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Chapter 1

The occurrence and economic impact of *Plasmodiophora brassicae* and Clubroot disease

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ABSTRACT

The significance of *Plasmodiophora brassicae* Woronin and the disease which it incites in members of the family *Brassicaceae* are reviewed as the focus for this Special Edition of the *Journal of Plant Growth Regulation*. Naming of the disease in vernacular terms indicates a well established importance in agriculture and horticulture from the Middle Ages onwards in Europe. Subsequent spread probably resulted from transfer on and in fodder used by colonists' livestock. Symptoms, worldwide distribution and economic impact are described. Clubroot disease devastates infected vegetable and agricultural crops. Particular importance is placed on the most recent reports of crop losses in tropical countries, albeit where the crops are grown in cooler altitudes and in the Canadian prairie land canola crops. This latter is of enormous importance since this crop is the single most importance source of vegetable oils used in human food and industrial lubrication.

Keywords: *Plasmodiophora brassicae*, Clubroot disease, vernacular names, symptoms worldwide distribution, economic impact

This Special Edition of the *Journal of Plant Growth Regulation* focusses detailed consideration on the microbial plant pathogen, *Plasmodiophora brassicae*, the causal agent of Clubroot Disease in members of the family *Brassicaceae*. This is the first monographic treatment, albeit of limited scope, for over 50 years. An *Acta Horticulturae* (no 706) contains a series of review papers and *Plant Protection Science* 45 (1) (in press) the abstracts of a conference held in 2008.

Introduction

The association of *P. brassicae* with the gross distortion of growth in its hosts makes it entirely appropriate to publish this digest of current research in the *Journal of Plant Growth Regulation*. Clubroot disease has been known on cultivated brassicas in Europe at least as far back as the 13th Century and ascribed to many causes. The early 19th century Scottish view that it resulted from "unsatisfactory soil conditions or unbalanced

fertiliser practices" was, in the absence of microbiological knowledge, a reasonable assertion. In 1873, the Russian biologist M S Woronin, commenced studies of the disease in St Petersburg and five years later identified *Plasmopipophora brassicae* Woron., as the causal agent of clubroot disease (Woronin, 1878; trans. Chupp, 1934). More than a century of subsequent study has unravelled much concerning the biology and host-pathogen relationships of *P. brassicae* and the *Brassicaceae*, briefly reviewed by Dixon (2006). As is very evident from ensuing chapters much is still unknown, not least the true taxonomic status of the microbe, its full life cycle, interactions with hosts and means of controlling a disease which is of considerable and increasing economic significance.

Vernacular Names

Common names for the disease are listed in Table 1. Probably in countries such as Belgium and Germany the multiplicity of names indicates a considerable degree of regionality and localisation reflecting the distinctly separate dialects used by farmers and growers living historically separate states. Naming also reflects the relative importance of the crops affected. In northern Europe cabbage was the major crop grown and hence diseased while further south in France cauliflower was of greater importance. Colonists from Europe took their names for this disease with them to the New World and at the same time this pathogen was exported in animal fodder such as swedes (*Brassica napus*) and turnips (*B. rapa*) used as sustenance for their beasts. In southern Europe the disease was viewed as a form of plant 'hernia' while further north as 'club foot', a term exported by German and Scandinavian colonists with their crops. In recent years the disease has generally been referred to as 'clubroot' as opposed to 'club root'. This reflects a desire for uniformity in the nomenclature of diseases stemming from European Union rules relating to crop cultivar assessment.

Table 1 Common Names for Clubroot

Common names	Country
Australia & New Zealand	Clubroot
Belgium	bosse, gross pied, kanker, klinger, knobbel, knoll, knoop, knotze, knuist, kwab, kwabbe, kwabbel, kwabbeziekte, kwadevoeten, oolen, tol, wratten, verrue,
Denmark	kaalbrok, kaolbrok
Finland	Mohojuuri
France	hernie du chou, gros pied, maladie digitoire, maladie du chou
Germany, Switzerland &	fingerkrankheit, galle, herniekrankheit, huas, kelch,

Austria	klumpenfuss, knotesucht, kohlernie, kohlkropf, krof des kohles, kropfkrankheit des kohles, kuss, nolle,
Great Britain	club root, finger-and-toe, anbury, banbury, clubbing
Italy	ernia, mal degosso dei cavoli, tuberculosi dei cavoli
Netherlands	Knolvoet
North America	club root, club foot, clump foot, finger-and-toe,
Norway	Klumprot
Russia	Kapoustnaja
South Africa	club root, finger and toe, club foot, dik voet
Spain	hernia, hernia de la col, potra
Sweden	Klumprotsjuka

