Report on
PLASMODIOPHORIDS and RELATED ORGANISMS

AN INTERNATIONAL WORKSHOP

held on Saturday 23rd August 2008

at

Jolly Hotel – Ambasciatori,
Corso Vittorio Emanuele II, 104 Torino, Italy

Organised by and under the aegis

of the International Clubroot Working Group (ICWG)

as a part of

International Congress of Plant Pathology (ICPP)

held on 24th–28th August in Torino (Italy)

CONTENTS

7. Race-differentiation and resistance genes in the *Plasmodiophora brassicae*–*Brassica napus* interaction

E. Diederichsen[1] and S. Werner[2]

[1]Institute of Biology, Departments and Central Institutes, Freie Universität Berlin, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany
[2]Plant Breeding Institute, Christian-Albrechts-University of Kiel, Olshausenstr. 40, D-24098 Kiel, Germany
E-mail: elked@zedat.fu-berlin.de

8. Managing plasmodiophorid pathogens on Australian vegetable farms

E.C. Donald, I.J. Porter, R. Faggian, N.S. Crump, R.F. de Boer, T.J. Wiechel, C.S. Scoble and A.K. O'Toole

Department of Primary Industries Victoria, Private Bag 15 Ferntree Gully Delivery Centre, Victoria, Australia 3156
E-mail: caroline.donald@dpi.vic.gov.au

9. Quantitative PCR-detection methods for mapping in-field variation of *Plasmodiophora brassicae* in *Brassica* crops

C. Almqvist[1], A-C. Wallenhammar[2] and A. Jonsson[1]

[1]Department of Soil Sciences, Section of Precision Agriculture, Swedish University of Agricultural Sciences, P.O. Box 234, SE-532 23 Skara, Sweden
[2]Hs Konsult ab, P.O. Box 271, SE-701 45 Örebro, Sweden
E-mail: ac.wallenhammart@hush.se

10. Experiences with clubroot on canola (oilseed rape) in Alberta, Canada

S.E. Strelkov, S.F. Hwang, R. J. Howard and J.P. Tewari

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
E-mail: stephen.strelkov@ualberta.ca

11. Soil treatments and amendments for management of clubroot on canola in Alberta, Canada

S.F. Hwang, S.E. Strelkov, G.D. Turnbull, V. Manolii, R.J. Howard and M. Hartman

Alberta Agriculture and Food, 17507 Fort Road, Edmonton, AB, Canada
E-mail: sheau-fang.hwang@gov.ab.ca

12. Temperature influences the incidence and severity of clubroot on Asian leafy *Brassica* vegetables

M.R. McDonald[1], S.M. Westerveld and B.D. Gossen[2]

[1]Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[2]Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2
E-mail: Mrmcdona@uoguelph.ca

13. Integrated control of *Plasmodiophora brassicae* – clubroot on brassicas crops in Poland

M. Staniaszek, J. Robak and W. Marczewski

Research Institute of Vegetable Crops, 96-100 Skierniewice, Poland
E-mail: jrobak@inwarz.skierniewice.pl

14. Calcium cyanamide – 100 years of successful integrated control

G.R. Dixon

University of Reading and GreenGene International, Hill Rising, Horsecastles Lane, Sherborne, Dorset DT9 6BH, England
E-mail: geoffrdixon@btinternet.com/geoffrey.dixon62@imperial.ac.uk

Introduction

This report contains summaries of the papers delivered at the Plasmodiophorids Workshop held in Turin, Italy on 23rd August 2008. This meeting was organised and funded by the The International Clubroot Working Group (ICWG). This Group was formed in 1974 as the means by which those few valiant scientists working with *Plasmodiophora brassicae* and the Clubroot Disease might informally discuss their work and exchange ideas and arguments. Because we are few and spread disparately around the globe such intercourse is vital. Over the decades ICWG has hosted a goodly number of meetings. These have been linked in the main with major congresses and symposia because it was recognised that obtaining finance simply to attend a 'clubroot meeting' was extremely difficult.

Exchange has become infinitely easier with the advent of email and the web. The youngsters among you may like to ponder on the world of 1970 where postal mail was still the major means of communication and if you wanted to inform colleagues of your work you 'sent a reprint'! In somewhat similar fashion the world of clubroot has changed and you need to look no further than the content of this Report for evidence of the magnitude of the change. More fundamentally however, there is a much greater fund of knowledge concerning both host resistance and virulence in *P. brassicae*. Drawing from basic research largely with *Arabidopsis thaliana* there is a much clearer, but still fragmentary, understanding of the host pathogen interaction at the level of molecular genetics. This is reinforced by considerable advances in determining interactions at the cellular and growth regulator levels.

