Plasmodiophora brassiciae in its environment

Chapter 3:

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Anderson (1855) "Mr Hunter of Haugh limed part of a field..... at the rate of sixty bolls per scotch acre and the turnips that were afterwards grown on this portion were free of disease..... Yet there are many reports where this failed to happen". This early comment presages the confusion in our understanding of the interaction of this microbe[1] and its environment which has continued for 150 years.**

ABSTRACT

***Plasmodiophora brassicae* Wor., is viewed here from the stand point of being a highly evolved and successful organism, well fitted for the ecological niche that it occupies. Physical, chemical and biological components of the soil environment are discussed in relation to their effects on the survival, growth and reproduction of this microbe. It is evident that *P. brassicae* is well equipped by virtue of its robust resting spores for survival through many seasonal cycles. Germination is probably triggered as a result of signals initiated by root exudates. The resultant motile zoospore moves rapidly to the root hair surface and penetration and colonisation follow. The short period between germination and penetration is one of greatest vulnerability for *P. brassicae*. In this phase survival is affected at the very least by:- soil texture and structure, its moisture, pH, calcium, boron, nitrogen content and the presence of active microbial anatagonists. These factors influence the inoculum potential (*sensu* Garrett, 1956) and its viability and invasive capacity. There is evidence that these effects may also influence differentially the survival of some physiological races of *P. brassicae*. Considering the interaction of *P. brassicae* with the soil environment from the perspective of its biological fitness is an unusual approach, most authors consider only the opportunites to destroy this organism. The approach adopted here is borne of several decades spent studying *P. brassicae* and the respect that has engendered for it as a biological entity. This review stops at the point of penetration, although some of the implications of the environment for successful colonisation are included since they form a continuum. Interactions with the molecular and biochemical cellular environment are considered in other chapters in this Special Edition.**

[1] **The term microbe is used as a short hand reference for *Plasmodiophora brassicae* reflecting its confused taxonomy**

Keywords: *Plasmodiophora brassicae*, clubroot, environment, inoculum potential, biological, chemical and physical interactions

***Plasmodiophora brassicae* Wor., the microbe causing Clubroot Disease[2] of the *Brassicaceae*, is very well fitted for successful life on three counts. Firstly, the robust, well protected and apparently long lived, soil borne resting spores allow this organism to withstand adverse conditions and yet these dormant structures appear to be capable of responding speedily once a compatible host arrives. Secondly, when that host is available the primary zoospores emerging from the perennating spores possess efficient and effective locomotion, penetration and invasive capacities. These features enable *P. brassicae* to exploit the soil environment and its interface, the rhizosphere, with the host to best advantage. Thirdly, once within the host environment the reproductive cycles of *P. brassicae* are shielded from adverse external conditions, allowing the production of multitudinous new resting spores that eventually rebuild the soil inoculum potential (*sensu* Garrett, 1956). During this phase the pathogen appears to have the capability of altering the host's metabolic activities to its own advantage (see chapter 4).**

For only short times and across minute distances in the soil are the primary zoospores exposed to hostile and adverse conditions. While in this soil phase the delicate and vulnerable single walled, zoospores equipped with twin flagellae swim through the soil moisture films from germinated resting spores to the outer surfaces of root hairs. This is the singularly most vulnerable part of the entire life cycle of *P. brassicae*. Yet, this phase has not received the scientific attention it deserves, probably because the tools needed for such study are either lacking or too crude. There are a few sign posts indicating the effects of chemical and physical soil components on *P. brassicae* itself, mostly these have been gathered through attempts to construct highly adverse conditions and hence stop host invasion thereby controlling the disease. Little is known of how *P. brassicae* interacts with its biological environment apart from a few studies of microbes that might offer elements in strategies for control. Indeed even Karling's monograph (Karling, 1968) is sparse on this topic. Perhaps this goes some way towards explaining why effective control of Clubroot Disease has proved so difficult to achieve. This Chapter outlines what is known and attempts to indicate why large gaps exist in our knowledge and how these might be filled. Comprehending the biological fitness of *P. brassicae* is the focus of this Chapter, elsewhere, for example Chapter 9, others have considered manipulating such understanding to the disadvantage of *P. brassicae*.

