

# 2018 INTERNATIONAL CLUBROOT WORKSHOP



August 7<sup>th</sup> – 9<sup>th</sup>  
Edmonton, Alberta  
**Canada**

# 2018 INTERNATIONAL CLUBROOT WORKSHOP

The organizers of the 2018 International Clubroot Workshop and the Canola Council of Canada would like to acknowledge the generous support of all our sponsors!

## PLATNIUM



## GOLD



## SILVER



## BRONZE



**GRAYMONT**

## STERLING



BIOVISION





## 2018 International Clubroot Workshop

In response to the critical global challenge of feeding a growing population, the **Canola Council of Canada** (CCC) developed a strategic plan to increase canola production to 26 million tonnes by 2025. **Keep it Coming 2025** is an ambitious strategic plan to increase canola yield to 52 bushels per acre by 2025 through a dynamic process of science-based solutions that promote a sustainable and growing canola industry. The CCC represents the interests of the Canadian canola industry through activities such as coordinating research programs that lead to cutting-edge management recommendations for growers and, ultimately, to a predictable market supply for processors and exporters.

The **Crop Production and Innovation** work plan focuses on initiatives around genetic improvement and four pillars of crop production which include plant establishment, fertility management, integrated pest management and harvest management. Disease management is vital to achieving the '52 by 25' collective canola industry goal, and clubroot is one disease which has the potential for devastating yield loss in canola (estimated up to 25%).

The CCC is hosting the 17<sup>th</sup> International Clubroot Workshop in Edmonton, Alberta from August 7<sup>th</sup> to August 9<sup>th</sup>, 2018. The workshop brings together a global network of leading researchers who are focused on the *Plasmodiophora brassicae* pathogen, the causative organism of clubroot in crucifer plants, as well as on clubroot management. *P. brassicae* is a soilborne pathogen which produces irregular swellings (or galls) on the roots of the canola plant which, in turn, impedes the flow of nutrients to the plant and can cause significant yield loss. In Canada, it was first found in 2003 around Edmonton, Alberta. Seed companies responded and the first canola varieties with excellent resistance to Alberta's pathotypes became available in 2009 and 2010. Resistant cultivars have continued to be the most effective tool for clubroot management, but it is now known that there is varietal resistance breakdown related to a shift in pathotypes.

The **Clubroot Mitigation Initiative** (CRMI), a federally funded program from 2008 to 2013, was a Canadian success story that came out of managing clubroot. The CRMI produced some of the best research on clubroot and catapulted Canadian researchers into the limelight on the international field. There has been a continuing, collaborative nature to managing clubroot internationally, with joint meetings being held and close ties to the **International Clubroot Working Group** (ICWG). The final CRMI report from April 2013 can be found at [www.clubroot.ca](http://www.clubroot.ca). In this report, it states: "*this clubroot research allowed the canola industry to get ahead of this disease. For the time being, the industry is ahead of clubroot – and that is a remarkable success. But this is a temporary state if we become complacent with our success.*" The current reality is that clubroot is spreading across the Canadian Prairie Provinces and varietal resistance, although still a major tool for canola producers is breaking down.



With clubroot resistance breaking down and new pathotypes being identified, these clubroot workshops with researchers who have extensive expertise in plant pathology, disease management and breeding are critical to the production of canola in Canada. The 2018 International Clubroot Workshop endeavors to promote complement research and to advance clubroot management through the collaborative efforts of the international scientific community. Sessions will be dedicated to the latest research on applied genomics, epidemiology, molecular plant-pathogen interactions and disease management. The workshop will be of significant relevance to growers, agricultural retailers, seed companies, agronomists, academics, government and other industry stakeholders: virtually anyone who has the potential to be affected by clubroot. It is expected to draw over 200 Canadian and international delegates.

**The primary objective of the 2018 International Clubroot Workshop is the risk management of clubroot in an integrated and sustainable manner to secure the production of canola in Canada. It is about science-based solutions to mitigate the risk of clubroot and to ensure that information and new technology gets into the hands of the Canadian canola grower.**

The CCC has played an important role in clubroot management from the coordination of research programs whose priorities include clubroot, to variety performance trials, to active involvement with the CRMI, to producing extension materials such as the clubroot video and various brochures on clubroot management, to promoting the all-important boots-in-the-field approach to scouting for clubroot, and to hosting the successful 2013 International Clubroot Workshop.

Hosting the 2018 International Clubroot Workshop is another activity undertaken by the CCC. The three-day program has international speakers attending, with research and field tours being planned. The event will be based on a cost-recovery scenario through registration and an industry sponsorship package. The CCC is providing administrative support to the event through venue selection and contracts, budget development, and administration of online registration. Another essential component to the development of the workshop program is the agronomy specialists with CCC and their unique ability to interface with researchers and industry. A 2018 International Clubroot Workshop Organizing Committee has been set up, with CCC staff on various sub-committees including Finance, Science Program, Local Arrangement, Sponsorship and others.

*The 2018 International Clubroot Workshop is an important event that will contribute to good science and progressive initiatives that will lead to the growth, profitability and sustainability of Canada's vibrant canola industry.*

# 2018 INTERNATIONAL CLUBROOT WORKSHOP

## Organizing Committee

### CO-CHAIRS:

**Bruce Gossen**, Research Scientist, Agriculture and Agri-Food Canada

**Dan Orchard**, Agronomy Specialist, Central Alberta North, Canola Council of Canada

### FINANCE & ADMINISTRATION:

**Chair - Gail Hoskins**, Crop Production Administrator, CARP Coordinator, Canola Council of Canada

**Bruce Gossen**, Research Scientist, Agriculture and Agri-Food Canada

**Ellen McNabb**, Research Administrator, Canola Council of Canada

### LOGISTICS:

**Chair - Dan Orchard**, Agronomy Specialist, Central Alberta North, Canola Council of Canada

**Gail Hoskins**, Crop Production Administrator, CARP Coordinator, Canola Council of Canada

**Ellen McNabb**, Research Administrator, Canola Council of Canada

**Brittany Hennig**, Graduate Student, University of Alberta & Research Administration, Alberta Canola

### SCIENCE/POSTER SESSION PROGRAM:

**Chair - Stephen Strelkov**, Professor, University of Alberta

**Murray Hartman**, Oilseed Specialist, Alberta Agriculture and Forestry

**Mary-Ruth McDonald**, Professor, University of Guelph

**Gary Peng**, Research Scientist, Agriculture and Agri-Food Canada

**Curtis Rempel**, Vice President, Crop Production and Innovation, Canola Council of Canada

**Jay Whetter**, Communications Manager, Canola Watch Editor, Canola Digest Editor, Canola Council of Canada

**Brittany Hennig**, Graduate Student, University of Alberta & Research Administration, Alberta Canola

### SPONSORSHIP:

**Co-Chair - Coreen Franke**, Manager, R & D Pathology Research, Crop Production Services

**Co-Chair - Clinton Jurke**, Agronomy Director, Canola Council of Canada

**Curtis Rempel**, Vice President, Crop Production and Innovation, Canola Council of Canada

**Delaney Ross Burtneck**, Executive Director, Manitoba Canola Growers Association

**Errin Willenborg**, Research Manager, SaskCanola

**Rick Taillieu**, Manager, Grower Relations and Extension, Alberta Canola Producers Commission

### TOURS:

**Co-Chair - Sheau-Fang Hwang**, Research Scientist, Plant Pathology, Alberta Agriculture and Forestry

**Co-Chair - Dan Orchard**, Agronomy Specialist, Central Alberta North, Canola Council of Canada

**Autumn Barnes**, Agronomy Specialist, Alberta South, Canola Council of Canada

**Rudolph Fredua-Agyeman**, Research Scientist, Pest Surveillance Section, Alberta Agriculture and Forestry

**Brittany Hennig**, Graduate Student, University of Alberta & Research Administration, Alberta Canola

### ADVERTISING:

**Chair - Gary Peng**, Research Scientist, Agriculture and Agri-Food Canada

**Dan Orchard**, Agronomy Specialist, Central Alberta North, Canola Council of Canada

**Stephen Strelkov**, Professor, University of Alberta

**Jay Whetter**, Communications Manager, Canola Watch Editor, Canola Digest Editor, Canola Council of Canada

### ENTERTAINMENT:

**Dan Orchard**, Agronomy Specialist, Central Alberta North, Canola Council of Canada

**Gregory Sekulic**, Agronomy Specialist, Peace, Canola Council of Canada



## WELCOME REMARKS

Dr. Elke Diederichsen, Institut für - Angewandte Genetik, Freie Universität/Berlin

Dr. Curtis Rempel, Canola Council of Canada, Canada

## KEYNOTE SPEAKERS

### GENETICS AND BREEDING

#### Maria Manzanares-Dauleux, PhD

Professor

Institute of Genetics

Environment and Plant Protection (IGEPP)

INRA/Agrocampus Ouest/University of Rennes, France



#### Biography

Dr. Maria Manzanares-Dauleux is a Professor of Genetics & Plant Breeding and Director of the Institute of Genetics, Environment and Plant Protection, a joint research unit of INRA/Agrocampus Ouest/University of Rennes, in France. She received an MSc from the University Complutense of Madrid, Spain, followed by a PhD degree in Biology and Agronomy from the University of Rennes, France. Her research aims to decipher plant response to pathogens. In particular, the Manzanares-Dauleux lab has worked for many years on the *Brassicaceae* (from *Arabidopsis* to Brassica crop species) - *Plasmodiophora* interaction combining epi-genetic, genomic, physiologic and metabolic approaches. Her research has enabled the identification and study of the diversity, genomic control and functions of the factors involved in quantitative resistance to clubroot. Current work in her lab is focused on the unravelling of the mechanisms underlying modulation of the plant response to clubroot infection by other biotic and abiotic factors (mainly nutritional constraint and hydric stress) and the contribution of epigenetic variability to plant quantitative resistance, both of which are needed to develop new resistant varieties adapted to different agro-ecological conditions and environments.

#### Presentation

Influence of nitrogen constraint on quantitative resistance to clubroot in *Brassica napus*

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### Zhongyun Piao, PhD

Professor  
Shenyang Agricultural University, China



#### Biography

Dr. Zhongyun Piao received his PhD in 2003 in Horticulture from Chungnam National University, Daejeon, Korea. He is a professor at Shenyang Agricultural University, Shenyang, China. His main research area includes mapping important traits and functional studies of genes of interest in *Brassica rapa* vegetables. One of his main interests is on clubroot resistance in *B. rapa* and *B. napus*, including marker development for marker-assisted breeding, *P. brassicae*-Chinese cabbage interactions, pathotype differentiation and *P. brassicae* diagnostics, and the molecular mechanisms of clubroot resistance.

### Chunyu Zhang, PhD

Professor  
Huazhong Agricultural University, China



#### Biography

Dr. Chunyu Zhang is a full professor at Huazhong Agricultural University. His research interests are focused on the genetics and breeding of canola. Over the past decade, the regulation of vitamin E metabolism for making more tocopherols in *Arabidopsis* was achieved, resulting in the successful vitamin E biofortification of canola via translation of the knowledge acquired from this model plant *Arabidopsis*. Given the increasing importance of clubroot as a disease of canola in China and worldwide, Dr. Zhang has dedicated most of his research efforts in recent years to understanding the genetics of and breeding for improved clubroot resistance in canola.

#### Combined Presentation

Clubroot resistance genes in *Brassica rapa*: the utilities in differentiation of *Plasmodiophora brassicae* pathotype and CR breeding in *Brassica* crops

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### Fengqun Yu, PhD

Research Scientist  
Agriculture and Agri-Food Canada, Saskatoon



#### Biography

Dr. Fengqun Yu received her PhD in 1995 in Crop Genetics and Breeding from Huazhong Agricultural University, Wuhan, China. She worked as an Associate Professor on canola resistance to *Sclerotinia* at the same university for two years, and then at the University of Manitoba as a Visiting Scholar for one year. She has been working with Agriculture and Agri-Food Canada at Saskatoon, SK, since 1999,

focusing on canola resistance to blackleg until 2011 as a NSERC Visiting Fellow and Research Biologist, and then on both clubroot and blackleg after 2011 as a Biology Study Leader in Plant Pathology. Fengqun identified and mapped several novel genes controlling resistance to blackleg, and produced advanced generations of canola germplasm carrying R genes, including the blackleg isogenic lines currently used worldwide. She leads studies on genetic mapping, molecular cloning and genome editing of clubroot resistance genes, developing SNP markers tightly linked to R genes and canola germplasm for resistance to clubroot and blackleg, and developing molecular tools for monitoring changes in the race structure of the blackleg pathogen.

### **Presentation**

Developing spring type *Brassica napus* lines containing single clubroot resistance genes

### GENOMICS

#### **Katsunori Hatakeyama, PhD**

Associate Professor  
Faculty of Agriculture  
Iwate University, Japan



### **Biography**

Dr. Katsunori Hatakeyama is an Associate Professor in the Faculty of Agriculture, Iwate University, Iwate, Japan. He earned his PhD in Agriculture (Plant Breeding) from Tohoku University, Sendai, in 1998, and was a researcher from 1995 to 2001 at the Research Institute of Seed Production Co. Ltd., Sendai. During this time, Katsunori focused his research on molecular genetic studies of self-incompatibility in *Brassica rapa*. After moving to the Institute of Vegetable and Floricultural Science (NIVTS), NARO, in 2001, where he was a senior researcher, he carried out molecular genetic studies on clubroot resistance, the development of molecular markers for breeding, and mapping of important traits such as the level of self-incompatibility and late bolting in *Brassica* vegetables. In 2015, Katsunori moved to his present position in the Plant Breeding Lab, Iwate University. His current research focus includes studies on the molecular genetics of important traits in *Brassica* vegetables, and one of his research interests is in the analysis of the molecular mechanisms of clubroot resistance.

### **Presentation**

Molecular genetics of the clubroot resistant genes in Chinese cabbage (*Brassica rapa* L.)

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### Satoru Matsumoto, PhD

Director

Vegetable Breeding Division

Institute of Vegetable and Floricultural Science (NIVTS)

NARO, Japan



#### Biography

Dr. Satoru Matsumoto is a leader in clubroot disease resistance research, including the development of DNA markers, isolation of resistance genes, and breeding of resistant cultivars at the Institute of Vegetable and Floricultural Science (NIVTS), NARO. In 2011, his research group bred the Chinese cabbage cultivar F1 hybrid, "Akimeki," that carries three resistance genes, *Crr1*, *Crr2* and *CRb*, and is highly resistant to four pathotypes of *Plasmodiophora brassicae*. At present, "Akimeki" is a leading cultivar in Ibaraki prefecture where Chinese cabbage production is the largest in Japan. Dr. Matsumoto is currently the Director of the Vegetable Breeding Division in NIVTS.

