizable, free software. Created 3 types of maps:
  - Static: made for paper publication, ggplot2. Could be fields/county which provides clear representation with clear measure of intensity in the county. Second option for static maps is infested fields on specific points on map: user can gain a better understanding of specific areas of where disease is as well as knowledge of distribution.
  - Animated: ggplot2 and ganimate, useful for showing sequence of changes over time and intensification of outbreak. Used for PowerPoint presentations.
  - Interactive: made for websites, can zoom in and out, change background (satellite, map), how you want to see data (county, GPS) and the type of data you want to see. Can contain sensitive information so users should be restricted.
- Example of a harmonized map for clubroot in AB and SK. Big difference between a number of cases in provinces. Limitation becomes the inability of provinces to share data (regulatory issues).
- Next steps:
  - Continue to improve maps. Add supplementary info (pathotypes, resistant breaking isolates). Make more interactive map (increase the number of selectable data)
  - Combine clubroot data with complementary data: crop rotation for each field (available 2009-1018), meteorological data, pedological data.

- Modeling using the clubroot data: infested field area, minimum convex polygon.

Questions & answers (Development of harmonized clubroot maps):

Q: Who would be able access this pinpoint info?
- **A:** These data are not public, nobody can access to them, and it's a local file.

Q: Did you consider adding pathogen detections?
- **A:** Yes we consider adding new information on our maps, like pathogen detection. However obtaining pathogen data for every new infested field requires a huge amount of work.

Clubroot management update – *Dr. Bruce Gossen*, Agriculture and Agri-Food Canada Saskatoon and *Dr. Mary Ruth McDonald*, University of Guelph

- Clubroot management research:
  - Whole genome sequencing and new virulent pathotypes (published)
  - Effects of grass cover crops and rotation crops on resting spore concentrations in soil
  - Fumigation and solarization
  - Boron as a soil amendment
- Whole genome sequencing (published in BMC Genomics):
  - Sequenced 43 collections including 9 single spore isolates, mostly from Canada. Phylogenetic tree found that they didn't cluster by pathotype or host. Some did cluster by geographic region (1 clade consisted of only collections from China).
  - Heat maps of SNPs: from same study, genomics of pathotypes changed a lot over time. Selection rather than single gene mutations responsible for the changes. Consistent with the idea of balancing selection, that *P brassicae* in a field would consist of more predominant genomes but there can be several different low levels of pathotypes as well. Seeing new pathotypes after we apply selection pressure in the field.
  - Using a micromanipulator to develop single spore isolates. In process of trying to find funding to continue this work and develop a collection of single spore isolates for researchers to use.
- Cover crops and rotation crops to stimulate germination of resting spores:
  - One of the recommendations for managing small patches of clubroot is seeding a perennial grass in that patch, some previous research has shown that the root exudates stimulate resting spore germination. Wanted to quantify that and see if there are some cover crops that are more effective than others.
  - Soil (combo of field soil and soil-less mix) inoculated with 5×10^5 resting spores per gram, crops grown 8 weeks, roots weighed, qPCR assessment of resting spores.
  - Crops: Shanghai pak choi (susceptible check), smooth bromegrass, meadow bromegrass, 3 varieties of perennial ryegrass.
  - Results: initial resting spore concentration was higher than intended. No correlation between resting spore concentration and root weight (wanted to see if more roots = more spore germination). Some reduction in resting spore concentration from grasses: Fiesta (perennial ryegrass), Fleet (meadow bromegrass), Common (smooth bromegrass). Getting close to recommendations for several grasses to use in fields.
  - What about rotational crops? Spring wheat is good rotation crop and may help to reduce resting spore numbers. Still lots of variability in the data. Looked at soybean, no plant,

barley, field pea, ryegrass, spring wheat. Soybean in this case seemed to increase resting spore concentration in the soil (will have to look at this in more detail)