(after Karling, 1968)

Crop hosts

All members of the family *Brassicaceae* are thought to be potentially host to *Plasmodiophora brassicae* and within them it is able to complete both the root hair and cortical stages of its life cycle (see Chapter 2). Cultivated crops appear especially susceptible. This includes all varieties (*sensu* Bailey, 1961) of *B. oleracea*, the Occidental Cole vegetables (Brussels sprout, cabbages, calabrese / green broccoli, cauliflower, culinary and fodder kale, kohlrabi); *B. rapa* (syn. *B. campestris*) including turnip, turnip rape, sarson and the enormous range of Oriental variants which provide leaf and root vegetables such as *Brassica rapa* var. *pekinensis* and *B. rapa* var. *chinensis* (Chinese cabbages); *B. napus* including swede (rutabaga), oil seed rape and fodder rape; seed, condiment (mustard) and vegetable crops derived from *B. carinata*, *B. nigra* and *B. juncea*. Related crops such as radish (*Raphanus*); cruciferous weeds, for example *Sinapis* and decorative ornamentals including stocks (*Matthiola* spp) and wall flower (*Cheiranthus cheiri*) may become infected. The model and rock garden plant *Arabidopsis* is also susceptible. Very few studies of infection have been made outside *Brassica*, *Raphanus* and *Arabidopsis* in the past half century and reliance is largely placed on the knowledge gathered by Colhoun (1958) and Karling (1968). *Raphanus* is credited with possessing a greater degree of resistance compared with *Brassica* but this may solely reflect a lower frequency of virulences in current pathogen populations. Certainly, where radish crops are grown intensively then clubroot disease rapidly becomes a major problem for crop producers. Of the few recent studies of *P. brassicae* on wild host populations probably the most extensive was that of Gustafsson (xxx). Despite intensive searching in the centres of origin of several *Brassica* hosts the microbe was difficult to locate. This leads to speculation that it may be a 'disease of cultivation',

ie increasingly intensive cultivation of brassicas and related crops provided conditions under which the pathogenic microbe can thrive and where it is less subject to predation by antagonistic organisms. In Japan Tanaka and others (2006) found clubroot on the cruciferous weed *Cardamine flexuosa* (bitter cress) in Hokkaido, Aomori and Okinawa. The pathogen appears to be spreading since these authors comment on its presence as new records in isolated island prefectures including:- Sado (Niigasaki), Oki (Shimane), Mishima (Yamaguchi), Tsushima, Iki and Goto (Nagasaki), Koshiki (Yakushima) and Tanegashima (Kagoshima). The host *C. flexuosa* is possibly an alien weed of cultivation having originated in Greece.

Disease Cycle

Primary stages of the life cycle have been recorded in root hairs and epidermal cells of some non - cruciferous hosts in the families, Papaveraceae, Poaceae and Rosaceae (Colhoun, 1958; Karling, 1968). The significance of these infestations in offering an additional opportunities for reproduction by *P. brassicae* are unresearched. These infections may simply be chance encounters providing no reproductive avenues for *P. brassicae* alternatively they could be part of systems as yet unrecognised and unquantified in terms of adding to the ultimate inoculum potential (*sensu* Garrett, 1956) of this microbe. The pathogen persists in soil as apparently very durable resting spores and is reputedly capable of remaining viable and dormant for at least 20 years (see Chapter 3). It is possible to speculate that wild hosts outside the *Brassicaceae* might offer means of maintaining this durability. This is only speculation. Yet *P. brassicae* is so enigmatic that it is only thanks to Dr Anne-Charlotte Wallenhammar's comparatively recent studies that its long term dormancy characteristics have been quantified (Wallenhammar, 1996).

In the disease cycle primary infection of the root hairs by zoospores formed from the resting spores leads to the deformation and curling of root hairs (Samuel & Garrett, 1945) and possibly some root 'epidermal' cells. Thereafter secondary zoospores are the vehicles for cortical infection which results in typical galling and clubbing of the main root systems. Symptoms vary according to whether the host produces a fibrous root system or the 'root' is mainly composed of a swollen hypocotyl as in swede and turnip. In both cases the net result is similar and root tissues become deformed and composed of massively disrupted cells filled by secondary plasmodia and eventually produces vast numbers of resting spores. Current thinking concerning the life cycle of *P. brassicae* is given in detail in Chapter 2 and the interaction of this microbe with the soil environment in Chapter 3.