#### Presentation

Development of a highly clubroot-resistant F1 cultivar Chinese cabbage (*Brassica rapa* L.) accumulating three resistance genes, *Crr1*, *Crr2* and *CRb*

### HOST-PATHOGEN INTERACTIONS

### Jutta Ludwig-Müller, PhD

Professor

Technische Universität Dresden, Germany



#### Biography

Dr. Jutta Ludwig-Müller is a Professor of Plant Physiology and Director of the Institute of Botany in the Faculty of Biology at the Technische Universität Dresden in Germany. Her research interests are plant pathology, especially clubroot, and plant hormones as well as plant secondary metabolites. At the TU Dresden, she is also active in teaching these topics in undergraduate and graduate courses and she has successfully supervised more than 10 PhD theses. She received her diploma and PhD from the Goethe Universität in Frankfurt/Main, Germany, where she also got her habilitation. She was a post-doctoral researcher at Case Western Reserve University, Cleveland, USA, and was a guest scientist at the Volcani Center in Israel and the United States Department of Agriculture in Maryland. Dr. Ludwig-Müller has published over 180 articles. She is a member of several editorial boards of scientific journals and has served as Editor-in-Chief of the Journal of Plant Growth Regulation for over 10 years. She is a member of several international scientific societies and her international achievements have been recognized by memberships on several international advisory boards.

## Presentation

Using knowledge on plant hormone metabolism by *Plasmodiophora brassicae* - a possibility to control the clubroot pathogen?

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### Gary Peng, PhD

Research Scientist

Agriculture and Agri-Food Canada, Saskatoon



### Biography

Dr. Gary Peng received his PhD in Plant Pathology from the University of Guelph in 1992. He is currently a Research Scientist at AAFC Saskatoon Research and Development Centre. His recent research has mostly been on clubroot and blackleg of canola, including identification of new resistance sources, host-pathogen interaction disease resistance mechanisms, blackleg pathogen race dynamics and disease management strategies on canola crops. Gary has authored/coauthored over 100 peer-reviewed papers in his career.

## Presentation

The effect and durability of incomplete resistance, based on two clubroot resistance genes, against a new pathotype of *Plasmodiophora brassicae*

## EPIDEMIOLOGY AND DISEASE MANAGEMENT

### Stephen Strelkov, PhD

Professor

University of Alberta, Canada



### Biography

Dr. Stephen Strelkov is a Professor of Plant Pathology at the University of Alberta. He received a BSc from the University of Alberta, followed by MSc and PhD degrees in plant pathology from the University of Manitoba. After a short stint as a NSERC Visiting Fellow with Agriculture and Agri-Food Canada, Dr. Strelkov accepted a faculty position at the University of Alberta, where his research is focused on diseases of field crops. Dr. Strelkov has authored or co-authored more than 150 peer-reviewed papers, with 20 graduate students successfully completing their degrees under his supervision. Dr. Strelkov has received the 'Outstanding Young Scientist' and 'Outstanding Achievements in Plant Disease Management' awards from the Canadian Phytopathological Society, and serves as Associate Editor-in-Chief of the Canadian Journal of Plant Pathology. He is also active in undergraduate teaching at the university, and his name has been placed on the Faculty of Agricultural, Life and Environmental Sciences 'Teaching Wall of Fame' 11 in years in a row. At present, Dr. Strelkov also serves as the Associate Chair, Graduate Programs, for the

Department of Agricultural, Food and Nutritional Science. He was selected as a 2016-2017 Killam Annual Professor at the University of Alberta.

### **Presentation**

The changing face of clubroot (*Plasmodiophora brassicae*) in Canada

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### **Sheau-Fang Hwang, PhD**

Research Scientist, Plant Pathology  
Alberta Agriculture and Forestry, Canada



### **Biography**

Dr. Sheau-Fang Hwang graduated from Washington State University with a PhD in Plant Pathology and has worked for over 20 years on soil-borne diseases of field crops at the Alberta Research Council. Dr. Hwang joined Alberta Agriculture as a research scientist in 2006, about the same time that the clubroot outbreak started. At Crop Diversification Centre North, she established a lab and field nurseries and began to develop methods to slow the spread of clubroot. More recently, she has initiated projects on blackleg and soil-borne seedling diseases of canola. Dr. Hwang became an Adjunct Professor at the University of Alberta in 2005, and has supervised many graduate students. Over her career, she has authored or co-authored more than 200 scientific papers and has led many successful research projects with both national and international collaborators. In 2011, Dr. Hwang received the Outstanding Research Award from the Canadian Phytopathological Society (CPS), which was followed in 2014 and 2016 with awards for Achievements in Plant Disease Management for her work on clubroot and pulse crop diseases, respectively. Throughout her career, Dr. Hwang has worked to integrate cultural practices, genetic resistance, fungicidal and biological methods for the sustainable management of diseases of field crops, particularly canola.

### **Presentation**

Management of clubroot of canola in Alberta, Canada

## DISEASE MANAGEMENT

### **Malgosia Jedryczka, PhD**

Deputy Director for Research  
Institute of Plant Genetics  
Polish Academy of Sciences, Poland



### **Biography**

Dr. Malgosia (Gosia) Jedryczka graduated from Poznan University of Life Sciences, Poland, with a degree in genetics and plant breeding. She received her PhD in 1995, was made an Associate Professor in 2007 and was promoted to full Professor in 2013. From 1984 until present, she has held a permanent position with the Institute of Plant Genetics, Polish Academy of Sciences in Poznan, Poland. From 2000 to 2012 she was the head of the Laboratory of Genetics of Resistance, and from 2013 to 2015 she was in charge of the Department of Pathogen Genetics and Plant Resistance. Since 2016, Dr. Jedryczka has been the Deputy Director at IPS PAS. Her research concentrates on diseases of oilseed rape. Her main focus is on the biodiversity of pathogens, methods for their early detection and the identification of sources of genetic resistance. This focus also concerns her studies of clubroot of winter oilseed rape. She has described pathotypes of *Plasmodiophora brassicae* in Poland and created maps of pathogen occurrence. Dr. Jedryczka also is interested in aerobiology and has created the System for Forecasting Disease Epidemics ([www.spec.edu.pl](http://www.spec.edu.pl)) - the biggest system for stem canker (*Leptosphaeria* spp.) detection worldwide. For this activity, she received an award from the Ministry of Agriculture and Rural Affairs in 2012. She is the Deputy President of the Polish Phytopathological Society and a member of the Board of the Polish Genetic Society.

### **Presentation**

Occurrence of *Plasmodiophora brassicae* in agricultural soils, pathotype variation and means of clubroot control in Poland

## AGRONOMY PROGRAM - PART ONE

### **Mary Ruth McDonald, PhD**

Research Program Director  
Plant Production Systems and Professor  
Department of Agriculture  
University of Guelph, Canada



### **Biography**

Dr. Mary Ruth McDonald is a professor in the Department of Plant Agriculture at the University of Guelph in Ontario. She is also a Research Program Coordinator at the university. Her research focuses on plant diseases and integrated pest management of vegetable crops, including Brassica vegetables and some field crops such as canola and pulse

crops. She is a member of the Clubroot Steering Group of the Canola Council of Canada. Mary Ruth teaches a graduate course on plant disease epidemiology. She has published over 75 scientific papers including several on clubroot, and has received national and international award for excellence in integrated pest management.

**Bruce Gossen, PhD**

Research Scientist

Agriculture and Agri-Food Canada, Saskatoon



**Biography**

Dr. Bruce D Gossen graduated with a PhD in Plant Pathology from the University of Saskatchewan in 1985. Since then, he has been employed as a research scientist with Agriculture and Agri-Food Canada at Saskatoon SK, specializing in management of diseases of field crops. Over the course of his career, he has worked on diseases of forages and turf, cereals, pulse crops, canola and vegetables. He has published more than 170 papers in refereed journals, plus hundreds of other articles and presentations. He has had a leadership role in many organizations, including a term as President of the Canadian Phytopathological Society, and has received numerous awards for his work, including two Golden Harvest Awards from AAFC and the Award for Outstanding Research from the Canadian Phytopathological Society. He currently leads studies on clubroot of canola and on root rot of field pea with colleagues across Canada and the USA, and continues to be active in supervision of graduate students and mentoring postdoctoral fellows.

**Combined Presentation**

Management of clubroot: an overview of the challenges

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Edmonton, Alberta, Canada

August 7th - 9th 2018

## WHAT LIES BENEATH...

DAY ONE, Tuesday August 7th 2018

7:30am - 8:15am	<b>Registration &amp; Breakfast</b>		
8:15am - 8:30am	<b>Welcome Remarks</b>	Dr. Curtis Rempel Dr. Elke Diederichsen	Canola Council of Canada Institut für-Angewandte Genetik, Freie Universität Berlin
<b>GENETICS &amp; BREEDING</b>			
8:30am - 8:55am	KS-1	Influence of nitrogen constraint on quantitative resistance to clubroot in Brassica napus	Dr. Maria Manzanares-Dauleux Agrocampus Ouest, INRA, Université de Rennes 1, France <i>Shengyang Agricultural University, China</i>
8:55am - 9:45am	KS-2	Clubroot resistance genes in Brassica rapa: the utilities in differentiation of Plasmodiophora brassicae pathotype and CR breeding in Brassica crops	Dr. Zhongyun Piao Dr. Chunyu Zhang <i>Huazhong Agricultural University, China</i>
9:45am - 10:10am	KS-3	Developing spring type Brassica napus lines containing single clubroot resistance genes	Dr. Fengqun Yu <i>Agriculture and Agri-Food Canada, Canada</i>
10:10am - 10:30am	<b>Nutrition Break</b>		
10:30am - 10:45am	VA-1	Characterization of clubroot resistance in Raphanus	<u>E.Diederichsen</u> , N. Gollinge, M. Schlathölder
10:45am - 11:00am	VA-2	Identification of clubroot resistant QTLs in radish (Raphanus sativus L.)	<u>C.Gan</u> , X.Deng, L.Cui, W.Yuan, X.Yu, Y.P.Lim, Z.Piao
11:00am - 11:15am	VA-3	Development of clubroot resistant interspecific hybrids of Brassica oleracea × Brassica rapa ssp. Rapa	S.Zhang, S.Shen, Q.Li, X.Ren, H.Song and <u>J.Si</u>
11:15am - 11:30am	VA-4	Transferring clubroot resistance by intergeneric hybridizations between Brassica napus and Raphanus sativus	<u>E. Diederichsen</u> , N.Gollinge, J.Mader, J.Schondelmaier, K.Lohgall & M.Schlathölder
11:30am - 11:45am	VA-5	Screening of Brassica accessions for resistance to 'old' and 'new' isolates of Plasmodiophora brassicae in Alberta, Canada	<u>Rudolph Fredua-Agyeman</u> , S.F.Hwang, S.E.Strelkov, Q. Zhou, V.P.Manolii & D.Feindel
11:45am - 12:00pm	<b>Free Time</b>		
12:00pm - 1:00pm	<b>LUNCH</b>		
<b>GENOMICS</b>			
1:00pm - 1:25pm	KS-4	Molecular genetics of the clubroot resistant genes in Chinese cabbage (Brassica rapa L.)	Dr. Katsunori Hatakeyama <i>Iwate University, Japan</i>
1:25pm - 1:50pm	KS-5	Development of a highly clubroot-resistant F1 cultivar Chinese cabbage (Brassica rapa L.) accumulating three resistance genes, Crr1, Crr2 and CRb	Dr. Satoru Matsumoto <i>Institute of Vegetable and Floriculture Science, NARO Division of Vegetable Breeding, Japan</i>
1:50pm - 2:05pm	VA-6	Gene similarity of Plasmodiophora brassicae collections from Canada	<u>A.Sedaghatkish</u> , B.D.Gossen, M.R. McDonald
2:05pm - 2:20pm	VA-7	Transcriptome changes in brassica napus cultivars upon interaction with Plasmodiophora brassicae pathotype 5x	<u>L.Galindo-González</u> , S.F.Hwang & S.E.Strelkov
2:20pm - 2:35pm	VA-8	Genetic analysis of clubroot resistance using multiple populations in Brassica rapa	<u>Y.P. Lim</u> , S.R.Choi & S.Park
2:35pm - 2:55pm	<b>Nutrition Break</b>		
<b>HOST-PATHOGEN INTERACTIONS</b>			
2:55pm - 3:20pm	KS-6	Using knowledge on plant hormone metabolism by Plasmodiophora brassicae – a possibility to control the clubroot pathogen	Professor Dr. Jutta Ludwig-Mueller <i>Technische Universität Dresden, Germany</i>
3:20pm - 3:45pm	KS-7	The effect and durability of incomplete resistance, based on two clubroot resistance genes, against a new pathotype of Plasmodiophora brassicae	Dr. Gary Peng <i>Agriculture and Agri-Food Canada, Canada</i>
3:45pm - 4:00pm	VA-9	Proto-oncogenes in a eukaryotic unicellular organism play essential roles in plasmodial growth in host cells	K.Bi, T.Chen, Z.C.He, <u>Z.X.Gao</u> , Y.Zhao, Y.P.Fu, J.S.Cheng, J.T.Xie & D.H.Jiang
4:00pm - 4:15pm	VA-10	Infection of canola with Plasmodiophora brassicae increases resistance to herbivory by Bertha armyworm, Mamestra configurata	<u>D.S.Weeraddana</u> , V.P.Manolii, S.E.Strelkov, A.P. de la Mara, J.J.Harynuk & M.L.Evenden
4:15pm - 4:30pm	VA-11	Screening important secreted proteins during the process in Brassica napus infected by Plasmodiophora brassicae	<u>Z.X.Gao</u> , Y.Zhao, K.Bi, J.T.Xie, J.S.Cheng, Y.P.Fu & D.H.Jiang
4:30pm - 6:30pm	<b>Ice Breaker Reception</b>		
5:00pm - 6:00pm	<b>Dedicated Poster Session</b>		

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August 7th - 9th 2018

## DIG A LITTLE DEEPER...

DAY TWO, Wednesday August 8th 2018

7:30am - 8:30am	Registration & Breakfast		
8:30am - 8:45am	Welcome Remarks		
<b>EPIDEMIOLOGY &amp; DISEASE MANAGEMENT</b>			
8:45am - 9:10am	KS-8	The changing face of clubroot ( <i>Plasmodiophora brassicae</i> ) in Canada	Dr. Stephen E. Strelkov <i>University of Alberta, Canada</i>
9:10am - 9:35am	KS-9	Management of clubroot in canola in Alberta, Canada	Dr. Sheau-Fang Hwang <i>Alberta Agriculture Forestry, Canada.</i>
9:35 am - 9:50am	VA-12	Epidemiology of clubroot disease and pathogenic variation among isolates of <i>Plasmodiophora brassicae</i> from oilseed rape growing in Europe	<u>N.Zamani-Noor</u> , E.Diederichsen, A.C.Wallenhammar, G.Cordsen-Nielsen, G.Orgeur, V.Konradová, F.Dissart, J.Smith & M.Jedryczka
9:50am - 10:05am	VA-13	Clubroot status in Colombia	<u>A.Botero-Ramirez</u> , F.L. Padilla-Huertas, L.Tarazona,
10:05am - 10:20am	VA-14	Impact, management and control of clubroot disease in the UK	<u>F.Burnett</u> & J.Smith
10:20am - 10:40am	Nutrition Break		
<b>DISEASE MANAGEMENT</b>			
10:40am - 11:05am	KS-10	Occurrence of <i>Plasmodiophora brassicae</i> in agricultural soils, pathotype variation and means of clubroot control in Poland	<u>Dr. Malgosia Jedryczka</u> <i>Institute of Plant Genetics, Polish Academy of Sciences, Poland</i>
11:05am - 11:20am	VA-15	Resting spores of clubroot detection service by LAMP method in Japan	<u>K.Wakayama</u> , T.Usui, M.Okada, Y.Kawahara
11:20am - 11:35am	VA-16	Molecular detection of <i>Plasmodiophora brassicae</i> the causal agent of clubroot of cruciferous crops	G.G.Guan, W.X.Pang, Z.Y.Piao & <u>Y.Liang</u>
11:35am - 11:50am	VA-17	Evaluation of various soil amendments to manage clubroot on canola in field condition	<u>V.Chapara</u>
11:50am - 12:00pm	Free Time		
12:00pm - 1:00pm	LUNCH		
1:00pm - 4:30pm	CDC North Research Facility Tour Load buses for tour and after tour to Galla Barbecue		
5:15pm - 9:00pm	University of Alberta Botanic Garden Barbecue Load buses back to Sutton Place Hotel		