[2] **The term Clubroot is reserved solely for the host symptoms resulting from infection by *Plasmodiophora brassicae***

Resting Spores

The resting spore of *P. brassicae* is an obvious point to commence considering environmental interactions. The structure of resting spores is described in Chapter 2. These robust spores have the purpose of providing long term survival and perennation for *P. brassicae* and consequently they have evolved to retain viability in the soil despite exposure to many seasons of adverse weather. Field studies indicate they have a half life of at least 3.6 years and some spores may exist for at least 18 years in the absence of suitable hosts before spore populations are eroded to undetectable levels (Wallenhammar, 1996). Apparently long term resting spore longevity perplexed early researchers such as Gibbs (1939), not least because of reports that cruciferous crops grown on land previously carrying permanent grassland leys could become rife with Clubroot Disease after one or two years of arable cropping with brassicas, frequently swedes (*Brassica napus*) grown for stock feed. Temperature, moisture content and position in the soil profile will influence spore longevity (Monteith 1924; Fedorintschik (1935). Soil pH apparently affects the rate of production of primary zoospores, numbers increased in acidic compared with alkaline soils (Bochow (1961) but without much change in total germination. Spore dormancy and the need for external stimulants form elements in the initial relations between *P. brassicae* and the environment (for example see Honig, 1931). Only a few spores on release from rotten roots germinate immediately (Ogawa and others 2001), forms of external stimulus (Ohi and others 2003; Hata and others 2002) could be needed to initiate the process. The readiness for germination of spores released from host roots was examined by a consecutive string of researchers, for example by:- Humphrey, 1892; Chupp, 1917; Naumov, 1925; Honig, 1931; and Colhoun, 1958; generally they concluded that bacteria and other organisms disintegrate the diseased host tissues and 'condition' spores for more effective germination. But these secondary microbes are not essential for the germination process itself. Unknown mechanisms present within the resting spore initiate germination and control its speed. It appears that these mechanisms within individual spores operate quite separately from other spores since not all spores germinate in synchrony.

Rain and flood water disseminate *P. brassicae* over quite substantial distances especially on sloping land. Wind disperses spores that are collected with light, dry, dusty soil particles over even greater distances. Earthworms (Gleisberg, 1922) and possibly moles, root nematodes and insects may be vectors (Chupp, 1924; Eriksson, 1936) for *P. brassicae* in soil. Spores are spread in manure (Gibbs, 1931b) and on farm animals themselves being capable of withstanding the gut environment. Farm animals and their food supplies sailing with European colonists to the New World and Australasia were probably vehicles for *P. brassicae* infesting virgin territory. The worldwide survey of physiological races completed by Toxopeus and others (1986) indicated a predominance of virulences for *B. napus* in these regions. At the more micro-geopraphical level it is thought likely that the pathogen was introduced into the previously uninfected soils of

intensively cropped vegetable holdings in Lincolnshire (UK) as a result of importing sheep to 'clean–up' unharvested broccoli (*B. oleracea* ssp *italic*) (P Corfield, personal communication). Dirty machinery, wheels, boxes and stillages all provide potential means for the spread *P. brassicae*. Wild and weedy members of the *Brassicaceae* and infested crop transplants harbour, spread and perpetuate the pathogen (see Chapter 8). Once established in a soil profile subsequent distribution is related to soil textural and structural properties and the frequency and intensity of husbandry operations. Soil compaction and panning by rototilling reduced the movement of spores into the subsoil as did a large 'A' horizon of top soil with an active rhizosphere (Murakami and others 2003). The population density of resting spores decreased at increasing soil depths, more than 97 % of the total of *P. brassicae* inoculum was present in the surface soil (0-5 cm depth) and few resting spores were found below 40 cm deep soil (Kim and others 2000). Since the density of resting spores is affected by soil type, pH and host susceptibility a combination of these factors determines intensity of the inoculum potential at a particular site. It follows that after germination in a specific environment the inoculum potential of *P. brassicae* produces dose–response curves (Murakami and others 2002) unique to that particular site.