- Fumigation and/or solarisation for a more rapid treatment
    - Fumigated in late June or July, chloropicrin, metam sodium, immediately covered with film (law in Canada). Left on for 2 weeks then seeded with susceptible pak choi. Higher organic matter soil (70%) that was naturally infected with clubroot.
    - Results: bioassay found 55% severity in untreated, significant reduction of clubroot in all others. Totally impermeable film was just as effective as the film plus the fumigant. Why? Solarization? Temp was warmer under the film (21.8 C vs 29.1 C) but shouldn't be hot enough to kill the clubroot spores. Is there some anaerobic effect? Usually needs more heat than that to kill. Will be continuing to explore this in 2020.
- Boron as a soil amendment
    - There has been work on both Ca and B in clubroot. Well document that B can suppress development of clubroot but B can be phytotoxic to some brassicas and other crops especially peas.
    - Boron insensitive vs boron sensitive varieties (*B. napus* and *B. rapa*). 8 kg/ha boron drench added, insensitive brassicas less susceptible to clubroot from the start. Sensitive varieties didn't have much of a difference in disease severity. 8 kg/ha is enough that it isn't toxic.
    - Assessing plants in the synchrotron (AAFC Saskatoon) to determine boron content of roots and leaves of Westar and an AAFC variety.

- Clubroot management conclusions:
    - Grass cover crops and rotation crops may reduce resting spores in soil faster than if soil was left fallow, however first results from field trials showed higher resting spores under perennial ryegrass.
    - New virulent pathotypes are selected from existing genotypes (not recent mutations). *P. brassicae* exhibits balancing selection to preserve many genotypes. Continuing to develop single spore isolates for research.
    - Solarization using totally impermeable film could be an approach to manage small patches of clubroot.
    - Could boron be used to suppress clubroot using boron insensitive lines of *B napus*?

Questions & answers (Clubroot management update):

Q: If the crops were only grown for 8 weeks and clubs form after 3 weeks, what would be expected if they were grown for 3 months? Or perennially?

- **A:** Field trials are underway to assess the effect of grass cover crops over several years. We expect that the root exudates from the grasses will continue to stimulate spore germination for as long as the grasses are present, but don't have the data yet to say for sure.

Q: Did you look at the effects of Oat in rotation? Or are you aware of any research that has?

- **A:** Not yet.

Q: When you inoculate with a single spore, does the resulting gall have just the one pathotype, or does it contain a mixture of pathotypes?

- **A:** As far as anyone knows, there is only one haploid copy of the DNA (=n) present in a clubroot spore, so we anticipate that there will only be one pathotype. However, we don't know the answer for that yet.

Q: At what rate does Boron become toxic? 8 kg/ha is much higher than Ontario and CCC would be willing to recommend?

- **A:** Boron is mobile in the soil, so there are issues with application rate, soil type and rainfall. Field peas are very sensitive and 8 kg would have been excessive if pea was seeded in the field the same year as canola. However, a study at Saskatoon where high rates (32 kg B /ha) were applied in one year did not produce phytotoxicity in a pea crop grown in the study area the following year. Phytotoxicity on the following crop may not be an important issue.

Q: How might the findings with resting spores on ryegrass affect "patch management" strategies?

- **A:** The main benefit of putting a grass crop in is that the pathogen doesn't get moved throughout the field. That doesn't change, even if some grasses do NOT strongly stimulate resting spore germination under field conditions.

Using RNA interference as a biological pesticide for clubroot in canola – *Dr. Laura Keffer-Wilkes*, University of Lethbridge

- This project is part of nationally funded collaborative research training experience (CREATE grant). Grant held jointly between Sherbrooke University (Quebec) and U of L. Hoping to foster relationships between researchers. Partnering with Corteva. Based on research and design capacity, able to align programs aims with their industrial needs/wants.
- Chose clubroot for this project because of its impact in Canada and the world, opportunity for innovation in management.
- Challenges in clubroot research: limited lab cultivation methods for clubroot spores, lack of annotated clubroot genome, difficulties with RNAi delivery mechanisms, unsolved mechanisms in clubroot life cycle, regulatory challenges in marketing GMO products.
- Objectives for this project:
    - Using RNAi to tackle clubroot: to reduce the virulence or population of *P brassicae* in the soil using RNA interference
    - Designing siRNAs against target genes: designing and characterization of siRNAs against potential genes which are identified in the clubroot genome.
- Overview of RNAi:
    - Process used to inhibit gene expression. Commercially available SmartStax Pro seeds contain RNAi (GMO product, not widely available). Minimal work reported on RNAi use for clubroot but promise shown that this is a viable option. RISC complex is present in clubroot genome (based on bioinformatics work conducted by our group).
- Screening of target genes using bioinformatics
    - Selected 7 genes from *P. brassicae* as potential targets based on likelihood of being involved in primary and secondary infection. 2 genes are related to zoospore primary infection. 5 genes are related to secondary infection of clubroot. Had initial discussions with Bonham Smith at University of Saskatchewan.
- *S. cerevisiae* as a model organism:
    - Single celled eukaryotic organism. Easy to manipulate yeast genetics.
    - Obtained genetically engineered yeast trains which contain the RISC complex.
    - Creating new yeast strain with *P. brassicae* gene in its genome by using homologous recombination. Constructed target gene with fluorescent reporter protein.