## BE LESS WRONG TOMORROW...

DAY THREE, Thursday August 9th 2018

7:30am - 8:30am	Registration & Breakfast		
8:30am - 8:45am	Welcome Remarks		
<b>AGRONOMY - PART I</b>			
8:45am - 9:35am	KS-11	Management of clubroot: an overview of the challenges	Dr. Mary-Ruth McDonald <i>University of Guelph, Canada</i> Bruce Gossen <i>Agriculture and Agri-Food Canada, Canada</i>
9:35am - 9:55am	VA-18	The influence of clubroot resistant canola on resting spore levels in the soil	<u>T.W.Ernst</u> , S.V.Kher, D.Stanton, D.C.Rennie, S.F.Hwang & S.E.Strelkov
9:55am - 10:10am	VA-19	Suppression of <i>Plasmodiophora brassicae</i> in infested oilseed rape farms by soil amendment with calcium cyanamide and burnt lime	<u>N.Zamani-Noor</u>
10:10am - 10:30am	Nutrition Break		
<b>AGRONOMY - PART II</b>			
10:30am - 10:45am	VA-20	Lime and limestone production, products and trends in history – a mining company's perspective	A.Nguyen & <u>B.Anderson</u>
10:45am - 11:00am	VA-21	Assessment of hydrated lime for the management of clubroot in canola	<u>Nicole M. Fox</u> , S.F.Hwang, V.P.Manolii, G.Turnbull & S.E.Strelkov
11:00am - 11:15am	VA-22	A recipe for managing small patches of infestation of clubroot in canola	<u>B.D.Gossen</u> , A.Sedaghatkish, S.F.Hwang & M.R.McDonald
11:15am - 11:30am	VA-23	Integrated management of clubroot – crucial for a sustainable oilseed rape production	<u>A.C.Wallenhammar</u> , Z.Omer & A.Jonsson
11:30am - 12:30pm	WORKING LUNCH "Opportunities that will Deliver Value"		Moderator: Jay Whetter Canola Council of Canada, Canada
12:30pm - 12:40pm	CLOSING REMARKS		
12:40pm - 1:00pm	Free Time (prepare for field tour)		
1:00pm - 4:30pm	Field Tour Load buses - some buses return to Sutton Place Hotel after the tour, others continue to farm tour		
5:00pm - 6:30pm	Farm Tour/Barbecue - Cost \$30 (optional, registration item) Load buses to return to Sutton Place Hotel		

# 2018 INTERNATIONAL CLUBROOT WORKSHOP

Edmonton, Alberta, Canada

August 7th - 9th 2018

## CONTRIBUTED POSTERS

August 7th, 5:00pm - 6:00pm

PA-1	Introgression of clubroot resistance and analyses of segregation distortion in two F <sub>2</sub> populations derived from <i>Brassica rapa</i> subsp. <i>rapifera</i> (ECD 02).	JUNYE JIANG, RUDOLPH FREDUA-AGYEMAN, SHEAU-FANG HWANG & STEPHEN E. STRELKOV
PA-2	Mapping of resistance loci associated with clubroot disease originally derived from RRCC in <i>Brassica napus</i> .	J. GONG, Z. ZHAN, J. WU, C. ZHANG
PA-3	Can <i>Brassica rapa</i> contribute a new clubroot resistance gene for use in the breeding of <i>Brassica napus</i> canola for Canada?	KAWALPREET KAUR & HABIBUR RAHMAN
PA-4	Fine mapping of <i>BraCrr5</i> , a novel gene conferring resistance to clubroot disease ( <i>Plasmodiophora brassicae</i> Woronin) in <i>Brassica rapa</i>	L. L.X. MAO, Y.X. YUAN, S.J. YANG, X.C. WEI, Y.Y. ZHAO, Z.Y. WAN, X.W. ZHANG & B.M. TIAN
PA-5	Identification of a novel clubroot resistance QTL in Chinese cabbage ( <i>Brassica rapa</i> L.).	JONG-IN PARK, RAWNAK LAILA, ARIF HASAN KHAN ROBIN, HOY-TAEK KIM, ILL-SUP NOU
PA-6	QTL-seq analysis identifies two clubroot ( <i>Plasmodiophora brassicae</i> ) resistance QTL in turnip ( <i>Brassica rapa</i> ssp. <i>rapifera</i> ).	H. ZHANG, S. J. ZHANG, F. CHENG, F. LI, S. F. ZHANG & R.S. SUN
PA-7	Screening of <i>Brassica oleracea</i> germplasm for resistance to <i>Plasmodiophora brassicae</i> pathotype 5X.	M. FARID, J. SI & H. RAHMAN
PA-8	Genetic diversity of rutabaga accessions as sources of clubroot resistance.	ZHIYU YU, RUDOLPH FREDUA-AGYEMAN, SHEAU-FANG HWANG & STEPHEN E. STRELKOV
PA-9	Marker-assisted introgression of <i>CRb</i> locus into elite Pol CMS restorer line for resistant hybrid production to clubroot in <i>Brassica napus</i> .	Q. LI, Y. ZHOU, T. FU, Z. PIAO & C. ZHANG
PA-10	Verification of two candidate genes resistance to <i>Plasmodiophora brassicae</i> in broccoli.	Z.S. LI, X.L. ZHANG, Y.M. LIU, Z.Y. FANG, L.M. YANG, M. ZHUANG, Y.Y. ZHANG & H. H. LV
PA-11	Genome-wide transcriptomic analysis used to identify putative effector proteins in <i>Plasmodiophora brassicae</i> .	E. PÉREZ-LÓPEZ, J.TU, M. WALDNER, A. J. KUSALIK, M. HOSSAIN, C. D. TODD & P.C. BONHAM-SMITH
PA-12	Modulation of abscisic acid and ethylene levels in response to clubroot disease in <i>Brassica napus</i> .	C.P. JAYASINGHEGE, V.P. MANOLII, J.A. OZGA, S.F. HWANG & S.E. STRELKOV
PA-13	Effects of root exudates on <i>Plasmodiophora brassicae</i> resting spore germination.	Y. WANG, B. KOOPMANN, P. KARLOVSKY, & A. VON TIEDEMANN
PA-14	Effect of canola cultivar rotation on <i>Plasmodiophora brassicae</i> pathotype population dynamics.	TIESEN CAO, VICTOR P. MANOLII, QIXING ZHOU, SHEAU-FANG HWANG & STEPHEN STRELKOV
PA-15	Decline in resting spores of <i>Plasmodiophora brassicae</i> in soil over 6 years.	B.D. GOSSEN, F. AL-DAOUD, T. DUMONCEAUX, J. DALTON, G. PENG, D. PAGEAU & M.R. MCDONALD
PA-16	Development of isolate-specific molecular marker for detecting Korean <i>Plasmodiophora brassicae</i> ; the causal agent of clubroot.	H.-T. KIM, J.-Y. JEONG, J.-I. PARK & I.-S. NOU
PA-17	Production of single-spore isolates of <i>Plasmodiophora brassicae</i> using micromanipulation of resting spores.	A. SEDAGHATKISH, B.D. GOSSEN, J. SINGH & M.R. MCDONALD
PA-18	Effect of <i>Plasmodiophora brassicae</i> inoculum density on yield of canola ( <i>Brassica napus</i> ).	A. BOTERO-RAMÍREZ, S.F. HWANG & S.E. STRELKOV
PA-19	Assessment of soils for the risk of clubroot disease of cruciferous vegetables using a simple dose response curve-dependent method.	A. FUKUNAGA, S. YOSHIDA & S. TSUSHIMA
PA-20	Isolation and evaluation of potential biocontrol agents against clubroot ( <i>Plasmodiophora brassicae</i> ) of <i>Brassica napus</i> .	L. ZHENG, Y.W. HE, M.L. ZHU, D.H. JIANG & J.B. HUANG
PA-21	Evaluating chemical disinfectants for their ability to inactivate <i>Plasmodiophora brassicae</i> resting spores using Evans blue staining.	M.W. HARDING, T.B. HILL, G.C. DANIELS, S.E. STRELKOV, S.F. HWANG & J. FENG



**DAY ONE: What lies beneath...**  
**Tuesday, August 7<sup>th</sup>, 2018**

## WELCOME REMARKS

**Dr. Curtis Rempel** – Vice-President, Crop Production and Innovation, Canola Council of Canada, Canada  
**Dr. Elke Diederichsen** – Co-Chair, International Clubroot Working Group

## GENETICS & BREEDING

**KS-1. Keynote Speaker: Influence of nitrogen constraint on quantitative resistance to clubroot in *Brassica napus*.** Dr. Maria Manzaneres-Dauleux, *Agrocampus Ouest, INRA, Université de Rennes 1, France*

Abiotic factors are known to influence quantitative resistance to plant pathogens, but underlying genetic and physiologic mechanisms are mostly unknown. In this work, we developed combined genetic and molecular physiology approaches to investigate the influence of nitrogen fertilization on quantitative resistance of *Brassica napus* to the clubroot causing agent *Plasmodiophora brassicae*. Disease response was studied in a panel of oilseed rape genotypes and *P. brassicae* isolates cultivated under low vs. high nitrogen supplies. This work highlighted that lower nitrogen input can modulate disease symptoms (from strong symptom inhibition to no effect), depending on both plant genotype and *P. brassicae* isolate. QTL analysis conducted in a 'Darmor-bzh' x 'Yudal' doubled haploid progeny showed that nitrogen deficiency exerted a major switch between the effects of two QTL involved in resistance toward the isolate eH. One low-nitrogen-dependent QTL identified on the chromosome CO2 was found to exert a major effect on the resting spore content in infected roots, but moderately influencing club symptom development. By contrast, the effect of a major QTL involved in resistance toward isolate K92-16 was unaffected by nitrogen fertilization. Combination of metabolomics and transcriptomics highlighted the putative role of nitrate transporter encoding genes, which are specially induced under the double biotic-abiotic stresses in the genotype 'Yudal' expressing low nitrogen-triggered resistance. Altogether, our results indicated that nitrogen fertilization influences clubroot disease in a QTL x isolate dependent manner. A better understanding of QTL x pathogen isolate x fertilization crosstalk may help to rationalize the use of clubroot quantitative resistance in breeding.

**KS-2. Keynote Speaker: Clubroot resistance genes in *Brassica rapa*: the utilities in differentiation of *Plasmodiophora brassicae* pathotype and CR breeding in Brassica crops.** Dr. Zhongyun Piao, Shengyang Agricultural University, China & Dr. Chunyu Zhang, Huazhong Agricultural University, China

Clubroot disease, one of the most devastating diseases in Brassica crops, is increasingly prevalent in Brassica vegetable and oil crops in China. *Plasmodiophora brassicae*, the causal agent of clubroot, undergoes rapid variation, leading to the fast erosion of clubroot resistance (CR). Therefore, accurate classification and identification of *P. brassicae* pathotypes is fundamental to guiding the breeding of CR cultivars, the most economic and efficient approach for clubroot management. So far, more than 10 CR loci showing pathotype-specific resistance were identified in *B. rapa*. Mining of CR germplasm provides an opportunity to differentiate *P. brassicae* pathotypes, in addition to the Williams' differential set and European Clubroot Differential set. Based on Williams' differential set, four pathotypes had been found in China. However, 12 pathotypes were differentiated with a newly developed Sinitic Clubroot Differentiation set consisting of different CR *B. rapa* germplasm with known or unknown CR genes. The different CR germplasm was successfully used to transfer CR genes into Chinese cabbage (*B. rapa*), Zhacai (*B. juncea*) and rapeseed (*B. napus*), and based on which new cultivars were released. Understanding the molecular mechanisms of clubroot resistance, the interaction between CR gene and *P. brassicae* pathotype, and genetic interaction between CR genes will accelerate the breeding process for durable clubroot resistance.

**KS-3. Keynote Speaker: Developing spring type *Brassica napus* lines containing single clubroot resistance genes.** Dr. Fengqun Yu, Agriculture and Agri-Food Canada, Canada

Clubroot, caused by *Plasmodiophora brassicae* Woronin, poses a serious threat to canola (*Brassica napus* L.) production in Canada. Pathotypes are characterized based on differential reactions on Brassica cultivars, but most of the differential cultivars are vegetable or fodder types. Ideally, a set of near-isogenic spring canola lines containing single clubroot resistance genes could be used to differentiate races of *P. brassicae*. Genetic mapping has identified more than 10 genes for clubroot resistance that occur in Brassica species. The objective of this study was to develop a set of near-isogenic lines of *B. napus* with single genes, suitable for identification of races of *P. brassicae*. Resistant lines of *B. rapa* carrying Rcr1, Rcr2, Crr1 to Crr4, CRa, CRb, CRc and CRk, and the European *B. napus* cultivar 'Mendel' carrying a clubroot resistance gene, were crossed with the susceptible spring type canola line DH16516. The resulting F<sub>1</sub> plants were crossed into the susceptible background to back cross generations 3 to 4 (BC<sub>3</sub> to BC<sub>4</sub>). SNP markers linked to most of the clubroot resistance genes were validated. Marker assisted selection was performed at each generation. Plants were tested with pathotypes 3H and 5X from Canada. Homozygous lines containing Rcr1 or Rcr2 in BC<sub>4</sub> were obtained. Plants carrying the other CR genes in BC<sub>3</sub> were developed. Microspore culture is being used to develop homozygous lines carrying the other CR genes.

**VA-1. Characterization of clubroot resistance in *Raphanus*.** E.Diederichsen, N. Gollinge, M. Schlathölter

*Raphanus* crops are important vegetables and break crops to control nematodes and N leakage. Despite a relatively high resistance level, clubroot affects also radish crops and even minor infections in oil radish receive public attention. Clubroot resistance (CR) in oil radish needs strong improvement to support the use of this break crop without increasing the clubroot risk for cash crops. Different sources of CR are studied in the frame of the RAPHKORE project. The known *Raphanus* CR that is effective against the prevailing pathotypes, which originate from Brassica crops, needs clarification of its inheritance and broadness. Newly collected *Plasmodiophora brassicae* isolates from oil radish crops were tested on different *Raphanus* and *B. napus* cultivars. So far, adaptation to this CR type is very seldom, and *Raphanus* cultivars usually show only few infections. One isolate has been identified showing strong virulence towards *Raphanus* CR. This isolate originates from a farm focusing on potato production with oil radish as a break crop. We used this isolate to screen *Raphanus* gene bank accessions and found a small number of resistant accessions. Resistant individuals were selected and their S1-progeny was often significantly more resistant than the original population. Future studies on the inheritance of CR from *Raphanus* will reveal the genetic potential to control clubroot in this crop.

**VA-2. Identification of clubroot resistant QTLs in radish (*Raphanus sativus* L.).** C.Gan, X.Deng, L.Cui, W.Yuan, X.Yu, Y.P.Lim, Z.Piao.