At the practical end of our studies recognition has emerged, that 'magic bullets' for clubroot control are very few and are never going to be the sole answer to disease management. In the last decade systems of integrated disease control have been developed that are based on sound science related to inoculum potential. These combine husbandry, nutritional, resistance and chemical elements combined to achieve disease management that allows successful crop production. Most excitingly it may well be that we are on the verge of possessing molecular diagnostic tools that will accurately quantify inoculum levels by testing in the field.

Our knowledge is now orders of magnitude better than when ICWG was first formed. But Plasmodiophora deserves much respect, it is spreading rapidly world wide and now threatens the Canadian canola crop, it is recorded now as a major problem in Northern India and parts of China. Reports also suggest it has substantial impact on crops in South America and Indonesia. Desperately knowledge is needed at the whole organism level to quantify the pathogens environmental interactions and much more is required from fundamental studies.

Two companies jointly sponsored this Workshop – ALZChem from Bavaria, Germany and Syngenta Seeds through their centre at Enkhuizen, the Netherlands. This support was vital in order to stage the Workshop and produce this resultant Report. Their generosity is very greatly appreciated. The papers contained in this Report offer a feast of information and I am most grateful to all of those who have agreed to contribute.

The Working Group is also sincerely grateful to the Editor-in-Chief, Professor A. Lebeda and the Executive Editor, Dr M. Braunová of Plant Protection Science for their ready agreement to publish these Abstracts. This provides a citable location recording the excellent science reported at the meeting. Since scientists in the Czech Republic have a long and distinguished history of research into Plasmodiophorid Organisms and the plant diseases that they cause, this is highly appropriated.

Professor Geoff R. Dixon
Chairman International Clubroot Working Group, January 2009

PAPER 1

The Importance of Seed- and Soil-related Inoculum for Powdery Scab Crop Infection

U. MERZ[1], A. KEISER[2], P.-Y. JAQUIÉRY[2] and T. OBERHÄNSLI[3]

[1]Plant Pathology, IIB, ETH Zurich, 8092 Zurich, Switzerland
[2]Swiss College of Agriculture, 3052 Zollikofen, Switzerland
[3]BIOREBA AG, 4153 Reinach, Switzerland
E-mail: ueli.merz@agrl.ethz.ch

Powdery scab of potato is caused by the biotrophic protozoan pathogen, *Spongospora subterranea* f.sp. *subterranea* (Sss). Its importance has increased in the last decades with new reports in several countries such as Malta, Korea and China. There are no efficient and economical control measures for this soil borne disease, mainly because of the longevity of the resting spores (sporosori) in soil. Thus, the most effective control is achieved when healthy seed is planted into clean soil. In Switzerland, the pathogen has already become established in many soils. An integrated control strategy should concentrate on prevention measures to stop soil infestation and the spread of the disease. Infected seed is the most common cause for disease dissemination. Powdery scab tolerance levels exist in the certification schemes but the inspectors often have difficulties to meet them. To estimate the impact of seed inoculum compared to soil inoculum field trials were made with either healthy seed into contaminated soil or infected seed into uncontaminated soil. Soil infestation was estimated and the crop infection with powdery scab was assessed. Results will be presented and discussed.

Keywords: powdery scab; *Spongospora subterranea* f.sp. *subterranea*; potato; integrated control; healthy seed; certification

PAPER 2

Significance and Occurrence of the Temperate Ribotypes of *Polymyxa* Species

M. J. SMITH, E. WARD, J. A. WALSH and M. ADAMS

Centre for Sustainable Pest and Disease Management, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ UK
E-mail: madeleine.smith@bbsrc.ac.uk

Polymyxa graminis and *Polymyxa betae* are obligate, intracellular, root-infecting organisms of cereals (*P. graminis*) and members of the *Chenopodiacae* (*P. betae*). Between the two species, they are known to transmit approximately 15 economically important plant viruses, which between them have a world-wide distribution. These viruses include *Soil-borne wheat mosaic virus*, *Barley yellow mosaic virus* and *Beet necrotic yellow vein virus*. Recent ribosomal DNA (rDNA) sequence data has shown that temperate isolates of *P. graminis* belong to two groups or 'ribotypes' based on sequence differences in the internal transcribed spacer (ITS) region. These ribotypes appear to differ in host range and ability to transmit viruses. It is hypothesised that particular ribotypes have different host specificities or preferences and are involved in the transmission of specific viruses. A number of studies have been undertaken to better understand the distribution and viral associations of the temperate ribotypes and to further clarify their taxonomic position within the order *Plasmodiophorales* and also their wider phylogenetic affinities.