    - Upon successful construction of yeast strain, want to use it for future experiments.
- Work plan: conditional expression of siRNA in yeast. Screen for potential siRNA molecule against target gene. Can control the induction of siRNA.
- Future directions:
    - Insertion of target genes in yeast genome.
    - Screen potential siRNAs antagonistic in *P. brassicae*.
    - Upon successful knockdown in yeast, expand the study to *P. brassicae*.

Questions & answers (Using RNA interference as a biological pesticide for clubroot in canola):

Q: What kind of delivery system?

- **A:** Working through it. First looking at yeast, looking at other organisms but speaking with Corteva for ideas.

Resistant sources and resources for clubroot at AAFC, Saskatoon – *Dr. Fengqun Yu*, Agriculture and Agri-Food Canada Saskatoon

- Resistance to clubroot in Brassica species varies. Brassica triangle diagram:
    - Limited resistance in canola itself. *B. nigra* = rich, *B. juncea* = no, *B. rapa* = strong R originating from turnip, *B napus* = varies (canola no rutabaga yes), *B. oleracea* = vegetables weak, *B. carinata* = no
- Identifying sources of resistance at AAFC: pathotypes 3 and 5x. More than 20 lines show resistance to 5x, 2 in *B. oleracea*, 7 in *B. nigra*
- Selected B napus lines tested for pathotyes 3A, 2B and 3D: 162 out of 845 B napus lines were tested. Lines resistant to clubroot (0 DSI) were identified: 3 resistant to all 4 pathotypes, 17 lines resistant to 1-3 pathotypes.
- 9 B napus lines were tested for resistance to more pathotypes. Found 3 napus lines resistant to all of the 13 pathotypes tested against.
- Introgression of clubroot resistance from turnip into canola. Microspore culture for developing double haploid (DH) *B napus*.
- Developing DH B napus lines carrying CR genes from turnip: seed from >800 Double haploid lines obtained. There's no vernalization need for all of the DH plants. Confirmation on the presence of each CR gene and identification of novel genes are in progress.
- Segregating for resistance and susceptibility in a DH pupation consisting of 84 DH lines from BC2 of DH16516 x Debra. Expected ratios 3:1, 1:1 and 1:3
- Brassica napus lines with clubroot resistance from *B. oleracea* were re-synthesized.
- Summary on sources and resources for resistance for Canadian pathotypes:
    - Sources of resistance: veggies (*B. rapa*, *B. oleracea*, *B. napus*) and mustard (*B. nigra*)
    - More than 30 lines with good resistance were identified from the species at AAFC Saskatoon.
    - Resources were developed by introgression of resistance from some of the sources into canola/*B. napus*.
    - Genetic resources including SNP markers developed at AAFC have been / will be distributed to canola breeders for developing cultivars for resistance to clubroot.

<u>In-field distribution of *Plasmodiophora brassicae* inoculum and its relationship to soil pH, Ca and B</u> - *Andrea Botero-Ramírez*, University of Alberta