Clubroot is a devastating disease caused by *Plasmodiophora brassicae* and results in severe yield losses in cruciferous plants, including the radish (*Raphanus sativus*). We firstly constructed a high-density linkage map using an F<sub>2</sub> population derived from the crossing between a partial clubroot resistant inbred line 'BJJ' and a susceptible inbred line 'XNQ'. The map consisting of 1148 SNPs developed by restriction-site associated DNA sequencing (RAD-seq) technology and spanning 794.3 cM with an average distance of 0.7 cM between adjacent markers. Based on this high-density linkage map, we evaluated clubroot disease severity degree in F<sub>3</sub> progenies with two independent inoculation tests for QTL analysis. A total of five QTLs were identified and accounting for 5.23 to 7.65 of the LOD value, 7.26 to 31.38% of phenotypic variance. The QTL RsCr1 on LG8 and other four QTLs on LG9, of these QTLs, RsCr2 overlap with RsCr5 were detected in different tests. This high-density genetic map of radish could provide the indispensable information in genetic and genomic research and the QTL mapping studies were the reference for effective gene exploration, and for marker assisted-breeding program.

**VA-3. Development of clubroot resistant interspecific hybrids of *Brassica oleracea* × *Brassica rapa* subsp. *rapa*.** S.Zhang, S.Shen, Q.Li, X.Ren, H.Song and J.Si

To develop new clubroot resistant Brassica germplasm resources, four interspecific crosses were made using three *B. oleracea* lines D<sub>3</sub>, E<sub>4</sub> and 545 as female and two *Brassica rapa* ssp. *rapa* lines WJ-1 and WJ-2 as male and interspecific hybrid plants were obtained through the application of embryo rescue technique. While comparing compatibility of these crosses, the formation of silique per pollinated flower was about 90% higher in E<sub>4</sub> × WJ-1 as compared to the other crosses; however, survival of immature embryos per pollinated flower was higher (3.09%) in D<sub>3</sub> × WJ-1. Number of interspecific hybrid plants obtained from the four crosses D<sub>3</sub> × WJ-1, E<sub>4</sub> × WJ-1, E<sub>4</sub> × WJ-2, 545 × WJ-2 were 5, 0, 0, and 0, respectively. These plants were identified to be true hybrids based on morphological and cytological observation, and had somatic chromosome number 2n = 19 and intermediate morphology of the two parents. Pollen fertility of the five plants derived from D<sub>3</sub> × WJ-1 was almost zero; however, they showed resistance to *Plasmodiophora brassicae* race 4. Results from this study demonstrated that clubroot resistant plants can be developed through interspecific hybridization between *B. oleracea* and *B. rapa* subsp. *rapa*.

**VA-4. Transferring clubroot resistance by intergeneric hybridizations between *Brassica napus* and *Raphanus sativus*.** E. Diederichsen, N.Gollinge, J.Mader, J.Schondelmaier, K.Lohgall & M.Schlathölter

Raphanus crops are important vegetables and break crops to control nematodes and N leakage. Despite a relatively high resistance level, clubroot affects also radish crops and even minor infections in oil radish receive public attention. Clubroot resistance (CR) in oil radish needs strong improvement to support the use of this break crop without risking the increase of clubroot inoculum. The RAPHKORE project aims at characterization and improvement of CR in Raphanus and studies different sources of CR. We have discovered a *P. brassicae* isolate from oil radish that shows strong virulence towards most Raphanus cultivars. On the other side, this isolate does not infect *B. napus* cultivars that were never considered to have clubroot resistance. This could indicate that virulence towards Raphanus is gained on the costs of virulence towards these *B. napus* cultivars. Combining these resistance sources could be a promising strategy to generate broad-spectrum resistance. We report on our results of intergeneric hybridizations between *B. napus* and *R. sativus*. To increase cross efficacy a combination of ovary culture and ovule culture was applied. From 130 initial seedlings, 107 were confirmed as true hybrids using SSR markers. Clubroot tests showed the efficacy of resistance from both parents in nearly 50% of the hybrids. A backcross program has been initiated to further transfer clubroot resistance into oil radish.

VA-5. Screening of Brassica accessions for resistance to 'old' and 'new' isolates of *Plasmodiophora brassicae* in Alberta, Canada. Rudolph Fredua-Agyeman, S.F.Hwang, S.E.Strelkov, Q. Zhou, V.P.Manolii & D.Feindel.

Genetic resistance is the main tool used to manage clubroot of canola (*Brassica napus*) in Canada. However, the emergence of new virulent strains of the clubroot pathogen, *Plasmodiophora brassicae*, has complicated canola breeding efforts. In this study, 386 Brassica accessions were screened against five single-spore isolates and 17 field isolates of *P. brassicae* to identify resistance sources effective against these new strains. The results showed that one *B. rapa* accession [CDCNFG-046, mean index of disease (ID) = 3.3%] and two *B. nigra* accessions (CDCNFG-263, mean ID = 3.1%; and CDCNFG-262, mean ID = 4.7%) possessed excellent resistance to all 22 isolates evaluated. Fifty other accessions showed differential clubroot reactions (resistant, moderately resistant or susceptible), including 27 (one *B. napus*, two *B. rapa*, four *B. oleracea* and 20 *B. nigra*) accessions that were resistant to 8 - 21 *P. brassicae* isolates with mean IDs ranging from 5.3-29.6%. The remaining 23 accessions (two *B. napus*, one *B. rapa*, five *B. oleracea* and 15 *B. nigra*) were each resistant to 3 - 13 isolates, but developed mean IDs in the range of 30.3-47.0%. The three accessions which showed absolute resistance and the 50 accessions which showed differential clubroot reactions could be used to breed for resistance to the new *P. brassicae* strains.

## GENOMICS

KS-4. Keynote Speaker: Molecular genetics of the clubroot resistant genes in Chinese cabbage (*Brassica rapa* L.). Dr. Katsunori Hatakeyama, Iwate University, Japan.

In *Brassica rapa*, at least 11 clubroot resistance (CR) loci have been identified and two of them successfully cloned. However, it remains to be uncertainties about the precise locations of the part of CR loci and the relationships between the CR loci and pathotypes of the clubroot pathogen. Therefore it is necessary to clone CR genes and evaluate their pathotype specificity. Recently we cloned and sequenced the genomic region of the CRb (CRb<sup>Kato</sup>) locus identified in the cultivar 'Akiriso' and demonstrated that this is a complex locus composed of at least six R genes in tandem with the same orientation and the gene with the CRb specificity and CRa were the same CR allele. Map-based cloning of the responsible genes for CRk and Crr1b is currently underway. Fine mapping of the CRk locus identified on *B. rapa* chromosome A03 revealed that two resistant loci were speculated to exist in the candidate region and show different pathotype specificity. By sequencing of three BAC clones covering the Crr1b, the candidate region was delimited to about 55 kb in the resistant line and at least four ORFs were speculated. Cloning of CR genes will contribute to improve the pathotype classification system and marker-assisted selection on CR breeding.

**KS-5. Keynote Speaker: Development of a highly clubroot-resistant F1 cultivar Chinese cabbage (*Brassica rapa* L.) accumulating three resistance genes, Crr1, Crr2 and CRb.** Dr. Satoru Matsumoto, *Institute of Vegetable and Floriculture Science, NARO Division of Vegetable Breeding, Japan.*

Clubroot-resistance (CR) Chinese cabbage cultivars play an important role in controlling the disease. However, the breakdown of the resistance of CR cultivars caused by the genetic variability of the pathogen has been observed. Four pathotypes (group 1 to group 4) have been identified in Japanese field isolates by using two commercial CR F<sub>1</sub> cultivars of Chinese cabbage as differential hosts. 'Parental line No.9' (PL9) carries CR loci, Crr1 (Crr1a and Crr1b) and Crr2, which were introduced from genetic resource 'G004' derived from European fodder turnip 'Siloga' and shows high resistance to clubroot isolates from pathotype group 1 which is pathogenic to most of Japanese Chinese cabbage F<sub>1</sub> cultivars, group 2 and group 4, but not group 3. The parents of F<sub>1</sub> hybrid 'Akiriso', T-line and V-line, were crossed with PL9, respectively. T-line harboring another CR gene, CRb (CRb<sup>Kato</sup>), confers resistance to pathotype group 3. The progenies were selected by DNA markers linked to Crr1 and Crr2, and backcrossed with each parent. Since CRb<sup>Kato</sup> is a dominant resistant gene, F<sub>1</sub> hybrid between the new parental lines showed highly resistant to all isolates from pathotype group 1 to group 4. CR breeding with DNA markers is also used for other vegetables and rapeseed (*Brassica rapa* and *B. napus*).

**VA-6. Gene similarity of *Plasmodiophora brassicae* collections from Canada.** A.Sedaghatkish, B.D.Gossen, M.R. McDonald.

Clubroot, caused by *Plasmodiophora brassicae* Wor., is generally managed using resistant cultivars, but new, virulent pathotypes are increasing rapidly on canola (*Brassica napus* L.) in Canada. Information on genetic similarity among pathogen populations could inform the development of sustainable management approaches. The objective of this study was to develop whole-genome sequences of pathogen collections from across Canada, assess their genetic similarity, and compare with collections from the USA, China and Europe. In total, 52 single-spore and field collections from clubbed roots were increased on the highly susceptible 'Mei Qing Choi' (*Brassica rapa* var. *chinensis*). DNA was extracted from clubs and *P. brassicae*-induced callus cultures. Sequencing reads were mapped to the published genome of *P. brassicae* isolate e3 (from Europe) to remove plant DNA sequences. A phylogenetic tree prepared using R software clustered the collections into five clades. The collections from Canada were separated into four clades: one from the Prairie provinces, one of new pathotypes from Alberta, and two clades from eastern / central Canada plus British Columbia. The collections from China formed the fifth clade. There was some overlap among clades. The similarity between samples collected from a site before and after a change of pathotype was generally low. Collections from the USA grouped with eastern / central Canada. This indicated that collections across much of North America differed from the initial collections in Alberta, which in turn differed from the new pathotypes.

**VA-7. Transcriptome changes in *Brassica napus* cultivars upon interaction with *Plasmodiophora brassicae* pathotype 5X.** L.Galindo-González, S.F.Hwang & S.E.Strelkov.

With over 2,700 fields of canola (*Brassica napus* L.) infested by *Plasmodiophora brassicae* Wor. in Alberta, clubroot disease has become a major concern. Resistant cultivars are used to mitigate the impact of the pathogen, but this resistance was first broken in 2013 by a novel variant of pathotype 5 (Williams differentials) known as pathotype 5X. RNA-seq was used to identify key differentially expressed genes (DEGs) over a time-course in two *B. napus* cultivars with contrasting responses to this pathotype. At 7 days after inoculation (dai), the number of DEGs in 'Laurentian' (resistant cultivar) was much greater than in 'Brutor' (susceptible cultivar), and most genes were upregulated. This pattern was inverted at 14 and 21 dai. At 7 dai, 'Laurentian' activated more genes related to stress responses (dehydration, wounding, heat shock proteins), protein turnover and signalling compared with 'Brutor'. At 14 dai the cultivars experienced a transition, with less DEGs but with some key genes (defense, hormones, cell wall, transcription factors) highly upregulated. Finally, at 21 dai, a strong downregulation in most genes was observed in 'Brutor', while 'Laurentian' still showed activity in numerous biotic stress-related genes. Candidate genes that can be modified by gene editing will be identified through this analysis to assess their roles in defense.

**VA-8. Genetic analysis of clubroot resistance using multiple populations in *Brassica rapa*.** Y.P. Lim, S.R.Choi & S.Park.

Clubroot disease is one of the most serious soil contagious diseases caused by *Plasmodiophora brassicae* in Brassicaceae crops. Traditional QTL mapping used to map with bi-parental mapping population and can find significant locus. In comparatively, a Genome-Wide Association Study (GWAS) is an approach that involves scanning genetic loci associated with traits across natural population and then might be screened out all of candidate loci contain in populations. In this study, we joint QTLs and association-mapping approach using 232 inbred lines with 3 years replications. As a result, association SNP markers identified. Most of these were located in chromosome A2 and A3 already known and several new candidate loci identified in A6 and A9. This study may help as basic information for the further study to identification of clubroot resistance or defense-associated genes, and as a marker-assisted selection for breeding.

## HOST-PATHOGEN INTERACTIONS

**KS-6. Keynote Speaker: Using knowledge on plant hormone metabolism by *Plasmodiophora brassicae* – a possibility to control the clubroot pathogen.** Professor Dr. Jutta Ludwig-Mueller, *Technische Universität Dresden, Germany*

The clubroot disease symptoms of Brassicaceae, caused by the soilborne obligate biotrophic pathogen *Plasmodiophora brassicae*, are determined by the modulation of plant hormones such as auxins and cytokinins inducing hypertrophies and metabolic sinks in root galls, but also defense hormones are altered. *Arabidopsis thaliana* is used as a model host to understand the molecular biology underlying these processes. The genome sequence of *P. brassicae* has opened up novel approaches to study the protist. We could identify several genes encoding putative plant hormone metabolizing enzymes, such as a SABATH-type methyltransferase, but also a GH3-family protein involved in the conjugation and thereby inactivation of indole-3-acetic and jasmonic acids. Further, a functional cytokinin oxidase was found. We have studied the question why the treatment of host plants with the defense compound salicylic acid (SA) did not result in increased tolerance and found that the methyltransferase is able to methylate mainly SA and to a lesser extent benzoic and anthranilic acids. Overexpression of this gene, PbBSMT, in *Arabidopsis* resulted in more susceptible plants to the clubroot pathogen. Selected mutants with higher constitutive levels of SA, however, were more tolerant to *P. brassicae*. The possible roles for the other plant hormone metabolizing enzymes will be discussed.

**KS-7. Keynote Speaker: The effect and durability of incomplete resistance, based on two clubroot resistance genes, against a new pathotype of *Plasmodiophora brassicae*.** Dr. Gary Peng, *Agriculture and Agri-Food Canada, Canada*.

Single-gene resistance against clubroot (*Plasmodiophora brassicae* Woronin) is often short lived. For example, in Canada almost all monogenic resistant canola (*Brassica napus* L) cultivars became susceptible to new pathotypes of *P. brassicae* only 3-4 years after introduction. The objectives of this study were to examine root infection in new canola hybrids carrying two clubroot resistance (CR) genes (A3, A8) that showed incomplete (moderate) resistance to the pathotype X of *P. brassicae*. The durability of resistance was also assessed by inoculating two of double CR-gene hybrids with the inoculum of a field pathotype X population from the prior planting cycle over five generational cycles (6 weeks per cycle) under controlled environment. Root infection was examined using fluorescent microscopy. At 10 and 35 days post inoculation (dpi), plasmodia, zoosporangia and/or resting spores were seen in both resistant and susceptible roots, but fewer were found in resistant roots, especially in cortical tissues at 35 dpi. Enhanced lignin autofluorescence was observed in the cell wall of resistant roots. In repeated resistance durability tests, harvested galls were let mature in damp soil for 3 weeks before being mixed thoroughly in recycled growth media. Resting spores in the media were quantified using qPCR just before each planting. After being exposed to the same pathotype population for five cycles, the two double CR-gene hybrids maintained the moderate level of resistance. The inoculum concentration increased slightly in the media of susceptible canola lines (controls), but decreased slightly in the media of resistant hybrids during the 5 generational cycles.