Keywords: *Polymyxa graminis*; *Polymyxa betae*; cereals; sugar beet; *Soil-borne wheat mosaic virus*; *Barley yellow mosaic virus*; *Beet necrotic yellow vein virus*; virus vector; ribotypes; taxonomy

PAPER 3

The selection and Characterisation of Resistance to *Polymyxa betae*, Vector of *Beet Necrotic Yellow Vein Virus*, Derived from Wild Sea Beet

M. J. C. ASHER[1], M. K. GRIMMER[2] and E. S. MUTASA-GOETTGENS[1]

[1]Broom's Barn Research Centre, Higham, Bury St Edmunds, Suffolk, IP28 6NP, UK
[2]Current address: ADAS Boxworth, Battlegate Rd, Boxworth, Cambridge, CB23 4NN, UK
E-mail: mike.asher@bbsrc.ac.uk

The plasmodiophoromycete *Polymyxa betae* is an obligate root parasite that transmits *Beet necrotic yellow vein virus* (BNYVV), the cause of sugar beet rhizomania disease. Currently, control of this disease is achieved through the use of cultivars with monogenic (Rz1) partial resistance to the virus. To improve the level and durability of this resistance, sources of resistance to the virus vector, *P. betae*, were sought. Over 100 accessions of the wild sea beet (*Beta vulgaris* ssp. *maritima*) from European coastal regions were evaluated for resistance in controlled environment tests. Quantification of *P. betae* biomass in seedling roots was achieved using recombinant antibodies raised to a glutathione-s-transferase expressed by the parasite *in vivo*. Several putative sources of resistance were identified and selected plants from these hybridised with a male-sterile sugar beet breeding line possessing partial virus resistance (Rz1). Evaluation of F_1 hybrid populations identified five in which *P. betae* resistance had been successfully transferred from accessions originating from Mediterranean, Adriatic and Baltic coasts. A resistant individual from one of these populations was backcrossed to the sugar beet parent to produce a BC_1 population segregating for *P. betae* resistance. This population was also tested for resistance to BNYVV. AFLP and SNP markers were used to map resistance QTLs to linkage groups representing specific chromosomes. QTLs for resistance to both P. betae and BNYVV were co-localised on chromosome IV in the BC_1 population, indicating resistance to the virus conditioned by vector resistance. This resistance factor (Pb1) was shown in the F_1 population to reduce *P. betae* levels through interaction with a second locus (Pb2) found on chromosome IX, a relationship confirmed by general linear model analysis. In the BC1 population, vector-derived resistance from wild sea beet combined additively with the Rz1 virus resistance gene from sugar beet to reduce BNYVV levels. With partial virus resistance already deployed in a number of high-yielding sugar beet cultivars, the simple Pb1/Pb2 two-gene system represents a valuable additional target for plant breeders.

Keywords: *Polymyxa betae*; *Beet necrotic yellow vein virus*; sugar beet; rhizomania; partial resistance; wild sea beet; *Beta vulgaris* ssp. *maritima*

PAPER 4

Hormone Signaling during the Development of the Clubroot Disease in *Arabidopsis thaliana* Roots

J. LUDWIG-MÜLLER

Department of Biology, Faculty of Science, Technical University Dresden, 01062 Dresden, Germany
E-mail: Jutta.Ludwig-Mueller@tu-dresden.de

The clubroot disease of Brassicaceae is one of the most damaging within this plant family. Caused by a soilborne obligate biotrophic pathogen, the interaction between *Plasmodiophora brassicae* and its host is difficult to analyse. Since *Arabidopsis thaliana* is a good host for *P. brassicae* we have used the ATH1 Affymetrix array to investigate host gene expression during root gall development. Auxins and cytokinins are directly involved in gall formation by contributing to cell division and enlargement in the hypertrophied host root, whereas other plant hormones, i.e. abscisic acid, ethylene or jasmonic acid constitute additional signals involved in the modulation of defense or stress responses. In auxin biosynthesis nitrilases play a role in gall formation, whereas early steps in the pathway to indole glucosinolates are not essential. Also

the GH3-gene family, which is involved in the conjugation of auxin and jasmonate, is differentially regulated, i.e. IAA conjugating family up and JA conjugating member down. Secondary metabolites such as flavonoids are also induced during gall formation. Since flavonoids are discussed to have different functions such as defense-related compounds, antioxidants or auxin transport inhibitors, the different possibilities for their role in clubroot development are currently investigated. Preliminary data show that they might act as modulators of auxin transport in developing young galls. The growing root gall constitutes a strong metabolic sink which could be induced by cytokinins. The pathogen is able to synthesise cytokinins, and in addition the degradation capacity of the host is down-regulated upon infection resulting in individual cells with high cytokinin levels. We use mutants and transgenic plants to elucidate the role for specific genes in this complicated regulatory network. A model to explain the function of plant hormones and secondary metabolites such as glucosinolates and flavonoids in clubroots will be presented.