- Epidemiological studies on spatial patterns of *P. brassicae* inoculum are scarce. May be useful for design & implementation of improved clubroot management strategies.
- Objective: to assess relationship between pathogen spatial patterns and soil chemical characteristics
- Clubroot levels in soil affected by many environmental factors. Is there any relationship between these factors and *P. brassicae* inoculum density in soil?
- Sampled four fields in central Alberta in 2017 = regular grid sampling (80 m x 80 m). In 2019 = intensification of sampling around locations that had positive samples in 2017.
- Soil samples: inoculum quantification by qPCR (all samples 2017 & 2019). pH (all samples 2017 & 2019), available Ca, Mg, B, Na (half of the samples from 2017, they were kept in regular grid of 160 m x 160 m)
- Spatial analysis: evaluation of spatial autocorrelation and clustering. Spatial models using Stochastic Partial Differential equations.
- Results:
    - Inoculum density & distribution varied. Patch size increased in every field over 2 years (average growth of 209 m).
    - No important relationship between inoculum density and pH, Ca, B.
- Conclusions:
    - *P. brassicae* inoculum: patchy distribution (sizes ranged 40 m – 586 m, average growth 209 m in 2 years). Increase in patch size was related to increased inoculum density and increased number of positive samples.
    - No effect of pH, Ca or B on the pathogen inoculum density was observed in any of the fields.
    - Observed spatial patterns may be explained by random spatial processes.

Questions & answers (In-field distribution of *Plasmodiophora brassicae* inoculum and its relationship to soil pH, Ca and B):

Q: What crops were produced in these fields from 2017-2019? Information on cropping history?

- **A:** We have information of the crops which were grown during the sampling years:

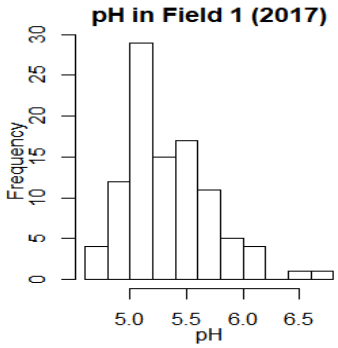
Field	Crop (2017)	Crop (2018)	Crop (2019)
F1	Canola	Wheat	Pea
F2	Wheat	Canola	Wheat
F3	Canola	Wheat	Canola
F4	Barley	Canola	Oats

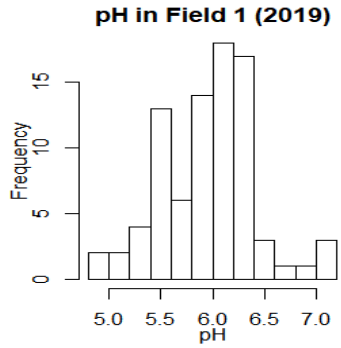
Q: What was the range of pH, Calcium or boron?

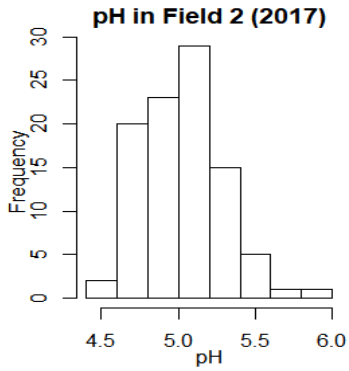
- **A:** (See table followed by graphs presenting the data distribution)

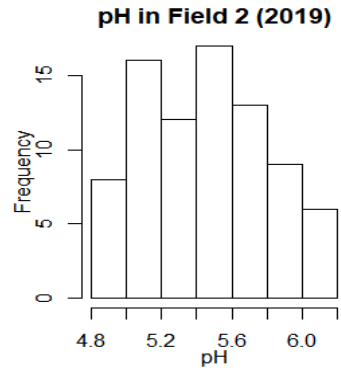
Field	Year	pH range	B (mg/kg) range	Ca (mg/kg) range
F1	2017	4.7-6.7	0.97-3	2990-5150
	2019	4.9-7.1		
F2	2017	4.5-5.97	1.6-3	3440-5880
	2019	4.8-6.0		
F3	2017	4.6-6.7	0.79-2.7	2090-5140

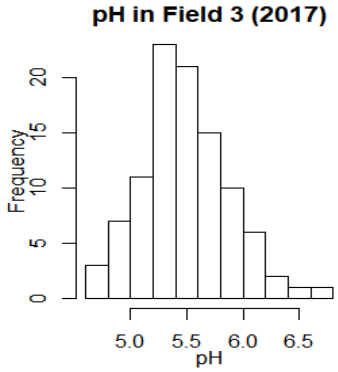
	2019	4.4-7.0		
F4	2017	4.9-6.9	1.2-4.2	1910-6190
	2019	5.2-7.4		