**VA-9. Proto-oncogenes in a eukaryotic unicellular organism play essential roles in plasmodial growth in host cells.** K.Bi, T.Chen, Z.C.He, Z.X.Gao, Y.Zhao, Y.P.Fu, J.S.Cheng, J.T.Xie & D.H.Jiang.

It is of great value to understand the interaction of *Plasmodiophora brassicae* and the host at the early infection stage. In this research we constructed a hydroponic rapeseed inoculation method and inoculated the rapeseed with resting spores of *P. brassicae*. The spores were collected from the roots and total proteins were extracted 48 hours after inoculation, and then the proteins were analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Proteins were annotated using mascot search engine (Matrix Science, London, UK; version 2.3.02) against database containing genome sequences of *P. brassicae*. Total 860 proteins were identified in the treatment (spores collected from inoculated rapeseed roots) and 1237 proteins in the control (resting spores). Two hundred and seven unique proteins were found only in the treatment, among which 11 proteins were predicted as secretory proteins. From the whole genome, 865 putative secretory proteins were predicted, and combined with transcriptome data, 23 were chosen for further research. Expressing the genes by TRV in tobacco, we found gene 01668 and 08125 could induce strong HR and gene 08307 had slight HR. Nine genes have been overexpressed in *Arabidopsis thaliana*. The study on the secreted proteins will help us to clarify the interactions between rapeseed and *P. brassicae*.

**VA-10. Infection of canola with *Plasmodiophora brassicae* increases resistance to herbivory by Bertha armyworm, *Mamestra configurata*.** C.D.S.Weeraddana, V.P,Manolii, S.E.Strelkov, A.P. de la Mara, J.J.Harynuk & M.L.Evenden.

Canola is an economically important crop grown in Canada. In the canola fields of the Canadian Prairies, clubroot disease, caused by a soil borne protist *Plasmodiophora brassicae* Woronin, has emerged as an important economic pest that impacts canola production. Bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore which is a significant insect pest of canola in Canada. Both *P. brassicae* infection and BAW infestation occur in the agroecosystems of Alberta canola fields, thus it is important to study the potential interaction between *P. brassicae* infected plants and BAW to properly manage both threats. The effect of *P. brassicae* inoculation on subsequent BAW oviposition and offspring performance was tested using *P. brassicae* susceptible and resistant canola. Fewer eggs were laid on susceptible canola inoculated with *P. brassicae* when compared to uninoculated susceptible canola, however, a similar number of eggs was laid on inoculated and non-inoculated resistant canola. Inoculation with *P. brassicae* influenced larval development as pupae weighed more when reared on non-inoculated as compared to inoculated susceptible canola. Higher levels of salicylic acid were found in *P. brassicae* inoculated susceptible canola as compared to non-inoculated canola. Analysis of the volatile organic compounds (VOCs) released from the variously treated plants showed that *P. brassicae*-inoculated plants emit a different VOC profile than uninoculated plants.

**VA-11. Screening important secreted proteins during the process in *Brassica napus* infected by *Plasmodiophora brassicae*.** Z.X.Gao, Y.Zhao, K.Bi, J.T.Xie, J.S.Cheng. Y.P.Fu & D.H.Jiang.

The eukaryotic unicellular protist *Plasmodiophora brassicae* is an endocellular parasite of cruciferous plants. In host cortical cells, this protist develops a unicellular structure that is termed the plasmodium. The plasmodium is actually a multinucleated cell, which subsequently splits and forms resting spores. The mechanism for the growth of this endocellular parasite in host cell is unclear. Here, combined de novo genome sequence and transcriptome analysis of *P. brassicae* strain ZJ-1 the top five significant enriched KEGG pathways of differentially expressed genes as Translation, Cell growth and death, Cell communication, Cell motility and Cancers were identified. We detected 171 proto-oncogenes from the genome of *P. brassicae* that were implicated in cancer-related pathways. Three predicted proto-oncogenes (Pb-Raf1, Pb-Raf2, and Pb-MYB), which showed homology to the human proto-oncogenes Raf and MYB, were specifically activated during the plasmodial growth in host cortical cells, demonstrating their involvement in the multinucleate development stage of the unicellular protist organism. Gene networks involved in the tumorigenic-related signaling transduction pathways and the activation of 12 core genes were identified. Inhibition of phosphoinositol-3-kinase relieved the clubroot symptom and significantly suppressed the development process of plasmodia. The results suggest that proto-oncogene-related regulatory mechanisms play an important role in the plasmodial growth of *P. brassicae*.

## **EPIDEMIOLOGY & DISEASE MANAGEMENT**

**KS-8. Keynote Speaker: The changing face of clubroot (*Plasmodiophora brassicae*) in Canada.** Dr. Stephen E. Strelkov, *University of Alberta, Canada*.

Clubroot, caused by *Plasmodiophora brassicae* Wor., has become an important disease of canola (*Brassica napus* L.) in western Canada. Despite efforts to slow its spread, the number of confirmed *P. brassicae* field infestations has increased from just 12 in 2003 to more than 2,700 by 2017. Management of clubroot relies mainly on the planting of clubroot resistant (CR) canola cultivars. Unfortunately, populations of *P. brassicae* capable of overcoming the resistance in most cultivars have been identified with increasing frequency since 2013. At present, the erosion or loss of resistance has been documented in 104 fields. Characterization of pathogen field isolates on the hosts of the Canadian Clubroot Differential (CCD) Set revealed the occurrence of 17 distinct pathotypes of *P. brassicae* in Canada, most of which are virulent on CR canola. These include pathotype 5X, which represents the first field isolates found to overcome resistance, and pathotype 3A, which is predominant among all collections examined from CR canola. An evaluation of genetic diversity within *P. brassicae* by restriction site-associated DNA sequencing showed that pathotype 5X is distinct from older pathotypes that do not cause clubroot on resistant canola. Testing of root galls using quantitative PCR assays developed to distinguish *P. brassicae* populations that are virulent or avirulent on CR canola indicated that virulent strains of the pathogen were present at a low frequency prior to the introduction of clubroot resistance. Collectively, these results indicate that the canola crop is still at risk from *P. brassicae* in Canada.

**KS-9. Keynote Speaker: Management of clubroot in canola in Alberta, Canada.** Dr. Sheau-Fang Hwang, *Alberta Agriculture Forestry, Canada*.

*Plasmodiophora brassicae*, the causal agent of clubroot of crucifers, poses a serious threat to Canadian canola production. Over the past 15 years, it has spread from 12 fields in central Alberta to over 2,700 fields across the province. The concentration of *P. brassicae* resting spores in the soil is a major determinant of clubroot severity, particularly for the new pathotypes. Control strategies have focused on reducing viable spore concentrations in the soil by rotating canola with non-host crops, planting bait crops, deploying resistant canola cultivars, and applying soil fumigants. Both non-host and bait crops reduced clubroot severity in bioassays relative to fallow treatments. A 2-3 year interval between canola crops reduced disease severity compared with a 1-year interval or no break between canola crops. Several 4-year rotation sequences with resistant canola cultivars reduced the severity of clubroot compared with continuous canola, although a 4-year non-host or fallow sequence reduced clubroot incidence to zero. Application of metam sodium or dazomet fumigants reduced clubroot severity and soil resting spore concentrations over a range of application rates (0.4–1.6 mL L<sup>-1</sup> soil), especially when applied into moist soil and incorporated with a rototiller. Covering the soil with a plastic moisture barrier after application enhanced fumigant efficacy.

**VA-12. Epidemiology of clubroot disease and pathogenic variation among isolates of *Plasmodiophora brassicae* from oilseed rape growing in Europe.** N.Zamani-Noor, E.Diederichsen, A.C.Wallenhammar, G.Cordsen-Nielsen, G.Orgeur, V.Konradová, F.Dissart, J.Smith & M.Jedryczka.

Clubroot, caused by *Plasmodiophora brassicae*, is one of the most destructive diseases in oilseed rape (OSR) cultivation and has become increasingly important in central Europe. The disease has been monitored by collaborators through field surveys in Czech Republic, Denmark, France, England and Scotland, Germany, Poland and Sweden. Infected plants and soil samples were collected randomly from clubroot-infested fields in different countries. Location, soil type, soil pH, plant genotype and rotation regime were recorded for each field. The presence of *P. brassicae*-resting spores in the soil was assessed by standard bioassays. Pathotype classification of the *P. brassicae*-populations was conducted on two or three known differential sets. Additionally, the degree of virulence of the collected isolates was analysed on the clubroot-resistant OSR cv. Mendel. Clubroot monitoring revealed that the disease occurred in different regions in all stated countries with different intensities. Especially it was a major issue when OSR is grown in a 2 or 3-year rotation which could lead to a rapid increase in clubroot severity. A slight significant negative correlation was found between soil pH and the disease incidence of infested fields. Variation in pathotype distributions was observed in different countries. In Czech Republic and Poland, there were nine pathotypes according to the evaluation system by Williams, four pathotypes based on Somé and 15 with the ECD set. In Germany, five pathotypes were found according to Somé and 20 virulence patterns according to the ECD set. In France, six pathotypes were classified according to the set of Somé. Although the population of *P. brassicae* appears to be very diverse in the UK, three pathotypes were found to be dominant when tested on the ECD set. In Sweden pathotypes were earlier evaluated according to Williams and four pathotypes were found. In 2010 a range of isolates originating from different Brassica species were evaluated by ECD in which different virulence patterns was observed. From all populations tested for virulence on cv. Mendel, several isolates were found to be moderately or highly virulent. These virulent populations were not restricted to a small geographical area in different countries.

**VA-13. Clubroot status in Colombia.** A.Botero-Ramirez, F.L. Padilla-Huertas, L.Tarazona, J.C.Gómez, J.S.Navas & C.García.

Clubroot, caused by *Plasmodiophora brassicae* Woron, is a major disease in Colombia. This first nationwide survey showed that the disease is spread along the main cruciferous productive areas with exception of the southernmost part of the country, with prevalence ranging from 29.4% to 88.9%. Under Colombian conditions, disease occurrence is favored by high contents in soil of calcium, boron, phosphorus and copper. Given the disease importance, yield losses, spread sources and in-field distribution of the pathogen have been evaluated to better understand its behavior in the country. When yield losses were evaluated, inoculum densities of  $10^3$  and  $10^6$  resting spores per gram of soil reduced yield by 43.9% and 74.5% in cabbage, 42.5% and 61.2% in broccoli, 33.3% and 70.1% in cauliflower. Regarding disease spread among fields, open irrigation systems can be a source of *P. brassicae* viable inoculum, however infection risks have not been assessed. A patchy distribution was observed, accompanied by a reduction in inoculum density as depth increased. Status of clubroot disease in Colombia shows that more efforts are required to better understand the pathogen behavior and provide effective solutions for its management in the short and long term.

#### VA-14. Impact, management and control of clubroot disease in the UK. F.Burnett & J.Smith.

The UK has a long history of vegetable crop production and of crop losses to clubroot, caused by *Plasmodiophora brassicae*. The introduction on oilseed rape, and its intensive inclusion in arable rotation has exacerbated this soil-borne problem. UK survey work shows that over 50% of arable soils in the UK are infested with clubroot. The practice of testing soils and selecting negative field is popular with growers but has proved unsustainable in the long term. In areas of intensive production clean fields are a diminishing resource and for arable farmers moving oilseed rape production to clean land is not practical as infected fields have to be managed within the individual farming units concerned. In this latter example, the use of resistant varieties of oilseed rape has been the preferred strategy. Historically in the UK these have been lower yielding and only deployed where infection levels are high enough to justify their use. Trial results show that in fields where resistance has not been deployed before it is highly effective but in fields where it has been previously deployed 2-3 times, efficacy has been eroded through the selection of pathotypes virulent against the only available resistance mechanism 'Mendel'. Yield loss measurements in susceptible and resistant varieties are similar at comparable infection levels. The use of soil amendments is also practiced with some efficacy in vegetable crop production but their use in oilseed rape crops has proved less effective and is seldom cost effective. Longer rotations are helpful but as oilseed rape is the most profitable crop after wheat in arable rotations as well as a useful break crop, rotations of one susceptible crop in three (or less) are common. Field mapping shows useful information on the patchy nature of the disease and the difficulties this poses in terms of setting economic decision thresholds for fields and offers some potential going forward that available solutions could be targeted to infected areas. Significant knowledge gaps exist, particularly on the long term cost benefits of management decisions, the resolution of which could inform long term rotational and treatment decisions in fields and would also inform higher level policy strategies on optimal, sustainable management of clubroot.

## DISEASE MANAGEMENT

**KS-10. Keynote Speaker: Occurrence of *Plasmodiophora brassicae* in agricultural soils, pathotype variation and means of clubroot control in Poland.** Dr. Malgosia Jedryczka, *Institute of Plant Genetics, Polish Academy of Sciences, Poland.*

*Plasmodiophora brassicae* occurs in agricultural soils as well as in low and high peat bogs exploited for the production of vegetables and ornamentals. The primary inoculum attacks several crops, but the secondary inoculum is limited to the plants of Brassicaceae family, causing the disease referred to as clubroot. Oilseed rape covers the highest acreage of Brassica crops and most of current cultivars of oilseed rape are susceptible to the disease. The disease occurrence has been monitored in Poland for the last several years and we have found clubroot in most regions of intensive cultivation of oilseed rape. The correlation between soil pH and the concentration of *P. brassicae* resting spores in the soil samples was weakly negative (-0.495). No spores were detected in three out of four soils of pH equal to or below pH 6.8, but a substantial amount of spores ( $4.7 \times 10^6$  resting spores/g soil) has been also found in the soil of pH 6.9. Moreover, the spores were also detected in soils of pH up to 7.6. Taking into account the longevity of resting spores lifespan and their high resistance to chemical treatments, a diagnosis of a level of *P. brassicae* infestation in soil before cultivation is one of the most effective way to avoid yield losses. Due to fast multiplication of the spores in brassica growing fields, early detection of the pathogen in soil allows to control the disease by avoiding the cultivation of susceptible crops what counteracts further spread of the disease. Therefore, our activities concentrated on the detection of the pathogen in plants, soil and small water reservoirs in fields of oilseed rape and agricultural soils in general. The detection was done using biotests as well as molecular tools, including PCR, Real-time PCR and LAMP techniques. Depending on the evaluation system we have detected up to nine pathotypes of *P. brassicae*. The presentation will cover differences between the pathotype evaluation in Europe and Canada. The list of cultivars of oilseed rape officially registered in Poland as resistant or tolerant to clubroot will be presented, as well as other ways and trials of farmers to combat the disease. We will also show short overview of the situation of clubroot on oilseed rape and vegetable brassicas in East Europe.

**VA-15. Resting spores of clubroot detection service by LAMP method in Japan.** K.Wakayama, T.Usui, M.Okada, Y.Kawahara.