Keywords: clubroot; *Plasmodiophora brassicae*; *Arabidopsis thaliana*; growth regulators; auxin; cytokinin; nitrilase; flavonoid

PAPER 5

Cytokinin is a Crucial Pathogenic Factor for Clubroot Development in A*rabidopsis thaliana*

N. GALFE[1], A.-A. BERGER[1], M. RIEFLER[2] and J. SIEMENS[1]

[1]Department of Biology, Molecular Biotechnology, Technical University Dresden, 01062 Dresden, Germany
[2] Department of Biology, Applied Genetics, Free University Berlin, 14195 Berlin, Germany
E-mail: Johannes.Siemens@tu-dresden.de

Arabidopsis thaliana is a host plant of the obligate biotrophic root parasite *Plasmodiophora brassicae***,** the cause of clubroot. Local changes in the cytokinin homeostasis by up-regulation of cytokinin receptor and down regulation of cytokinin-oxidases has been shown to be linked to pathogenesis (Siemens *et al.*, 2006, Molecular Plant-Microbe Interaction, **19**: 480–494). Three sensor histidine kinases, AHK2, AHK3, and CRE1/AHK4 of *A. thaliana* have been shown to be cytokinin receptors, which revealed partially redundant functions (Riefler *et al.*, 2006, Plant Cell, **18**: 40–54). The interaction with *P. brassicae* of loss-of-function mutants of all three receptors has been analysed. Single mutants showed wildtype clubs, whereas the development of clubroot in the root of the double mutant AHK3/AHK4 is hampered. These double mutants showed a reduced gall size. Histological analysis revealed an inhibiton of the development of the pathogen. Thirty days after infection the root of the double mutant AHK3/AHK4 is colonised by vegetative secondary plasmodia of the pathogen but no mature spores can be detected in contrast to wildtype plants. In order to get further insights into the mechanism of this apparent locking and the dependence of pathogen development on cytokinin-mediated signal transduction of host cells whole genome expression analysis were compared between mutants as well as compatible and incompatible interaction.

Keywords: clubroot; *Plasmodiophora brassicae*; *Arabidopsis thaliana*; cytokinin; receptor; histidine kinase; signal transduction

PAPER 5A (supplied after the Meeting)

RPB1-mediated Clubroot Resistance in *Arabidopsis thaliana*

F. REHN[1], A. ARBEITER[2], N. GALFE[1], S. REINHARDT[1] and J. SIEMENS[1]

[1]Department of Biology, Molecular Biotechnology, Technical University Dresden, 01062 Dresden, Germany
[2]Department of Biology, Applied Genetics, Free University Berlin, 14195 Berlin, Germany
E-mail: Johannes.Siemens@tu-dresden.de

Arabidopsis thaliana is a host plant of the obligate biotrophic root parasite *Plasmodiophora brassicae*, the causal agent of clubroot disease. A monogenically inherited resistance phenotype has been found in *A. thaliana* ecotypes Tsu-0, Ze-0, Ta-0, and RLD. The dominantly inherited *RPB1* gene mediates a hypersensitive response like reaction to *P. brassicae***.** The *RPB1* gene has been identified by transformation of susceptible ecotypes with DNA fragments from resistant ecotypes, the expression of *RPB1* has been shown by RACE. According to the full-length cDNA sequence the encoded small protein has three trans-membrane domains but no features of classical resistance genes. The genomes of resistant ecotypes either contain a single copy (RLD) or two closely linked copies of *RPB1* (Tsu-0, Ze-0, Ta-0). Furthermore a small number of putative genes which would encode proteins of size and structure very similar to *RPB1* has been found in the genomes of resistant *A. thaliana* ecotypes**,** the susceptible ecotype Col-0, and *Brassica rapa***.** Genetic data revealed no influence of the hormones salicylic acid, jasmonic acid and ethylene, but a mutation of *SGT1a* (At4g23570) reveals to be epistatic to *RPB1***.** The genome wide expression data of the incompatible interaction pinpoint to protein degradation, transport, and cell wall modifications as important parts of clubroot resistance mechanism. Furthermore, the AP2/EREBP and NAC domain transcription factors are consistently up- or down-regulated, respectively.