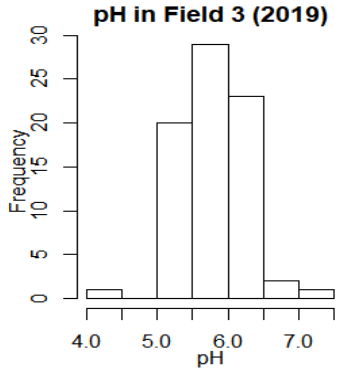


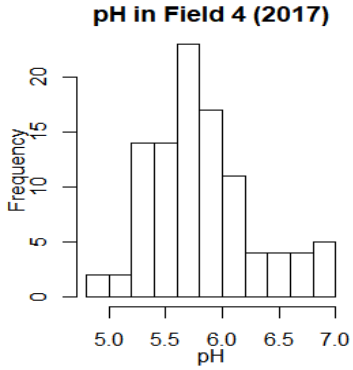


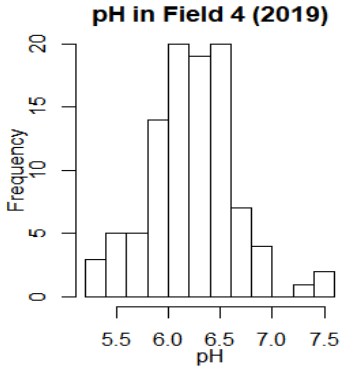


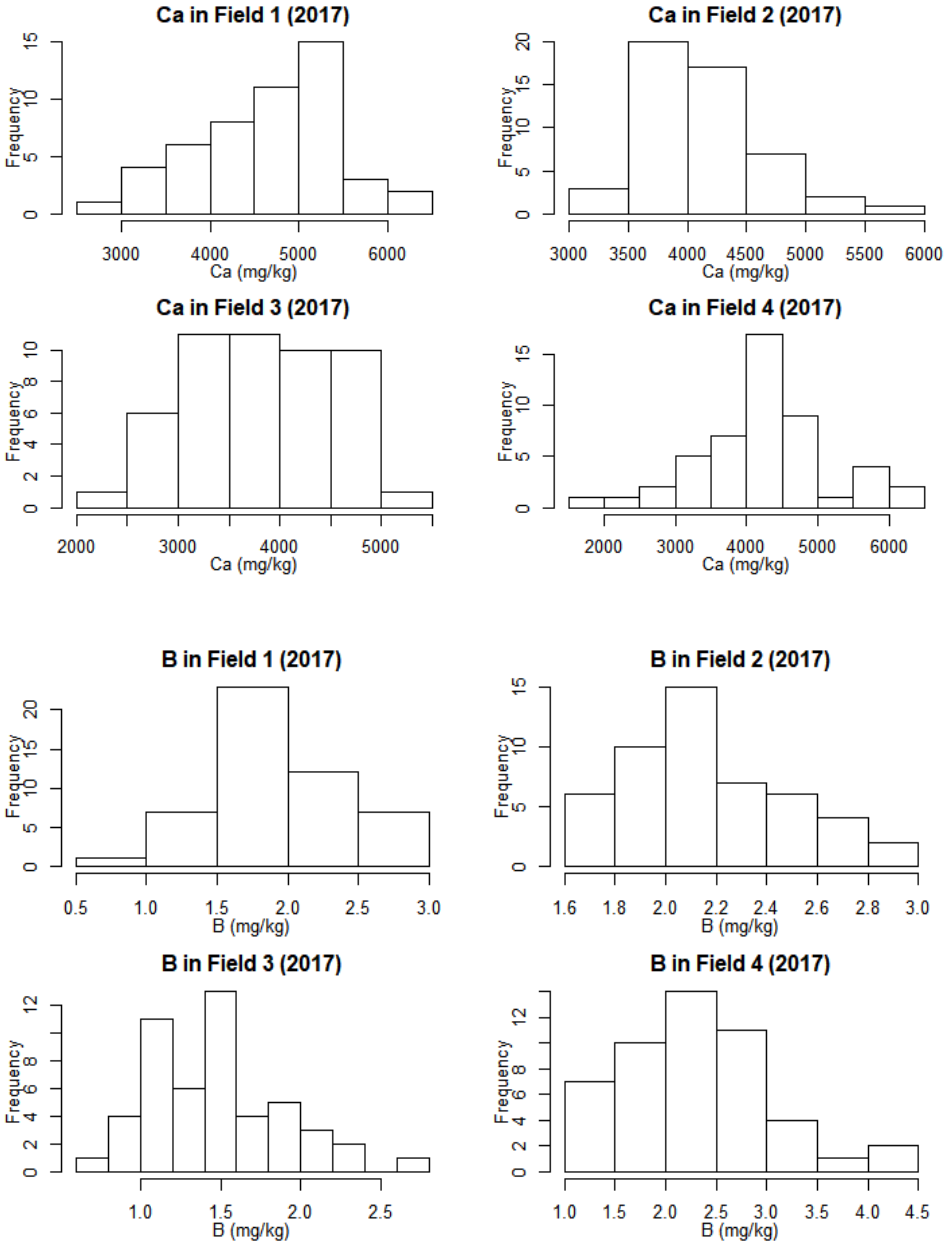












Q: Did you look at the direction of prevailing winds as a possible explanation for spatial distribution?

- **A:** No, we did not include this variable into our analysis

<u>Harnessing Plant Immunity for Durable Clubroot Resistance</u> – *Dr. Edel Pérez-López*, University Laval

- Identified 32 *P. brassicae* proteins of interest.
- Cysteine protease inhibitor – first defense for plant. Been identifying also what is the protein in canola and Arabidopsis interacting with the effector.
- The EdeLab is working to improve plant resistance to *P. brassicae* through elucidation of the mechanisms used by clubroot pathogen to infect its hosts and to escape plant immunity.

- Want to use a culture surrogate and then use Arabidopsis as the model: screening of effector by effector without 'bodyguard' mechanism interfering on the NLR receptors recognition of *P. brassicae* effectors. Unveiling of new R genes sources of resistance that can be exploited to generate resistant canola.
- Clubroot tracker idea: working with bioinformatics to develop maps. Showed example interactive GPS map for tracking *P. brassicae* around the world. Don't have any data right now but could build and implement a registration feature to know who uploads data.
- EdeLab & clubroot Mexico:
- Identifying and characterizing *P. brassicae* affecting cruciferous crops in Mexico.
- Expanding genomic information available for *P. brassicae*.
- Contributing to identification of an accurate *P. brassicae* effectoromic.

Biocontrol potential for clubroot by *Acremonium alternatum*- chances and challenges – *Dr. Jutta Ludwig-Müller* and *Dr. Susann Auer*, Technische Universität Dresden