*Plasmodiophora brassicae*, the cause of clubroot, is a serious disease of cabbage, Chinese cabbage, broccoli, and of other crops in Brassica family in Japan. If this pathogen becomes established in the soil throughout a field, serious yield loss can occur. Pesticides exclusively for clubroot of more than 3 billion yen/year are used for control in Japan. The aim of this work was to social implement fast and reliable screening method to detect *P. brassicae*. By quantifying the clubroot in the soil, we eliminate unnecessary control, propose efficient control as to what kind of control is appropriate. If necessary, and create a contamination map, it is to prevent spread of pollution before it happens. To achieve the Loop-mediated isothermal DNA amplification (LAMP) technique has been selected. The detection with LAMP proved its usefulness; it was easy, fast and accurate. Measurement of clubroot is conducted at reasonable cost, applying LAMP protocol capable of detecting and quantifying *P. brassicae*. After sample arrival, the analysis result will be replied within about one week. The detection limit was 1,000 spores/g soil, so LAMP was the same sensitive as quantitative PCR tests reported. The method is simple, so it is a good alternative, when it comes to practical use and the assessment of numerous samples.

**VA-16. Molecular detection of *Plasmodiophora brassicae* the causal agent of clubroot of cruciferous crops.** G.G.Guan, W.X.Pang, Z.Y.Piao & Y.Liang.

Clubroot caused by *Plasmodiophora brassicae* is one of the most destructive diseases on cruciferous crops. The pathogen produces numerous resting spores that can remain pathogenicity in soil for years. Pathogen dissemination and causing epidemics were mainly through the movement of resting spores on seed, diseased plants, contaminated soil and farm equipment. In this study, a pair of PCR primers was designed according to the internal transcribed spacer (ITS) sequence (GenBank accession xxx). This primer pair could be used in end-point PCR and quantitative PCR (qPCR). Specificity assay indicated that this primer pair could produce an amplicon against DNA extracted from *P. brassicae* but not from other plant pathogenic fungi, bacteria and nematodes as well as the endophytic bacteria isolated from the clubbed root. Sensitivity tests indicated that the end-point PCR could amplify a product from  $1 \times 10^{-6}$  ng of total *P. brassicae* DNA and DNA derived from  $1 \times 10^3$  resting spores. On the other hand, the sensitive levels of qPCR were  $10^{-9}$  ng of total *P. brassicae* DNA and 10 resting spores. The study provided an accurate and reliable method for detection of *P. brassicae* in host plants, soil and seed samples, which will be useful to forecast clubroot on cruciferous crops.

**VA-17. Evaluation of various soil amendments to manage clubroot on canola in field condition. V.Chapara.**

Clubroot of canola (*Brassica napus* L.), caused by *Plasmodiophora brassicae* W., is a primitive living organism with characteristics of fungi, plasmodium and slime mold. These characteristics make the pathogen difficult to control with any pesticide group. Research indicates that soil amendments that increase soil pH show significant control of clubroot. A field study was conducted to evaluate the efficiency of amendments that alter soil pH, plant defense mechanisms and fungicidal properties against clubroot pathogen. The experimental design was a randomized complete block design with four replications. Treatments of wood ash, pelletized lime, beet lime and gypsum were applied seven days before planting. Likewise, cyazofamid, fluazinam, penta chloro nitro benzene, zinc nano-particle, and a non-ionic surfactant were applied just prior to planting. The treatments were mixed thoroughly into soil with a rototiller to a four inch depth after each application. A clubroot rating scale of 0-3 described by Strelkov was used. Soil samples were collected before and after the application of treatment for pH assessments. Clubroot incidence and severity were significantly lower in the wood ash treatment followed by beet lime and pellet lime compared to the other treatments tested. Likewise, increase of soil pH from acidity to alkalinity in the treatments amended with wood ash, beet lime and pelletized lime was observed.



## AGRONOMY PART I

**KS-11. Keynote Speaker: Management of clubroot: an overview of the challenges.** Dr. Mary-Ruth McDonald, University of Guelph, Canada & Bruce Gossen, Agriculture and Agri-Food Canada, Canada.

Management of clubroot [*Plasmodiophora brassicae* Wor.] is a challenge worldwide, wherever brassica crops are grown. Major gene resistance has been an effective short-term strategy, but is not sustainable because of the high risk of erosion of resistance. To reduce this risk, populations of resting spores in soil must be monitored and kept low. However, spore populations in soil are difficult to quantify. Molecular approaches have been developed to quantify total or viable resting spores in soil, but these cannot reduce the high levels of variability that are present both horizontally and vertically within the soil profile. For example, resting spore concentration ranged from  $10^3$  to  $10^6$  within a 0.4 ha spot in a heavily infested field. Also, spore levels in heavily infested fields can be so high ( $> 10^7$ ) that a reduction of 99% would leave enough spores to cause severe clubroot, which makes assessment of management strategies more difficult. Management strategies need be used in combination to be effective. These include rotation and / or stacking of major resistance genes and quantitative resistance genes, in combination with crop rotation, seeding into cool soil (5-14 °C), soil amendments with lime, boron or calcium cyanamide, solarization, fumigation, and use of non-host crops to stimulate the germination of resting spores. None of these approaches are effective on their own, so effective combinations must be identified that are economical and sustainable alternatives for producers.

**VA-18. The influence of clubroot resistant canola on resting spore levels in the soil.** T.W.Ernst, S.V.Kher, D.Stanton, D.C.Rennie, S.F.Hwang & S.E.Strelkov.

*Plasmodiophora brassicae* Wor. causes clubroot, an important soilborne disease of canola (*Brassica napus* L.) and other crucifers. The pathogen produces large numbers of resting spores, which can remain viable in the soil for many years. Clubroot is managed mainly by the planting of clubroot resistant (CR) canola cultivars. This study examined the impact of the cultivation of CR canola on *P. brassicae* resting spore concentrations in commercial cropping systems in Alberta, Canada. Resting spore concentrations were monitored by quantitative (q)PCR analysis of soil samples collected pre-seeding and post-harvest, at multiple geo-referenced locations within 17 *P. brassicae*-infested fields, over periods of up to 4 years. Resting spore concentrations were measured by quantitative PCR analysis. A subset of samples also was evaluated in greenhouse bioassays with a susceptible host. The cultivation of CR canola in soil with quantifiable levels of *P. brassicae* DNA resulted in increased inoculum levels. There was a lag in the release of inoculum after harvest, with quantifiable *P. brassicae* inoculum peaking in the year following CR canola cultivation. Rotations that included a  $\geq 2$ -year break from host plants resulted in significant declines in *P. brassicae* resting spore concentrations. A strong positive relationship was found between the bioassays and qPCR-based estimates of soil infestation. The results suggest that CR canola should not be used to reduce soil inoculum loads, and crop rotations in clubroot-infested fields should include breaks of at least 2 years away from *B. napus*

**VA-19. Suppression of *Plasmodiophora brassicae* in infested oilseed rape farms by soil amendment with calcium cyanamide and burnt lime. N.Zamani-Noor.**

Clubroot, caused by *Plasmodiophora brassicae*, is an important disease of oilseed rape (OSR), causing serious losses in Germany. The detection of 124 new *P. brassicae*-infested fields during 2013-2017 across several federal states in Germany suggests that clubroot disease maybe more widespread in oilseed rape fields than previously thought. At the present study, calcium cyanamide and burnt lime used with cultivar resistance were evaluated for their potential in suppression of clubroot. Multifactorial field trials with natural infection were conducted on three locations in Germany from 2014 to 2016. The plots consisted of two OSR cultivars differing in their levels of resistance to clubroot and subplots of two soil amendments which were applied at different time points. Calcium cyanamide (300kg/ha; 50% calcium oxide) and burnt lime (1500kg/ha) were distributed evenly to the soil surface one day prior to the sowing or when the OSR had reached the growth stage 11-12. Soil's moisture, temperature and pH at two depths were measured at regular intervals over the growing season. Clubroot incidence and severity were assessed visually for the development of root galls. The results showed that the incidence and severity of clubroot disease varied across locations and years. The most severe disease was observed in all locations in 2014 in which the clubroot-resistant OSR also showed strong infection in one field. In other two fields, the resistant cultivar provided up to 90% disease control. In general, a slight increase in soil pH, about 0.2-0.5 unit higher than the natural soil suspension pH, was observed after application of calcium cyanamide or burnt lime. However, some days later the soil pH decreased again and was as equal as control plots. Changing the time of fertilizer's application had a significant effect on the final severity of the disease. Relative to untreated controls, clubroot incidence and severity were decreased by application of fertilizers at later growth stages. In comparison with calcium cyanamide, burnt lime application has a smaller effect. Nearly 30% yield losses were recorded in susceptible cultivar in non-treated plots in compare to the treated ones.

## **AGRONOMY PART II**

**VA-20. Lime and limestone production, products and trends in history – a mining company's perspective. A.Nguyen & B.Anderson.**

This presentation will discuss lime and limestone products, outline how lime and limestone are mined and manufactured, the properties, characteristics and differences between lime (both quick lime and hydrated lime) and limestone as they relate to adjusting pH in soils. There will also be information on current uses of lime and limestone products such as in other agricultural applications.

The presentation will also describe the efficiency factor of various limestone size fractions, the effective neutralizing value (ENV) and the calcium carbonate ( $\text{CaCO}_3$ ) equivalent (CCE) as it relates to pH adjustment in soils. It will address some common misunderstandings of lime and limestone as it pertains to the agricultural community such as nomenclature and chemical specifications.

There will be some discussion on the research work Graymont does independently and with partners to better understand the needs and requirements of the industry.

**VA-21. Assessment of hydrated lime for the management of clubroot in canola.** Nicole M. Fox, S.F.Hwang, V.P.Manolii, G.Turnbull & S.E.Strelkov.

Clubroot, caused by *Plasmodiophora brassicae* Wor., is a soil-borne disease that has become a constraint to canola (*Brassica napus* L.) production in Alberta, Canada. The disease is managed primarily by the planting of clubroot resistant cultivars, but resistance has been overcome in >100 fields in the province. Clubroot development is favoured in acidic soils; therefore, increasing soil pH may reduce disease severity in infested soils and serve as another management tool. The efficacy of hydrated lime products in reducing clubroot was assessed in replicated field plot experiments in central Alberta in 2017. The addition of moderate to high rates of hydrated lime significantly reduced clubroot severity and increased above-ground biomass in a susceptible canola cultivar at 8 weeks after planting. At the highest application rate, lime treatment reduced the clubroot disease severity index by 35-91%, while increasing above-ground plant biomass by 58-116%. The field trials are being repeated in 2018. A greenhouse study also was conducted to assess the efficacy of hydrated lime in reducing clubroot severity in susceptible and moderately resistant canola cultivars, under different application rates and concentrations of inoculum. In the control treatments at all inoculum levels, the susceptible canola developed severe clubroot (92-100%) while the moderately resistant canola developed mild clubroot (9-13%). In contrast, neither cultivar developed visible symptoms of clubroot when treated with four rates of hydrated lime. Quantitative PCR analysis is underway to measure the impact of the treatments on *P. brassicae* inoculum levels in the soil and proliferation in host tissues.

**VA-22. A recipe for managing small patches of infestation of clubroot in canola.** B.D.Gossen, A.Sedaghatkish, S.F.Hwang & M.R,McDonald.

Clubroot of canola (*Brassica napus*), caused by *Plasmodiophora brassicae* (Wor.), is spreading rapidly on the Canadian prairies. Genetic resistance can be extremely effective against clubroot but breaks down quickly under high disease pressure. A recipe for treating small areas of infestation has been proposed, as follows: mark the area ( $\geq 2\times$  the area where symptoms occurred), apply lime (quick lime for rapid effect + standard lime for longer-term maintenance) to increase the soil pH to  $\sim 7.4$ , then seed a perennial grass crop. pH above 7.2 suppresses clubroot and grass crops such as perennial ryegrass (*Lolium perenne*) and smooth brome grass (*Bromus inermis*) further reduced resting spores in soil. A grass cover also minimises the movement of spores from the treated area. Soil samples from the centre of the patch are used to determine when the resting spore levels drop below economic thresholds. Alternatives for reducing resting spore populations are solarisation or fumigation. Solarisation, achieved by covering the patch with totally impermeable film (TIF) for 16 days, increased mean soil temperatures by about 10 °C and reduced clubroot severity to 0% in 2016, but in 2017 only reduced severity from 81% to 35%. Addition of fumigants (chloropicrin or metam sodium) did not further reduce clubroot severity, but each was also effective on its own. However, application of fumigants requires specialized equipment and licences, and so is not available to most producers on the Canadian prairies. Crop rotation can also be effective, but takes longer.

**VA-23. Integrated management of clubroot – crucial for a sustainable oilseed rape production.**  
A.C.Wallenhammar, Z.Omer & A.Jonsson.

Clubroot is a serious threat to OSR production in Sweden and genetic resistance is the most important factor in a cropping strategy. A new project aiming at developing a concept for integrated production of winter OSR supported by DNA technology started in 2017. The objective is to provide an improved decision support and guidelines for growing winter OSR in fields where *Plasmodiophora brassicae* DNA occurs. Infestation levels and yield of clubroot resistant (CR) and susceptible cultivars grown in field trials with different infection levels of *P. brassicae* are investigated at four sites established in 2017 in south and central Sweden. Three CR resistant cultivars of winter OSR were selected together with a mixture of susceptible cultivars. The selected fields showed *P. brassicae* at levels ranging from 5000 to 2.5 million target copies g<sup>-1</sup> soil at presampling in July. Soil samples were then collected plotwise immediately prior to seeding the trials. Quantification of *P. brassicae* by qPCR was performed and bioassays were carried out to ensure optimal infection of the tested cultivars. Plants were sampled plot-wise in late autumn and roots were assessed for disease symptoms. Preliminary results will be discussed as soil DNA-analyses will be correlated with disease severity and yield.

**Panel Presentation – “*Opportunities that will deliver value*”** Moderator: Jay Whetter, *Canola Council of Canada, Canada*

## POSTER ABSTRACTS (PA)

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**PA-1. Introgression of clubroot resistance and analyses of segregation distortion in two F<sub>2</sub> populations derived from *Brassica rapa* subsp. *rapifera* (ECD 02).** J. Jiang, R. Fredua-Agyeman, S.F Hwang & S.E. Strelkov.

Resistance breeding is the most effective way to manage clubroot, a soilborne disease of crucifers caused by *Plasmodiophora brassicae* Wor. In this study, *Brassica rapa* L. subsp. *rapifera* (European Clubroot Differential, ECD 02), which possesses broad spectrum resistance to many pathotypes of *P. brassicae*, was crossed with two clubroot-susceptible *B. rapa* accessions to produce two F<sub>2</sub> populations of 1103 (Popl#1) and 464 (Popl#2) individuals. A Chi-square test for the goodness of fit for the F<sub>2</sub> plants of Popl#1 screened with pathotypes 5X (7R : 9S,  $\chi^2 = 0.8372$ ,  $df = 3$ ,  $P = 0.8406$ ) and 5G (6R : 10S,  $\chi^2 = 5.1933$ ,  $df = 3$ ,  $P = 0.1578$ ) indicated a variation of the 9:3:3:1 segregation ratio expected for control of resistance by two dominant genes. Similarly, a Chi-square test for the F<sub>2</sub> plants of Popl#2 screened with pathotype 5G (11R: 5S,  $\chi^2 = 0.2402$ ,  $df = 3$ ,  $P = 0.9709$ ) suggested a variation of the ratio expected for two dominant genes. In contrast, the 3:1 segregation ratio obtained for the F<sub>2</sub> plants of Popl#2 screened with pathotype 5X ( $\chi^2 = 0.4099$ ,  $df = 1$ ,  $P = 0.5271$ ) was consistent with genetic control of resistance by a single dominant gene. Preliminary molecular marker analysis suggests that one dominant gene and possibly one QTL control clubroot resistance introgressed from ECD 02.