Keywords: clubroot; *Plasmodiophora brassicae*; *Arabidopsis thaliana*; monogenic; resistance; *Brassica rapa*; protein degradation; transport; cell wall

PAPER 6

Integrative Analysis of the *Arabidopsis thaliana–Plasmodiophora brassicae* Interaction: Deciphering Mechanisms Associated With Partial Resistance

M. JUBAULT, A. GRAVOT, Ch. LARIAGON, C. DELEU, A. BOUCHEREAU, R. DELOURME and M. J. MANZANARES-DAULEUX

INRA, Agrocampus Rennes, Université Rennes 1, UMR118, BP 35327, 35653 Le Rheu, France
E-mail: maria.manzanares@agrocampus-rennes.fr

Clubroot, caused by the obligate biotroph *Plasmodiophora brassicae***,** is one of the economically most important diseases of *Brassica* crops in the world. The development of resistant cultivars is currently the most efficient way to control clubroot in all *Brassica* crops. However, successful strategies for breeding and management of durable host-plant resistances require knowledge of clubroot resistance gene functions and associated mechanisms. The model plant *Arabidopsis thaliana* is also a host for clubroot and shows natural variation in the responses to clubroot. The present work aims to determine the genetic factors and metabolic pathways associated with partial resistance, using the *A. thaliana–P. brassicae* pathosystem. A quantitative trait locus approach was carried out using two segregating populations ($F_{2/3}$ and recombinant inbred lines) from crosses between the partially resistant accession Bur-0 and the susceptible one Col-0. Four additive QTLs and four epistatic regions controlling partial resistance to clubroot were identified. A functional genomic approach, using the CATMA whole genome microarray, was then applied to measure changes in gene expression associated with partial quantitative resistance. We showed that partial clubroot resistance response was characterised by an induction of classical plant defense responses, an active

inhibition of cell enlargement and proliferation and a reduced metabolic diversion by the pathogen. In particular, this work highlighted the involvement of arginine metabolism in partial clubroot resistance. We demonstrated at the transcriptional, enzymatic and metabolic levels that polyamine metabolism and arginine catabolism are induced during the later stages of disease in compatible interactions. However, susceptible and partially resistant plants showed strikingly different arginine metabolism signatures. Susceptible plants were characterised by a transient agmatine production, a massive induction of arginase and a strong accumulation of proline, which could represent a diversion of host metabolism in favour of the pathogen. Partially resistant plants showed a continuous agmatine production and a weaker arginase pathway activity than the susceptible genotype suggesting that the pathogen influence on the host-metabolism was attenuated or delayed. These data will be then used to characterise precisely quantitative resistances previously identified in *Brassica* and to optimise the management of the different resistances.

Keywords: clubroot; *Plasmodiophora brassicae*; *Arabidopsis thaliana*; resistance; plant defense; metabolism; agmatine; arginase; proline; quantitative trait loci; QTL; epistatic

PAPER 7

Race-differentiation and Resistance Genes in the *Plasmodiophora brassicae–Brassica napus* Interaction

E. DIEDERICHSEN[1] and S. WERNER[2]

[1]Institute of Biology, Departments and Central Institutes, Freie Universität Berlin, Albrecht-Thaer-Weg 6, D-14195 Berlin, Germany

[2]Plant Breeding Institute, Christian-Albrechts-University of Kiel, Olshausenstr. 40, D-24098 Kiel, Germany

E-mail: elked@zedat.fu-berlin.de

Clubroot caused by *Plasmodiophora brassicae* has gained increasing importance in major oilseed rape growing areas. Interspecific hybridizations and breeding efforts did lead to the release of the clubroot resistant *B. napus* cultivar Mendel in many European countries. The resistance in Mendel is race-specific and monogenic, and therefore, demands resistance management. To check for the occurence of compatible pathotypes in infected oilseed rape crops, greenhouse tests were made including also differential hosts and reference material. Results from differential testing will be presented giving a more detailed picture of Mendel's resistance. To evaluate additional resistance sources, a set of different *B. napus* lines representing major race-specific resistance QTL from a DH mapping population has been tested with different *P. brassicae* collections. A summary of map positions and race-specificity will be given. In contrast to previous reports, a clear differentiation into major QTL from *B. rapa* and minor QTL from *B. oleracea* could not be found. At one chromosome clubroot resistance was explained by only one major QTL originating from the susceptible parent, although clubroot resistance has never been observed before in this cultivar. As a general conclusion the genetics of clubroot resistance in *Brassica* appears to be more complex than earlier models did propose. So far, race-specificity seems to be the rule, with some QTL having a broader efficacy than others.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica napus*; race-specific; monogenic; resistance; quantitative trait loci; QTL; double haploid; DH

PAPER 8

Managing Plasmodiophorid Pathogens on Australian Vegetable Farms

E. C. DONALD, I. J. PORTER, R. FAGGIAN, N. S. CRUMP, R. F. DE BOER, T. J. WIECHEL, C. S. SCOBLE and A. K. O'TOOLE

Department of Primary Industries Victoria, Private Bag 15 Ferntree Gully Delivery Centre, Victoria, Australia 3156
E-mail: caroline.donald@dpi.vic.gov.au