Symptoms

Infection of seedlings may lead to plant death but invasion in later growth stages rarely kills. Infected plants typically exhibit reversible, foliar wilting when under slight water stress. As the disease progresses the leaves become reddened, chlorotic, necrotic and abscise. Plants become stunted flowering is accelerated with the formation of poor quality curds or spears in cauliflower and calabrese (green broccoli) and small unthrifty hearts in Chinese cabbage. Heavy framed plants such as Brussels sprout become

physically unstable and crops may lodge badly. Seed number and oil quality are depressed in oil seed and turnip rape crops while the roots of swede and turnip exhibit contusions and excrescences on their surfaces. Crop consistency is impaired as a consequence of erratic maturity. Yield, quality, palatability and storability are reduced and land capital value diminished (Dixon, 2007, 1984). The latter effect frequently fails to receive the attention that it deserves. In all countries high quality land capable of producing *Brassica* vegetables is prized and valuable. Once infested with *P. brassicae* then the asset value drops substantially and wealth is reduced. The physiological and biochemical effects of *P. brassicae* are described in detail in Chapter 4. The root malformations produced may be confused with the symptoms of insect damage, especially that of the turnip gall weevil (*Ceutorhynchus pleurostigma*) and on swede and turnip, with the hard swellings of uncertain origin, termed 'hybridisation nodules'.

Handling

Plasmodiophora brassicae cannot be cultured axenically. Galls containing resting spores may be stored at -20 °C for several years with minimal loss of viability. When required the galls are brought to room temperature, macerated either mechanically or with a pestle and mortar. The resultant suspensions are filtered through nylon or muslin sheets removing host debris and then centrifuged at approximately 3,000 rpm for 10 minutes, this yields pellets of resting spores. The supernatant liquid is decanted and discarded. Resting spore pellets are resuspended in sterile water and recentrifuged until clean preparations, free from starch granules, are obtained. Inoculum may be applied directly to seedling roots by dipping, or may be mixed with compost into which seed or seedlings are placed or aliquots of standardised concentration of spores (normally 10^4 - 10^5 resting spores ml^{-1}) applied to the surface of compost in which seedlings are grown (Dixon, 1976a). Inoculated test plants should be maintained with a basal temperature of 20 to 25 °C for up to 6 weeks when galling symptoms will be apparent and these may be assessed by the method of Dixon (1976b). Worldwide physiological specialisation was analysed by Toxopeus and others (1986). On the microgeographical scale there is substantial physiological variation while on the macro-scale it is more limited and reflects the dominant forms of *Brassica* cultivated in the Region.

Economic Significance

Plasmodiophora brassicae is widespread worldwide, especially in mild, moist temperate regions. Epidemics develop quickly as the intensity of *Brassica* cultivation increases. It is thought that movement of the pathogen from Europe resulted from transport of diseased fodder taken by colonists travelling to America, Australasia and other similar centres of settlement. Unpublished information indicates the presence of *P. brassicae* in several other countries, notably Indonesia, Java and Zambia.

List of Countries (based on data from the Commonwealth Mycological Institute, Egham, Surrey UK (Anon, 1977) and the European Plant Pathology Organisation, Paris, France (Anon, 1996)).

Europe

Austria: widespread
Belarus: present,
Belgium: present,
Bulgaria: widespread
Channel Islands: present,
Czechoslovakia (formerly):
Denmark: widespread
Estonia: present,
Faeroe Islands: present,
Finland: present,
France: present,
Germany: widespread
Greece: present,
Hungary: restricted distribution
Iceland: present,
Ireland: widespread
Italy: present,
Sardinia: present,
Latvia: present,
Lithuania: present,

Netherlands: present,
Norway: present,
Poland: present,
Portugal: present,
Azores: present,
Romania: present,
Russian Federation: present,
Russia (Europe): restricted distribution
Siberia: present,
Spain: present,
Canary Islands: present,
Sweden: widespread
Switzerland: widespread
United Kingdom: widespread
England: present,
Northern Ireland (UK): present,
Scotland: present,
Wales: present
Yugoslavia: present,