**PA-2. Mapping of resistance loci associated with clubroot disease originally derived from RRCC in *Brassica napus*.** J. Gong, Z. Zhan, J. Wu, C. Zhang.

Breeding of new resistant species plays an important role in the integrated control of clubroot disease of cruciferous crops. Interspecific hybridization is an effective method and tool for transferring desirable traits between crop species. Most of Radish species (*Raphanus sativus* L.) are highly resistance to clubroot disease. A synthesized allotetraploid Brassicoraphanus (RRCC, 2n=36), a cross between *R. sativus* cv. HQ-04 (2n=18, RR) and *B. oleracea* var. *alboglabra* (L.H Bailey) (2n=18, CC) exhibited strong resistance against a number of pathotypes of *P. brassicae*, therefore this valuable material has a great potential of improving the clubroot resistance for *Brassica napus*. The average values of pollen fertility of the two randomly selected BC<sub>3</sub>F<sub>1</sub> lines were 97.77% and 98.15%, respectively, indicating that the fertility of the BC<sub>3</sub>F<sub>1</sub> generation was basically restored. Whereas, the results of inoculation of one of the BC<sub>3</sub>F<sub>1</sub> population showed segregation with clubroot resistance, about 16.19% of them was immune, 19.05% of them were susceptible and the rest of them showed intermediate between resistant and susceptible, respectively. Subsequently, the mapping results of resistant gene derived from RRCC revealed by using bulk segregant analysis (BSA) and resequencing approach with two extreme mixing pools of DNA from resistant and susceptible lines, respectively, will be reported in this work.

**PA-3. Can *Brassica rapa* contribute a new clubroot resistance gene for use in the breeding of *Brassica napus* canola for Canada?** Kawalpreet Kaur & Habibur Rahman.

Clubroot disease of crucifers, caused by *Plasmodiophora brassicae*, is a threat to Brassica crop production. Various pathotypes of this pathogen have been reported in different countries and this disease causes about 10-15% yield loss worldwide. This disease has been reported in canola fields in North America in 2003; therefore, the development of clubroot resistant canola cultivars has been a priority to the canola breeders. Different Brassica accessions have been reported to carry resistance to different pathotypes. The objective of this research was to introgress resistance to pathotype 3 from *B. rapa* into *B. napus* canola. For this, interspecific cross between spring a *B. napus* canola and a *B. rapa* var. *rapifera* was made and conventional breeding was followed. The self-pollinated populations were grown in a greenhouse and selection for resistance to *P. brassicae* pathotype 3 was done from where several resistant families were developed. Genotyping of this population will be done with simple sequence repeat (SSR) markers, including the published markers reported to be associated with resistance, to identify the markers linked to the clubroot resistance gene for use in marker-assisted selection. The materials developed in this study can be used in breeding to develop clubroot resistant *B. napus* canola cultivars.

**PA-4. Fine mapping of BraCrr5, a novel gene conferring resistance to clubroot disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa*.** L. L.X. Mao, Y.X. Yuan, S.J. Yang, X.C. Wei, Y.Y. Zhao, Z.Y. Wan, X.W. Zhang & B.M. Tian.

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an important disease of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) in China and worldwide. In this study, a novel CR resistance gene BraCrr5 from ECD01-1 (European Clubroot Differentials inbred line) was identified and preliminary mapped the interval of 0.5Mb between 0.45 and 1.95 Mb on chromosome A08 via BSA-seq (Bulked Segregant DNA Sequencing). Three LRR-RLK (Leucine-rich repeat receptor-like protein kinase) genes (BraA08000045, BraA08000049 and BraA08000154) were identified in the target region. 3379 single-nucleotide polymorphisms (SNPs) sites were detected between one pooled resistant (R) and one pooled susceptible (S) samples in the region. 133 polymorphic SNP sites are nonsynonymous among them. A segregating population consisting of 520 plants was analyzed with 12 SNP sites in the region using the Kompetitive Allele Specific PCR (KASP) method, further narrowing the candidate region to the interval between 0.4 and 1.1 Mb. Five robust SNP markers were developed associated with BraCrr5. These markers could provide an effective and robust basis for introgression of BraCrr5 into Chinese cabbage using MAS.

**PA-5. Identification of a novel clubroot resistance QTL in Chinese cabbage (*Brassica rapa* L.).** J.I. Park, R. Laila, A.H.K. Robin, H.T. Kim, I.S. Nou.

*Plasmodiophora brassicae* is a soil-borne plant pathogen that causes clubroot disease, which results in crop yield loss in cultivated Brassica species. Here, we investigated whether a quantitative trait locus (QTL) in *B. rapa* might confer resistance to a Korean pathotype isolate, Seosan. We identified and mapped a novel clubroot resistance QTL using a mapping population that included susceptible Chinese cabbage and resistant turnip lines. We crossed resistant and susceptible parental lines and analyzed the segregation pattern in 348 F<sub>2</sub> plants. A 3:1 ratio was observed for resistant: susceptible genotypes, which is in accordance with Mendelian segregation. Further, 45 resistant and 45 susceptible F<sub>2</sub> plants along with their parental lines were used for double digest restriction site-associated DNA sequencing (ddRAD-seq), which identified a new locus, CRs, on chromosome A08 that was different from the clubroot resistance (CR) locus, Crr1. The newly identified locus is novel since the Akimeki cultivar bearing the Crr1 locus was susceptible to the Seosan isolate. These results could be exploited to develop molecular markers that can be helpful to develop Seosan resistant Chinese cabbage cultivars.

**PA-6. QTL-seq analysis identifies two clubroot (*Plasmodiophora brassicae*) resistance QTL in turnip (*Brassica rapa* ssp. *rapifera*).** H. Zhang, S. J. Zhang, F. Cheng, F. Li, S. F. Zhang & R.S. Sun.

European fodder turnips (*Brassica rapa* ssp. *rapifera*) were identified as sources of clubroot resistance (CR) and have been widely used in Brassica resistance breeding. In this study, an F<sub>2</sub> mapping population derived from a cross between a resistant turnip and a susceptible Chinese cabbage was used to determine the inheritance and locating the resistance gene(s). The parents showed very resistant/susceptible to three field isolates of clubroot from Yunan (Pby), Henan (Pbh) and Liaoning province (Pbl) in China. After inoculation with Pbh (pathotype 4), the 206 F<sub>2</sub> individuals showed a 9:7 segregation ratio in resistance, indicating that clubroot resistance is controlled by multiple genes with complementary effect in this population. Next generation sequencing based QTL-seq was used to locate resistance genes. Each of twenty seven very resistant (R)/susceptible (S) individuals were selected to construct R/S bulks, respectively. SNP index and  $\Delta$ (SNP-index) graphs identified base on bioinformatics information of *Brassica rapa* genome. Two regions on chromosome A03 (upstream of Crr3) and A08 (downstream of Crr1) showed significant different, respectively. There are four and five genes in A03 and A08 candidate region, respectively.

**PA-7. Screening of *Brassica oleracea* germplasm for resistance to *Plasmodiophora brassicae* pathotype 5X.** M. Farid, J. Si & H. Rahman.

Clubroot disease of brassica, caused by *Plasmodiophora brassicae* Woronin, is a concern to the canola (*Brassica napus* L.;  $2n = 38$ , AACC) growers in Canada. Management of this disease through cultural practices is a challenging task; growing clubroot resistant cultivars in an appropriate crop rotation is, therefore, considered to be the cost-effective and environmentally friendly way of managing this disease. To date, several clubroot resistance genes have been identified in the A genome of *B. rapa* and used in the breeding of *B. napus* canola; however, very limited efforts have been made to use the resistance of the C genome of *B. oleracea*. Identification of resistance in the C genome and use in the breeding of *B. napus* canola will broaden the genetic base of resistance in this crop for sustainable production from a long-term perspective. To date, breeding efforts mainly focused on the use of the genotypes conferring resistance to pathotype 3. Recently, new virulent pathotypes have evolved in the canola fields in Alberta; among these, the pathotype 5X is a threat to canola production. The focus of this study was to evaluate a global population of the diploid species *B. oleracea* (CC) and a set of *B. napus* canola lines for resistance to *P. brassicae* pathotype 5X. Results from this research are expected to provide valuable information for the development of clubroot resistant canola cultivars.

**PA-8. Genetic diversity of rutabaga accessions as sources of clubroot resistance.** Zhyu Yu, Rudolph Fredua-Agyeman, Sheau-Fang Hwang & Stephen E. Strelkov.

Rutabaga or swede (*Brassica napus* sp. *napobrassica*) is a root crop derived from the hybridization between turnip (*B. rapa*) and cabbage or kale (*B. oleracea*). It is cultivated as a minor crop in Europe and North America for use as a table vegetable and as fodder for animals. Rutabagas vary in root and leaf shape and colour, foliage growth habits, quality parameters and maturity date. They also have been used in breeding programs as sources of clubroot resistance. Despite these differences, no in-depth molecular research has been conducted on the genetic diversity of rutabaga accessions. In this study, 134 rutabaga accessions from Scandinavia (Sweden, Norway, Denmark, Finland and Iceland) were genotyped with a 15K Brassica SNP array from TraitGenetics. After excluding markers that did not amplify genomic DNA and also those with >20% missing data, 118 accessions were genotyped with 6000 SNP markers. Both UPGMA and NJ methods clustered the accessions into two major groups, with each branching into three sub-groups. Rutabaga accessions from Norway, Sweden and Finland were the most diverse and were distributed across the two major groups. In contrast, accessions from Denmark and Iceland clustered into each of the two major groups. Clubroot screening will establish if resistance is correlated with geographic distribution.



**PA-9. Marker-assisted introgression of CRb locus into elite Pol CMS restorer line for resistant hybrid production to clubroot in *Brassica napus*.** Q. Li, Y. Zhou, T. Fu, Z. Piao & C. Zhang.

Clubroot disease spreads rapidly these years in China. It forcibly occupies more than 3 million mu fertile farms in Sichuan province only. Now it is spreading faster than ever following the development of mechanized farming in China. This disease threatens to destroy canola industry in China because of lack of resistant varieties. Here, we report the marker-assisted introgression of clubroot resistant (CR) locus to improve rapeseed resistance to clubroot. Both F1 and BC1 show dominant resistance to clubroot different race (race2, 4, 7, 10) in greenhouse. Near iso-lines of Pol CMS restorer line containing a single locus CRb was obtained through foreground and whole genome background selection by using different molecular markers. NILs containing CRb resistance gene displayed immune resistance to different isolates in China, such as Yichang, Zhijiang, Huanshan, Penxian, Deyang, etc. Finally, this improved restorer line was used successfully for making hybrid (named as Huayouza62R) with good performance that resistant to clubroot and with high seed yield in different regions and was widely used as valuable donor plant for clubroot resistant breeding in China.

**PA-10. Verification of two candidate genes resistance to *Plasmodiophora brassicae* in broccoli.** Z.S. Li, X.L. Zhang, Y.M. Liu, Z.Y. Fang, L.M. Yang, M. Zhuang, Y.Y. Zhang & H. H. Lv.

Clubroot is one of the most devastating diseases to the Brassicaceae family. In this study, two candidate genes resistance to *Plasmodiophora brassicae* in broccoli was detected based on transcriptome analysis method. Two genotypes of broccoli, one clubroot-resistant wild cabbage B863 (*Brassica macrocarpa* Guss.) and another clubroot-susceptible broccoli B196 (*Brassica oleracea* var. *italica*), were inoculated with *P. brassicae* and sequenced in young plants during two weeks previously. The result revealed that two genes of Bol R1 and Bol R2 with highly gene expression difference between two resources in the infection process, might be defense responses to *P. brassicae*, and both of them were located in scaffold 000004 on chromosome C09. Bioinformatics and genome annotation of cabbage presents that Bol R1 belongs to NBS gene family including three gene CDSs, and Bol R2 is with no annotation including eight gene CDSs. Now, two candidate genes of Bol R1 and Bol R2, were both verified by agrobacterium-mediated genetic transformation in *Arabidopsis thaliana* and broccoli respectively, and positive plants were obtained from T0 generation of *Arabidopsis thaliana*. The result would provide new insight into clubroot resistance in Brassica plants.

**PA-11. Genome-wide transcriptomic analysis used to identify putative effector proteins in *Plasmodiophora brassicae*.** E. Pérez-López, J.Tu, M. Waldner, A. J. Kusalik, M. Hossain, C. D. Todd & P.C. Bonham-Smith.

*Plasmodiophora brassicae* (Wor.) is an obligate plant pathogen, which makes study of its biology and mechanism of infection difficult. Identification of effector proteins could be an important key to understanding the interaction between this pathogen and the genes involved in host resistance. *Arabidopsis thaliana* ecotype Columbia (Col.0, susceptible to clubroot), was used as a model plant host. Each 13-day-old seedling was inoculated at the base of the stem with 400 µL of  $5 \times 10^7$  resting spores/mL from clubbed roots of canola plants. Root tissue from inoculated and control plants were collected at 17, 20 and 24 days post-inoculation (dpi) for RNA-seq analysis, with 24 plants per biological replicate, 2 biological replicates, and 3 technical replicates. After RNA extraction and library construction, library sequencing was conducted from both ends on an Illumina HiSeq 2500. More differentially expressed genes (DEGs) were identified at 24 dpi relative to the two earlier time points. RNAseq data and a bioinformatics pipeline were used to identify 32 small secreted proteins of *P. brassicae* that were highly expressed in *Arabidopsis*. BLAST2GO, 3D structure, and phylogenetic analysis indicated that at least a quarter of these proteins were involved in cell division and cell cycle regulation. Further studies will be performed to identify the subcellular localization of the proteins and to assess their role in infection and subsequent symptom development.

**PA-12. Modulation of abscisic acid and ethylene levels in response to clubroot disease in *Brassica napus*.** C.P. Jayasinghege, V.P. Maolij, J.A. Ozga, S.F. Hwang & S.E. Strelkov.

The plant defense mechanisms associated with clubroot (*Plasmodiophora brassicae* Wor.) disease of crucifers are not well understood. The plant hormones abscisic acid (ABA) and ethylene play direct and indirect roles in plant defense and stress responses. In this study, we compared the levels of ABA, ABA metabolites, and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in the roots of a clubroot susceptible and a clubroot resistant cultivar of canola (*Brassica napus* L.). Hormone profiling was conducted by high-performance liquid chromatography-tandem mass spectrometry at 4, 14 and 21 days after inoculation (DAI). At 4 and 14 DAI, there were no or minimal differences in ABA and ACC levels between the two cultivars and inoculated and non-inoculated plants. By 21 DAI, however, the ABA level was 5-fold higher in the susceptible inoculated plants vs. the non-inoculated controls, indicating that the infected plants were experiencing drought stress conditions. Concomitantly, water demand in the susceptible, inoculated plants started to decline compared with the non-inoculated controls. Also at 21 DAI, the levels of ACC were 2.5- and 4-fold higher in inoculated plants of the susceptible and resistant cultivars, respectively, than in the non-inoculated controls. This suggests a role of ethylene in the clubroot-associated stress responses and disease resistance of *B. napus*.

**PA-13. Effects of root exudates on *Plasmodiophora brassicae* resting spore germination.** Y. Wang, B. Koopmann, P. Karlovsky, & A. Von Tiedemann.