Plasmodiophora brassicae and *Spongospora subterranea* are two important horticultural pathogens causing clubroot of vegetable brassicas and powdery scab of potatoes respectively. An integrated program to control and prevent the spread of *P. brassicae* is widely used on Australian vegetable brassica farms. The program uses a 'whole of production' approach and incorporates pathogen detection, eradicant and preventative methods to manage inoculum below the threshold required for disease. In commercial nurseries a PCR assay has been used to identify sources of inoculum contamination. In the field, the inoculum threshold for disease depends upon a range of site specific factors such as soil and crop type, climate and cultural practice but it is generally approximately 1000 spores per gram of soil. Real-time PCR has been used to quantify the inoculum load in soils and predict expected yield loss. Manipulation of soil pH (by application of burnt lime, CaO), application of calcium and boron, and strategic placement of the fungicide fluazinam (1.5 l a.i./ha in 500 l water/ha) have been used alone (on low risk sites), or together, as part of an integrated clubroot control strategy (on moderate-high risk sites). A similar strategy is now being developed for *S. subterranea*. In field trials fluazinam has consistently caused a significant reduction in the incidence and severity of powdery scab on tubers. In spite of their taxonomic similarities, there are key differences between these pathogen/host interactions, including the duration of cropping and infection sites (root and tuber) and this influences the effectiveness of different management strategies. The implication of these differences is discussed in relation to the interpretation of predictive soil DNA tests and the method and timing of application of fluazinam.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica*; powdery scab; potato; *Spongospora subterranea* f.sp. *subterranean*; integrated control; detection; eradication; prevention; inoculum threshold

PAPER 9

Quantitative PCR-Detection Methods for Mapping In-field Variation of *Plasmodiophora brassicae* in *Brassica* Crops

C. ALMQVIST[1], A.-C. WALLENHAMMAR[2] and A. JONSSON[1]

[1]Department of Soil Sciences, Section of Precision Agriculture, Swedish University of Agricultural Sciences, P.O. Box 234, SE-532 23 Skara, Sweden
[2]Hs Konsult ab, P.O. Box 271, SE-701 45 Örebro, Sweden
E-mail: ac.wallenhammart@hush.se

Soil borne diseases are responsible for annual yield losses in many agricultural crops. A prerequisite for successful development of a sustainable plant production is the availability of efficient analysis of soil-borne diseases such as *Plasmodiophora brassicae* causing club root in oilseed rape and cabbage crops and *Vericillium longisporum* causing verticillium wilt in oilseed rape. In Swedish crop rotation sequences oilseed brassicas are encouraged to be grown every 5^{th}–6^{th} years with respect taken to the presence of soil-borne diseases. This project started in 2006 and is aiming at adjusting and developing molecular diagnostic methods for an efficient quantitative PCR-detection of *P. brassicae* for an effective plant disease management within field variations and variations between fields in the occurrence of *P. brassicae* are determined on farms in south and central Sweden. Spatial variability within fields and variations between

fields in the occurrence of *Plasmodiophora brassicae* were determined on farms in south and central Sweden using quantitative PCR-assays. The molecular methods are validated by traditional bioassay techniques. Soil has been sampled using GPS from fields where the disease occurred and the results presented as an interpolated disease map. Relations between the occurrence of pathogens and soil parameters such as pH, soil type, clay content, plant available macro- and micro-nutrients are evaluated. Species-specific primers and Taqman fluorogenic probes were designed to amplify small regions of *P. brassicae* ribosomal DNA. Total genomic DNA was extracted and purified from soil samples using commercial kits, the amount of pathogen DNA was quantified using a standard curve generated by including reactions containing different amounts of a plasmid carrying the *P. brassicae* target sequence. Regression analysis showed that the assays were linear over at least 6–7 orders of magnitude ($r^2 > 0.99$) and that the amplification efficiency was > 95%. A considerable (100–1000 times) variation in DNA – content was observed in the sampled fields for *P. brassicae.* The level of pathogen was correlated to soil pH-value, concentration of elements such as potassium, phosphorus and calcium in the soil sample. Molecular methods for routine diagnosis will enable producers to respond to market opportunities by securing a more intensive crop rotation. The results will constitute basic data for an evaluation of the benefit of a systematic detection of soil borne pathogens by a quantitative PCR-method in combination with chemical soil mapping aiming at an efficient application in precision agriculture.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica*; oilseed rape; cabbage; *Verticillium longisporum*; molecular diagnosis; quantitative-PCR; polymerase chain reaction; bioassay

PAPER 10

Experiences with Clubroot on Canola (Oilseed Rape) in Alberta, Canada

S. E. STRELKOV, S. F. HWANG, R. J. HOWARD and J. P. TEWARI

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
E-mail: stephen.strelkov@ualberta.ca