- The defense compound salicylic acid can be derivatized by an effector of *P. brassicae*. Plasmodiophora has a protein with homology to plant SABATH methyltransferases
- PbBSMT can methylate salicylic acid, benzoic acid and anthranilic acid
- Tried several more substrates but found that only some other chlorobenzoic acids are methylated. Some agents are not methylated. Neither disease index nor infection rates were affected after treatment. This approach doesn't seem to work. Nevertheless, we believe we can induce this pathway by an endophyte.
- Can we use plants with constitutively activated SA mediated defense? Looking to downregulate gall size. Has been shown that galls smaller in Arabidopsis. Not a good idea because these plants are dwarf. SA is good way to induce clubroot defense, might be regulated by PbBSMT and we can induce clubroot resistance but unfortunately only on dwarf plants.
- Recent work – clubroot and oilseed rape: Susann Auer.
- Co-inoculation with Aa resulted in positive effect on disease index and biomass of plants in *B. napus*.
- Clubroot suppression in *B. napus* cv. Ability by *Acremonium*: 27% reduction in DI after treatment with *Aa* cell wall extract (CWE)
- *Acremonium* increased stem length and yield of 'Ability' and had earlier flowering in greenhouse
- Cultivars have their own microbiomes. High microbial diversity suppresses pathogens: plants can exploit microbial consortia from soil for protection. Breeding can hamper possibilities for positive BCA interaction.
- What do we think is important for clubroot research in the future?
    - Conditions more close to field: using field soil if only greenhouse trials are possible.
    - Testing of more canola cultivars with more *P. brassicae* isolates should be made.
    - Identification of current clubroot differential sets that are active in soils right now & timely testing of isolates. Easier/faster access to genome/transcriptome data
- More collaboration possibilities between remote labs