Asia

Brunei Darussalam: present,
China: present,
Anhui: present,
Fujian: present,
Gansu: present,
Guangdong: present,
Guangxi: present,
Hong Kong: present,
Hubei: present,
Hunan: present,
Jiangsu: present,
Jiangxi: present,
Taiwan: present,

Yunnan: present,
Zhejiang: present,
India: widespread,
Israel: present,
Japan: present,
Korea, DPR: present,
Korea, Republic: present,
Malaysia: present,
Peninsular Malaysia: present,
Philippines: present,
Sri Lanka: present,
Turkey: present,

Africa

Angola: present,

South Africa: present,

Western Hemisphere

Argentina: present,
Brazil: present,
Rio Grande do Sul: present,
Sao Paulo: present,
Canada: widespread

Chile: present,
Guyana: present,
Mexico: present,
Puerto Rico: present,

Trinidad and Tobago: present,
USA: widespread,
Alaska: present,
Hawaii: present,
Venezuela: present,

Oceania

Australia: present,
New South Wales: present,
Queensland: present,
South Australia: present,
Tasmania: present,

Victoria: present,
Western Australia: present,
New Zealand: present,
Papua New Guinea: restricted distribution.

There are several European reports of what appears to be clubroot disease in the 16th and 17th centuries and some illustrations of cruciferous plants by noted artists appear to have swellings which might represent symptoms elicited by *P. brassicae* on the roots. The Agricultural Revolution in the 18th century encouraged the use of root crops such as the turnip as part of the four-course-rotation possibly increased the incidence of disease. The frequency of reports rises substantially in 19th century Europe as agricultural intensity rose in order to feed burgeoning populations serving the Industrial Revolution. In the latter part of the century disease incidence was being noted in North America, Asia and Australasia. Presumably as a result of spread along with colonisation of the mid west and west coast of North America. In north-eastern Europe where cabbage represented a significant part of the diet and was conserved in a pickled form (sauerkraut) to carry populations through the winter the disease appears to have reached severe proportions. In the 20th century clubroot was recognised as a major constraint to the production of cruciferous vegetables on all continents. Records are of variable quality as the disease diagnosis is relatively simple and hence frequently not formally noted in plant pathologists' records. In rural areas where sheep formed a major segment of the agrarian economy the effects of clubroot disease were devastating. In the northeast of Scotland for instance, swede and turnip formed the backbone of winter fodder supplies and Morrison (1977) estimated that 50% of the acreage was infested with *P. brassicae*. In Asia crops of Chinese cabbage in all its multiplicity of variant forms which are of major importance in the diet of local people has been and still is at serious risk from this pathogen. The importance of this pathogen is demonstrated by the intensive breeding of resistant Chinese cabbage cultivars from the 1960s onwards at the National Institute for Vegetables, Ornamentals and Tea (NIVOT) in Japan (see Chapters 5, 6 and 7). Breeding has been supplemented by a successful joint industry-Japanese government search for agrochemicals competent to control the microbe. More recently in the Republic of Korea breeding year round Chinese cabbage cultivars has been accompanied by the search for clubroot resistance. Here there is a significant parallel between Chinese cabbage (*B. rapa*) and European heading cabbage

(*B. oleracea* var. *capitata*) in the Korean pickle Kimshi which was used to carry the population through the winter months. Clubroot disease in China it is recognised as of major significance in depressing the yield of their vast *Brassica* crops such as Chinese cabbage (Daowang and others, 2004). Recently, Jing and others (2008) quantified losses to oil seed rape in China as 17% loss of young growing plants and 15% at maturity with 10.2% loss of yield. They report significant losses to plant height, numbers of siliquae and seed production.

Clubroot is now spread throughout the world wherever *Brassica* crops are grown and is assessed as the major source of disease - induced loss. The severity of infestation and symptom expression increases with the intensity of crop production. The only scientifically valid worldwide attempt to establish the frequency of infestation was Crête (1981). His survey looked at infection in individual *Brassica* crops. High levels of infestation (>10%) were recorded in *B. oleracea* in Australia, Canada, Czechoslovakia, Finland, Germany, Ireland, Netherlands, Norway, Poland, Scotland and Wales; in *B. rapa* in Germany, New Zealand and the United States of America; in *B. napus* in Finland, New Zealand, Scotland and Wales. In total Crête's survey covered 6 million hectares of *Brassica* and related crops and indicated an overall mean infection of 11%. This concurs with the general estimate of in-field losses to individual crop pathogens of 10-15%.