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is one of the most devastating soil-borne diseases of cruciferous crops throughout the world. Germination of dormant *P. brassicae* resting spores is essential for the formation of the clubroot disease. Root exudates of several host and non-host plants can stimulate resting spore germination in laboratory experiments. A hydrophobic root exudates trapping system based on a hydroponic sand substrate system was established for continuous trapping of exudates from undisturbed living roots. The system provided sterile growth conditions for the experimental plants in order to exclude any modulations of root exudates by microbes. Hoagland's solution was continuously circulated through the root system and a XAD8 resin column, followed by a XAD4 resin column. Extracellular hydrophobic metabolites were selectively adsorbed by the resin, while inorganic nutrients were recycled to sustain plant growth. Metabolite profiling by HPLC-MS was conducted on root exudates of the host plant oilseed rape and non-host plants tomato and ryegrass. Candidate substances were selected and their effect on the germination of resting spores will be tested in bioassays. The objective of this study is to find out the substances that trigger the *P. brassicae* resting spore germination.

**PA-14. Effect of canola cultivar rotation on *Plasmodiophora brassicae* pathotype population dynamics.** T. Cao, V.P. Manolij, Q. Zhou, S.F. Hwang & S.E. Strelkov.

In Alberta, Canada, clubroot (*Plasmodiophora brassicae* Wor.) is managed mainly by planting clubroot resistant (CR) canola (*Brassica napus* L.) cultivars. However, multiple new pathotypes of *P. brassicae* have emerged recently which are virulent on CR canola. To understand the impact of cultivar rotation on pathotype population dynamics, greenhouse experiments were conducted in which different canola rotations were grown in a soil mix containing equal amounts of pathotypes 5X and 3, which are virulent and avirulent, respectively, on CR canola. Three treatments were assessed: T1, the same susceptible cultivar planted over 4 cycles; T2, the same CR cultivar planted over 4 cycles; and T3, different CR cultivars planted in each cycle. Clubroot severity increased from cycles 1 to 4 in all treatments, with the exception of one CR cultivar in T3 that may carry a different resistance source. Pathogen populations were recovered with a susceptible bait crop and pathotyped on the differentials of Williams plus a CR host. The proportion of galls classified as pathotype 5X in T1 declined to 6.7% over the course of the experiment. In contrast, the proportion of pathotype 5X increased to 66.7% and 70% in T2 and T3, respectively. Pathotype 5X-specific quantitative PCR analysis of the soil mix indicated a significantly higher amount of 5X-DNA in T2 vs T1. The results suggest that continual planting of CR canola favours the proliferation of virulent pathotypes of *P. brassicae*, as evidenced by the increase in pathotype 5X observed in this study.

**PA-15. Decline in resting spores of *Plasmodiophora brassicae* in soil over 6 years.** B.D. Gossen, F. Al-Daoud, T. Dumonceaux, J. Dalton, G. Peng, D. Pageau & M.R. McDonald.

The concentration of resting spores of *Plasmodiophora brassicae* Wor. in a canola (*Brassica napus* L.) field has a major effect on clubroot severity and the risk of a breakdown in resistance. This study was carried out on soil samples collected from a long-term rotation study at Normandin, QC in 2014, with replicated plots representing a 0-, 1-, 2-, 3-, 5- or 6-year break following a susceptible canola crop heavily infested with clubroot. Five molecular techniques for estimating *P. brassicae* resting spores in soil were assessed, including quantitative polymerase chain reaction (qPCR), competitive internal positive control PCR (CIPC-PCR), droplet digital PCR (ddPCR), loop-mediated isothermal DNA amplification (LAMP), as well as propidium monoazide PCR (PMA-PCR) to assess spore viability. For several of the techniques, calibrations needed to be developed using spiked soil samples, where a known amount of resting spores were added. Each of the assays provided a similar pattern of spore decline over time. This result supported the conclusion of a previous study at this site, that resting spore numbers declined rapidly over the first 2 years after a susceptible crop, but the rate of decline was substantially lower in subsequent years (type III survival curve). CIPC-PCR and ddPCR provided better estimates of resting spore numbers in soil compared with those from qPCR alone or LAMP. Estimates of viable spores from PMA-PCR were much lower than the estimates of spore numbers from the other techniques in the first year, but were similar thereafter.

**PA-16. Development of isolate-specific molecular marker for detecting Korean *Plasmodiophora brassicae*; the causal agent of clubroot.** H.T. Kim, J.Y. Jeong, J.I. Park & I.S. Nou.

Clubroot, caused by an obligate parasite *Plasmodiophora brassicae*, is one of the most economically important diseases of Brassicaceae family. In Korea, at least five races namely, race 1, 2, 4, 5 & 9 of the pathogen have been detected that fall under four pathotype groups. This study was planned to develop isolate-specific markers by exploiting genomic sequence variations. A total of 119 markers were developed based on unique variation in genomic sequences of each of the races. Only 12 markers were able to detect *P. brassicae* strains under each isolates/races/pathotypes. Ycheon9 and Ycheon10 markers were specific to Yeoncheon isolate (race 2, pathotype 3); Ycheon14 markers was specific to race 2-isolates Yeoncheon and Hoengseong; ZJ1-3, ZJ1-4 and ZJ1-5 markers were specific to Haenam2 (race 4) isolate; ZJ1-35, ZJ1-40, ZJ1-41 and ZJ1-49 markers were specific to Hoengseong isolate; and ZJ1-56 and ZJ1-64 markers were specific to Pyeongchang isolate (race 4, pathotype 3). The PCR based SCAR markers developed in this study are able to detect five Korean isolates of *P. brassicae*. These markers can be utilized in identifying Korean *P. brassicae* isolates from different regions. Additional effort is required to develop isolate specific markers for remaining Korean isolates.

**PA-17. Production of single-spore isolates of *Plasmodiophora brassicae* using micromanipulation of resting spores.** A. Sedaghatkish, B.D. Gossen, J. Singh & M.R. McDonald.

Clubroot, caused by *Plasmodiophora brassicae* Wor., is an important disease of Brassicaceae crops. The molecular basis of clubroot pathogenicity and resistance are poorly understood. Extraction of high quality DNA of single spores for genomic and genetic studies is a challenge because *P. brassicae* often occurs as a mixture of pathotypes, is associated with other soil microbes, and cannot be cultured in the absence of host tissue. The objective of this study was to isolate single spores for inoculation and high quality DNA for downstream applications. A technique was developed to isolate and culture single resting spores of *P. brassicae* using micromanipulation. Spores were extracted from clubbed roots, were isolated using a micromanipulator and inoculated individually onto the root of three-day-old seedling of the highly susceptible 'Mei Qing Choi' (*Brassica rapa* var. *chinensis*) grown in sterile Hoagland's solution media. Clubroot formation was visible in 8% of inoculated plants at 6 weeks after inoculation. This is a high rate of success compared with other published methods for inoculation with single spores. The approach was also effective for inoculation of root tissue sections that had been plated onto solid MS media. This method is fast and efficient and results in clean single-spore isolates of *P. brassicae* for molecular and genomic studies.

**PA-18. Effect of *Plasmodiophora brassicae* inoculum density on yield of canola (*Brassica napus*).** A. Botero-Ramírez, S.F. Hwang & S.E. Strelkov.

Clubroot, caused by *Plasmodiophora brassicae* Wor., is a major threat to canola (*Brassica napus* L.) production on the Canadian Prairies. However, reliable estimates of the relationship between yield and *P. brassicae* inoculum density under western Canadian conditions are lacking. This research aimed to evaluate the effect of clubroot disease on the yield of two canola hybrids with different clubroot resistance levels under field conditions in Alberta. The susceptible canola hybrid '45H31' and the resistant hybrid '45H29' were grown in soil inoculated with pathotype 5X at rates equivalent to  $10^7$ ,  $10^6$ ,  $10^5$ ,  $10^4$  and  $10^3$  resting spores of *P. brassicae* per plant. Clubroot incidence and severity, along with yield parameters including seed weight, productive branches and pods per plant, were measured at 79 and 122 days after planting. It was found that as *P. brassicae* inoculum density increased, so did the incidence and severity of clubroot on both the resistant and susceptible hybrids, although no statistical differences were observed at the lower inoculum densities ( $10^3$  -  $10^5$  resting spores per plant). Yield was not affected by *P. brassicae* inoculum density in the resistant hybrid, but was reduced by 31-51% in the susceptible hybrid. Regression analysis indicated that a second-grade polynomial equation best described the inoculum density effect over the yield in the susceptible hybrid.

**PA-19. Assessment of soils for the risk of clubroot disease of cruciferous vegetables using a simple dose response curve-dependent method.** A. Fukunaga, S. Yoshida & S. Tsushima.

Incidence of clubroot disease occurrence of cruciferous vegetables, caused by *Plasmodiophora brassicae*, has tended to increase in Japan. Development of methods for assessing soils for the risk of the disease occurrence are significant to reduce excessive soil sterilization using agricultural chemicals. Since it is significant to evaluate risk of disease occurrence of soils for the success of HeSoDiM (Health checkup-based Soil-borne Disease Management) practice, we developed a simple assessment system using plug tray-planted seedlings. Cabbage (indicator crop) seeds were sown into the soils containing 0 to  $10^6$  g<sup>-1</sup> soil resting spores of *P. brassicae* using a stepwise density distribution in the plug trays (25 individual cells, 44-mm-length × 44-mm-width × 49-mm-depth). After cultivation for 5 weeks, the disease index of each seedling was determined to obtain a dose-response curve (DRC) pattern and it needs only half of soil and the planting area that used conventional method. Although this method was slightly sensitive to the disease occurrence, comparing to that by conventional method using potted seedlings, we obtained DRC patterns in the plug trays system similar with those by the conventional system. Therefore, this simple assessment system is suggested to be applicable for evaluation of soils for the risk of the disease occurrence.

**PA-20. Isolation and evaluation of potential biocontrol agents against clubroot (*Plasmodiophora brassicae*) of *Brassica napus*.** L. Zheng, Y.W. He, M.L. Zhu, D.H. Jiang & J.B. Huang.

*Plasmodiophora brassicae* is the causal agent of clubroot disease. This soil pathogen causes one of the most damaging diseases within the family Brassicaceae in China. Microorganisms and their metabolites have attracted attention as potential biocontrol agents to reduce fungicide use. In this study, total 667 microbial strains were isolated from the rhizosphere soil of rapeseed in severely diseased fields in Dangyang City, Hubei Province, China. These included 323 strains of bacteria, 253 strains of fungi, and 91 strains of actinomycetes. As previously reported, strains of *Fusarium oxysporum* and *Magnaporthe oryzae* could be used as indicators for *P. brassicae* in the first round screening of biocontrol agents. Fifty-four strains with a inhibition zone width of more than 3 mm by dual culture test were obtained. Among these, two potential biocontrol strains F85 and T113, which were identified as *Bacillus* spp., were found to have a control efficiency of more than 60% through the pot experiments. Strains F85 and T113 significantly inhibited the infection of root hair, and reduced the differentiation of primary plasmodium of *P. brassicae* as well as formation of secondary zoosporangium. These two *Bacillus* strains F85 and T113 could be tested further in naturally diseased fields.

**PA-21. Evaluating chemical disinfectants for their ability to inactivate *Plasmodiophora brassicae* resting spores using Evans blue staining.** M.W. Harding, T.B. Hill, G.C. Daniels, S.E. Strelkov, S.F. Hwang & J. Feng.

*Plasmodiophora brassicae* Woronin, the causal agent of clubroot, has become an important disease on canola in Alberta. Its rapid spread across much of the Province has been due, in large part, to movement of infested soil on equipment. The most effective practice for avoiding the spread of *P. brassicae*-infested soil is equipment sanitization. Sanitization involves cleaning or washing away soil and plant material, followed by treatment with a chemical disinfectant. However, the thick-walled resting spores produced by *P. brassicae* are known to survive exposures to physical and chemical treatments, making it challenging to predict which disinfectants may be effective. In order to make accurate recommendations for equipment sanitization it was important to know which disinfectants, if any, can quickly and effectively inactivate resting spores. Evans blue is a vital stain that can discriminate viable from non-viable resting spores. *P. brassicae* resting spores were treated with six concentrations (1%, 10%, 25%, 75% and 100%) of each of ten chemical disinfectants. After a 15 min exposure, the disinfectants were neutralized using a universal neutralizer solution. Spores were rinsed three times with sterile water and evaluated for their viability using the Evans blue staining method. Repeated experiments, and comparisons of spore staining results with paired plant bioassays, indicated that only two of the disinfectants tested were capable of achieving greater than 95% inactivation of resting spores; sodium hypochlorite and ethanol. Additional chemical disinfectants will be evaluated, but at the time of this presentation, a 2% sodium hypochlorite solution was found to be the most reliable, inexpensive, safe and effective treatment for inactivation of *P. brassicae* resting spores.



## RESOURCES

*Effective technology transfer is needed to address the evolving challenges and opportunities within the canola industry. The Canola Council of Canada has several resources available that connect industry, markets and research.*

### ONLINE:

Canola Council of Canada Website: <http://www.canolacouncil.org/>

\*Clubroot Website: [www.clubroot.ca](http://www.clubroot.ca)\*

Canola Research Hub: <http://www.canolaresearch.ca>

Canola Watch is an e-publication which is free, unbiased, timely and research-focused:

<http://www.canolawatch.org/>

Canola Encyclopedia: <http://www.canolacouncil.org/canola-encyclopedia/>

Crop Production Videos: <http://www.canolacouncil.org/media/video-gallery/crop-production-videos/>

Crop Production Publications: <http://www.canolacouncil.org/publication-resources/print-resources/crop-production-resources/>

Canola Calculator tool: <https://www.canolacalculator.ca/>

Canadian Canola Biotechnology: [www.canolastory.ca](http://www.canolastory.ca)

Keep it Clean: [www.spraytoswath.ca](http://www.spraytoswath.ca)

Canola Performance Trials: <http://www.canolaperformancetrials.ca/>

Ultimate Canola Challenge: <http://www.canolacouncil.org/crop-production/ultimate-canola-challenge/>

Canola Diagnostic Tool: <http://www.canoladiagnostictool.ca/>

Canola and Bees: <http://www.canolacouncil.org/markets-stats/industry-overview/bees-and-canola-a-sweet-relationship/>

CanolaInfo Program: [www.canolainfo.org](http://www.canolainfo.org)

Canola Meal: [www.canolamazing.com](http://www.canolamazing.com)

### QUARTERLY PUBLICATIONS:

**Canola Digest:** The official publication of Canada's canola growers. It covers a range of topics from agronomics to marketing to the latest developments in the canola industry: <https://canoladigest.ca/>

**The Annual Canola Digest: Science Edition** issues are also available on this page.

### GROWER GROUPS:

Alberta Canola Producers Commission: <http://www.albertacanola.com>

Manitoba Canola Growers Association: [www.canolagrowers.com](http://www.canolagrowers.com)

SaskCanola: [www.saskcanola.com](http://www.saskcanola.com)

British Columbia Grain Producers Association: [www.bcgrain.com](http://www.bcgrain.com)

Ontario Canola Growers Association: [www.ontariocanolagrowers.ca](http://www.ontariocanolagrowers.ca)

Canadian Canola Growers Association: <http://www.ccca.ca>