At germination the resting spore volume increases as vacuoles enlarge and the walls thicken becoming more transparent (Woronin, 1878; Favorski, 1910; Chupp, 1917). A single swarm zoospore is liberated from each resting spore leaving behind residual cytoplasm. Germination is characterised by a loss of refractile globules characteristic of stored reserves in dormant spores and probably indicating the enzymic mobilisation of these resources. Immersion of spores in water may encourage germination (Chupp, 1917, Bawden 1948). Ayers (1944) obtained germination in 1 to 10 days using tap water and speed of germination appeared to be dependent on spore maturity. The absolute need for a host stimulus could be questionable as Honig (1931) induced germination below 21^0 C in the absence of seedling roots. Optimal temperature for resting spore germination was established as 24 ^oC and pH 6.0–6.7 with an upper lethal temperature of 45 ^oC and with visible light inhibiting germination. Spores may be stored as dense suspensions at 3-4^oC for 3 years without loss of viability (Macfarlane, 1958) apparently withstanding anaerobic conditions and are not killed by exposure to -20 ^oC for 3 days. It is standard practice to store galls at -20 ^oC for several years as stock inoculum (Dixon, 1976). These few fragments of information are sufficient to identify the resting spores of *P. brassicae* as being very robust, capable of coping with very adverse conditions. Comparative experiments aiming to establish the effects of temperature on resting spore germination, motility and host infection require that spore maturity, age and hydrogen ion concentration in the immediate vicinity of the host-microbe interaction are known, standardised and reproducible. All too frequently this has not been the case.

Evidence for an impact of the host on resting spore germination is provided by Niwa and others (2008) who reported a significant increase in the percentage of germinated spores (lacking a nucleus) in rhizospheres where the host *B. rapa* var. *perviridis* (neep

greens) was present. The involvement of root exudates as stimulants for resting spore germination was postulated and eventually confirmed by:- Chupp, (1917); Hooker and others, (1945); Macfarlane & Last, (1957), Bochow, (1963, 1965) and Macfarlane, (1970). Substantial studies by Kowalski & Bochow (1996) concluded that the stimulant effect for germination is non-specific and could come from exudates emanating of many species and is not confined solely to those from hosts of *P. brassicae*. This was supported by the evidence of Craig (1989) who found that root exudates from both calabrese and perennial ryegrass stimulated spore germination. Some specific stimulants effects were suggested by Ohi and others, 2003 and Hata and others, 2002. Greatest germination (75%) was found to be induced with root exudates from susceptible cabbage hosts. Suzuki and others, (1992) established that an abiotic stimulant could be present in root exudates particularly those from susceptible and resistant Chinese cabbage cultivars. Complex carbohydrate compounds found in the exudates of cabbage stimulated germination (Mattey & Dixon, unpublished) *in vitro*. Possibly several factors may sequentially influence germination, Yun and others, (2007). Yano and others, (1991) established that a release of calcium ions from spores induced their germination. Host plant exudates stimulated resting spore germination which in turn released a second stimulatory factor, encouraging further activity. The environment in which the host plant grows affects the composition of exudates, drought for example, encourages a release of amino acids. Page (2001) identified calcium as a factor in generating soil suppressiveness to *P. brassicae* and hence adversely affecting germination but recognised that this element does not operate in isolation from the effects of soil microbial flora. Similar findings were reported by Stewart (2007) who used a comparable range of calcium sources. The number of resting spores present was adversely affected by adding highly calcareous converter furnace slag to soils in Japan (Shinoda and others, 2005). Direct evidence that the inhibition of spore germination is a primary cause of pathogen suppression under neutral pH comes from Niwa and others (2008). Numbers of germinated resting spore in soil correlates with levels of root hair invasion. When soil calcium declined so did the number of germinated spores and level of root hair invasion. It seems therefore, that not only do host exudates affect germinability but the numbers of spores available for germination in the first instance relates in some way to calcium availability in soil. Potentially calcium and pH may affect the longevity and viability of the resting spore *in situ* in soil. Calcium–rich compost or calcium carbonate changing soil pH from 6.0 to 6.9 and 6.2 to 7.1 respectively significantly reduced the percentage of germinated spores in the rhizosphere and the number of root–hair infections. This research provides direct evidence that spore germination and subsequent root–hair colonisation is retarded by the presence of calcium and alkaline pH values. Earlier Niwa and others, (2007), found the addition over 15 years of large amounts of organic matter raised soil calcium concentration, changed pH to alkaline values resulting in previously clubroot disease conducive soil becoming suppressive. Organic matter suppressed infection by *P. brassicae* and the finer particle fractions (< 5 mm) changed pH most effectively. Calcium hydroxide, calcium carbonate and potassium hydroxide also suppressed