Country	Average % Infection	Country	Average % Infection
Australia	6	Japan	5
Canada	0.11	Netherlands	10
Czechoslovakia	10	New Zealand	15
Denmark	5	Norway	12
England	6	Poland	4
Finland	4	Scotland	48
France	3	Sweden	1
Germany	8	United States of America	10
Ireland	17	Wales	45

Abstracted from: Dr René Crête, Québec, Canada and published in Clubroot Newsletter no. 11 (1981)

The only subsequent surveys have been those of private and public plant breeders used in setting objectives for their programmes of work. These indicate that producing clubroot resistant cultivars is rated highly (see chapter 7) but nonetheless one beset with substantial scientific and technical problems.

The disease is becoming of substantial economic importance in India as the following statement from demonstrates. "Club root disease is the main constraint for oilseed rape and mustard cultivation in Eastern part of India mainly in West Bengal bordering Bangladesh and sporadic occurrence in Orissa states where yellow sarson *Brassica campestris* (*B. rapa*) var. *yellow sarson* has been found to be highly susceptible. Clubroot disease is also a serious problem on cruciferous vegetables in Darjeeling Hills in the eastern Himalayan region and north eastern region states like Manipur, Meghalaya, Mizoram and Arunachal Pradesh bordering China. Bangladesh and China are now

both considered high risk zones for clubroot disease" (personal communication from Dr Indrabrata Bhattacharya, 2008). While in Nepal clubroot disease has been observed since 1993 (Timila and others, 2008). Severe and widespread outbreaks have been seen since 2004 in Bhaktapur, Kathmandu, Lalitpur and the Palung Valley. Disease is especially severe in the Kathmandu Valley and Palung / Dama area of Makwanpur District. Cauliflower appears to be the crop most at risk with 40% overall loss to clubroot. In some parts production was reduced from 5-6 metric tonnes per household plot of 1,500 m² prior to 2004 to 300 kg per plot. A report from Indonesia (Cicu, 2006) comments on the serious nature of clubroot disease but fails to quantify this statement. This reinforces the report of Yamada and others (2004) for West Java, Indonesia.

Possibly of greatest concern from an economic perspective are the recently reported outbreaks of clubroot in Canadian canola crops (*B. napus*) which have raised the profile of this problem especially as legislation now prohibits cultivating this valuable crop on infested land for five years.. This disease has been known for many years in the vegetable crops near Toronto, Montreal and Newfoundland, for example see McDonald and others (2004), Belec and others (2004). Reports of infection in oil seed rape from the area near Quebec in the east (Pageau and others, 2006) indicated the susceptibility of the canola crop. Testing cultivars they found losses in grain yield of 80, 91 and 85% in Argentinian cultivar over 1998-2000 seasons and 69, 96 and 89% in Polish cultivars in the same period. Now clubroot has reached the Great Plains which carry 6 million ha of canola (oil seed rape) (mostly *B. napus* with *B. rapa* to a lesser extent). Appreciable losses in yield of canola are reported by Tewari and others (2005) in Alberta. Previously Donald & Porter (2003) indicated the upsurge in importance of oil seed rape (*B. napus*) as part of Australia's broadacre agricultural economy (\$_{Au} 545 million) indicating the risks posed by clubroot based on the losses sustained in field vegetable brassicas. In Australia these authors estimate clubroot causes losses of at least 50% (1.6 t ha⁻¹) in diseased crops of oil seed rape.

Sources of Information

The International Clubroot Working Group (ICWG) was founded in 1975 and published Newsletters until 1985, thereafter reports from its meetings have been contained in the *Cruciferae Newsletter*. Meetings of ICWG are usually arranged in conjunction with *Brassica* Symposia of the International Society for Horticultural Science (ISHS) and Crucifer Genetics Workshops, International Horticultural Congresses or International Congresses of Plant Pathology. Recently, there has been sufficient research interest to permit free standing meetings such as those held in London (2006) and Turin (2008) (see photograph). Abstracts of the latter are published in *Plant Protection Science* 44 (1). Seed of the European Clubroot Differential (ECD) Series which was developed by ICWG in 1975 is available on request from THE Gene bank, Warwick University Horticulture Research International (Warwick-HRI), Wellesbourne, Warwickshire CV35 9EF, UK.

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