Clubroot, caused by *Plasmodiophora brassicae* Woronin, was initially found on *Brassica napus* L. canola (oilseed rape) in the Edmonton, Alberta region in 2003, in the first report of this disease on canola in the Canadian prairies. Surveys conducted from 2005 to 2007 indicate that clubroot is spreading and more widespread than originally thought, with several hundred clubroot-infested fields identified in eight counties in central Alberta, the centre of the outbreak. The disease has also been recently found in canola fields in a county in southern Alberta. The primary mechanism of spread between fields is the movement of infested soil on farm machinery. Yield losses ranging from 30% to 100% have been reported in severely infested canola fields. The occurrence of clubroot is not restricted to fields with acidic soils, but there is a significant negative correlation between disease severity and soil pH. Evaluation of the virulence of populations and single spore isolates of *P. brassicae* on differential hosts has revealed the presence of at least three, and possibly four, pathotypes in Alberta. Pathotype 3, as classified on the differentials of Williams, is predominant and highly virulent on all canola cultivars currently available in Canada. At this time, there are few management options for farmers except extended crop rotations, although studies are underway to assess the efficacy of various fungicides and soil amendments for clubroot control. The Province of Alberta recently made *P. brassicae* a declared pest under the Agricultural Pests Act, as part of its Clubroot Management Plan.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica napus*; canola; oilseed rape; Alberta; Canada; disease management; legislative control; pathotype

PAPER 11

Soil Treatments and Amendments for Management of Clubroot on Canola in Alberta, Canada

S. F. HWANG, S. E. STRELKOV, G. D. TURNBULL, V. MANOLII, R. J. HOWARD and M. HARTMAN

Alberta Agriculture and Food, 17507 Fort Road, Edmonton, AB, Canada
E-mail: sheau-fang.hwang@gov.ab.ca

Clubroot, caused by *Plasmodiophora brassicae* Woronin, has appeared in many canola crops near Edmonton, Canada. With a half-life of four years, this pathogen represents a long-term challenge to canola production in central Alberta. Field plots were established in infested soils near Leduc and St. Albert, Alberta, to determine the effects of soil amendments and chemical soil treatments on crop damage due to clubroot. Clubroot severity was significantly lower compared to the inoculated control in soils treated with Terraclor 75% WP. This treatment also resulted in reduced seedling mortality, increased plant cover, increased plant height and increased emergence in severely infested soils. Percentage plant cover and height also responded positively to treatment with Ranman at 7.5 l/ha in less severely infested soils. Amendment of infested soils with calcium carbonate, wood ash, or calcium cyanamide did not result in changes in clubroot severity, compared to the untreated control. In severely infested soils, amendment with wood ash at 7.5 t/ha or with calcium carbonate at 5.0 or 7.5 t/ha resulted in greater plant height and crop cover compared to the untreated control. Results indicate that Terraclor 75% WP and treatment with high levels of calcium carbonate or wood ash have the potential to reduce the effect of *P. brassicae* on canola.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica napus*; oil seed rape; canola; fungicide; disease management; pH; calcium

PAPER 12

Temperature Influences the Incidence and Severity of Clubroot on Asian Leafy *Brassica* Vegetables

M. R. McDONALD[1], S. M. WESTERVELD and B. D. GOSSEN[2]

[1]Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1
[2]Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada, S7N 0X2
E-mail: Mrmcdona@uoguelph.ca

Clubroot of crucifers (*Plasmodiophora brassicae* Woronin) is a limiting factor in the production of leafy *Brassica* vegetables in the muck soil of the Holland/Bradford Marsh region of Canada (44°5'N, 75°35'W), but only at certain times of the year. To determine the effects of soil and air temperatures on the development of clubroot on these crops, Shanghai pak choy (*Brassica rapa* L. subsp. *chinensis* (Rupr.) var. *communis* Tsen and Lee) and flowering (*B. rapa* L. subsp. *chinensis* (Rupr.) var. *utilis* Tsen and Lee) were seeded into organic soil, naturally infested with the clubroot pathogen in May, June, July, and August of 1999, 2000, 2001, and 2007. Data from 22 trial years were used to compare disease incidence and disease severity index (DSI) with weather conditions during crop development. Clubroot was highest on crops seeded in June and July and lowest for the August seedings. In general, air temperatures were a better predictor than soil temperatures recorded by a nearby weather station. Mean daily air temperatures during the 10 days before assessment ranged from 13–27°C and were positively correlated with disease incidence and DSI (r = 0.70–0.84). There is a potential to predict levels of clubroot based on daily air temperatures in the crop. Crop management methods to keep soil temperatures cool may suppress clubroot.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica rapa*; Chinese cabbage; pac choy; temperature; crop management

PAPER 13

Integrated Control of *Plasmodiophora brassicae* – Clubroot on Brassicas Crops in Poland

M. STANIASZEK, J. ROBAK and W. MARCZEWSKI

Research Institute of Vegetable Crops, 96-100 Skierniewice, Poland
E-mail: jrobak@inwarz.skierniewice.pl

One of the most dangerous and most frequent diseases in cruciferous crops cultivation is clubroot caused by the *Plasmodiophora brassicae* Wor. fungus. The best method of controlling the pathogen is the introduction of resistant varieties or throught analysis of soil healthiness used for cruciferous crops cultivation. A nested PCR and a bioassay in Chinese cabbage cv. Granaat seedlings were used for the detection of the fungus *Plasmodiophora brassicae* in roots of white cabbage and cauliflower, and field soil and peat moss substrate. The specific 507 bp DNA fragment was amplified in all infected roots tested. It was identified in field soil and peat moss substrate when the resting spore concentration was higer than 10^3 of spores per 1 g of growth media. In the biological test the pathogen was detected in the field soil and peat moss substrate in which the concentration of spores was 10^6 and 10^3 spores per 1 g, respectively. The treatment of field soil and peat moss substrate with fungicide IBE3878 or steam autoclaved to be sufficient to decrease the spore level of *Plasmodiophora brassicae* less than this requires to detect the fungus by bioassay and the PCR method. The new chemical products such as: amisulbrom, IBE 3919 (coded) and fluazynam and methods of their application has been investigated for clubroot control on head cabbage growing in open field. The primary results are very promising.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica oleracea*; *Brassica rapa*; cabbage; cauliflower; nested-PCR; polymerase chain reaction; bioassay; fungicide

PAPER 14

Calcium Cyanamide – 100 Years of Successful Integrated Control

G. R. DIXON

University of Reading and GreenGene International, Hill Rising, Horsecastles Lane, Sherborne, Dorset DT9 6BH, England
E-mail: geoffrdixon@btinternet.com/geoffrey.dixon62@imperial.ac.uk

Calcium cyanamide fertiliser celebrates a century of successful use in agriculture and horticulture in 2008. It is manufactured by a straighforward electrochemical process from limestone, coal and atmospheric nitrogen. It is therefore a significant source of calcium for crops and supplies nitrogen fertiliser in a form that is not easily leached into ground water. Benefits accruing from the use of calcium cyanamide are consistently greater than might be anticipated from solely a calcium and nitrogen fertiliser. Use of this nutrient source is associated with improved soil fertility in terms of increased benign microbial activity. Associated with this property is a reduction in the pathogenic activity of several soil borne microbes particularly *Plasmodiophora brassicae*, the causal agent of clubroot disease in brassicas and *Sclerotinia sclerotiorum*, a cause of stem and root rot in an extensive range of crops. These effects are well documented by detailed research published by independent workers worldwide since manufacture commenced in 1908. Well established keys to the successful use of calcium cyanamide are: continuous application over seasons, timing relative to seed sowing and transplanting and particle size. *Brassica* crop production is increasing in significance world wide for several reasons: recognition that consumption is associated with reduced risk from cancer and coronary diseases, major source of vegetable oils for culinary and industrial purposes and a biofuel energy source. Intensification of brassica production brings attendant problems of increased incidence of crop diseases especially those caused by soil borne microbes such as *P. brassicae*. Clubroot disease has been a longstanding and intractable scourge of vegetable brassica crops and is now also established as the cause

of significant losses in oil seed crops. Only a few agrochemical pesticides have ever been demonstrated as effective against clubroot and their licensing for use is restrictive especially in Europe. The general thrust of agricultural policy world wide is to reduce pesticide use. Consequently a worldwide research aim is the development of crop protection systems which safeguard the environment and reduce human exposure to pesticides. One avenue for achieving such goals is the use of integrated control. The control of clubroot disease is now recognised as requiring the careful use of integrated control for which calcium cyanamide is a pivotal ingredient. Research completed over the last decade or so has amply demonstrated that clubroot can be effectively controlled by using integrated systems. These require carefully formulated combinations of husbandry measures, manipulation of soil structure, water table and alkalinity, control of calcium, boron, nitrogen and irrigation applications, use (where feasible) of resistant cultivars and pesticides. Each ingredient is added to the control strategy in relation to the soil inoculum potential. Hence as the risk of severe disease increases so greater numbers of control measures are required. The regular and consistent use of calcium cyanamide applied to soils with low inoculum potentials becomes a major means of constraining disease risk at low levels compatible with profitable crop production.

Keywords: clubroot; *Plasmodiophora brassicae*; *Brassica*; *Sclerotinia sclerotiorum*; white rot; calcium cyanamide; biofertiliser; integrated control; calcium; nitrogen; nitrate vulnerable zones; sustainable; environment