infection with potassium hydroxide being the least effective (see also Webster, 1986). Adding sulphuric acid to a suppressive soil promoted infection by acidifying it. It is concluded that soil pH has major influence on the processes of infection and calcium contributes separately to these influences with both factors operating in unison. Resting spores from 'unnatural' sources such as callus cultures are less capable of germination than those from galls from whole plants (Matsumiya., 1989, quoting the late T. Naiki, personal communication). This may result from such spores differing physiologically from those grown under natural conditions perhaps as a result of the callus culturing system as suggested by D S Ingram (personal communication). Resting spores have been estimated by the methods of Shinoda and others (2003) and this topic is further explored in Chapter 8. Resting spore numbers per diseased plant increased with low values of disease severity but thereafter, remained almost constant for plants with category '3' symptoms and beyond (Dixon & Doodson, 1970, 1971, Dixon 1977, 1984b). Mean numbers of resting spores per diseased plant ranged from 9^3 to 10^9 regardless of the value of the disease index having apparently passed a saturation threshold. When resting spore load in soil reaches even modest concentrations disease severity increases (Murakami and others 2004).

Zoospore Motility and the Processes of Invasion

Direct knowledge of the movement of zoospores is very limited relating as it does to behaviour after liberation from the resting spore to the point of encystment on root surfaces within soil. This could be termed *Colhoun's Dilemma* (Colhoun, 1958) since *in vivo* studies are obscured by soil and *in vitro* studies are limited by the problems associated with culturing a minute biotrophic microbe which defies axenic culture. The *Dilemma* continues to restrict knowledge even 50 years after Colhoun's labours. Possibly flagellae are those parts of the zoospores primarily affected by soil pH, moisture, calcium, temperature and interactions with other microbes. The motility of other flagellate organisms is known to be affected by such factors, but little information is available for *P. brassicae*. There is a helpful treatment for some other flagellate organisms in Amos & Duckett (1982), while de Weger and others (1987) confirmed that removal of flagellae from the bacterium *Pseudomonas fluorescens* impaired subsequent colonisation of potato roots. Attributing a combined function of locomotion and location to the flagellae of *P. brassicae* is supported by Dick (1997). Less environmental specialization was suggested in the primary zoospores as compared with secondary stages Dixon (1984b).

Soil Moisture

Soil moisture was from the earliest studies of *P. brassicae* viewed empirically as the medium by which the host is reached. In practice of course, the impact of seasonal water supply varies, thus while clubroot is regarded as a disease of wet soils there are many reports of its severity increasing during dry seasons or on dry sites. This perhaps reflects a loss of productive root systems which renders foliage highly susceptible to

water stress in periods of soil deficit. Clubroot disease is however, considered as associated with low lying, poorly drained soils and disease is severest after wet weather. For this reason soil moisture is classed as a dominant environmental factor in interactions with *P. brassicae*. This contention is supported by only limited serious scientific experimentation.

Colhoun at variance to Monteith (1924), Naumova (1933) and Larson and Walker (1934), obtained infection up to pH 8.2 where moisture content was at 70% maximum water holding capacity (Colhoun, 1952, 1953). Thereby, he demonstrated that plants grown under alkaline conditions were vulnerable to clubroot disease when other environmental factors are weighted heavily in favour of the pathogen. In support of this contention, infection developed in 10 to 18 hours with an excessively moist soil (Wellman, 1930). Hence, when soil moisture content rises above 50% soil water holding capacity disease develops very quickly demonstrating indirectly the speed at which primary zoospores travel. Variations in the effects of soil moisture content may well reflect differences in the textures of soil used by differing researchers. Texture could affect the motility of *P. brassicae* zoospores as suggested by Samuel & Garrett (1945) since in their experiments sand–soil mixtures produced the highest levels of infection. Infection developed at moisture levels as low as 9% in mineral soils whereas 60% was necessary with organic soils (Hamilton & Crête, 1978). Where soil moisture rises from 50% of maximum water holding capacity up to saturation (Dixon, 1981, 1984b) disease severity escalates. Lange & Olson (1983) emphasised the dependence of zoosporic microbes on free water existing between the soil crumbs for the movement of zoospores. Free water is critically important for the formation, discharge and dispersal of zoospores and may influence the encystment and penetration processes at the root-hair surface. The distances travelled by soil borne zoospores are relatively short, probably between 10–20 mm judging by information for *Olpidium brassicae* or *Synchitrium endobioticum* both relatives of *P. brassicae.* Invasion of root hairs occured up to 75mm from the source of *P. brassicae* infection in soils where water mass movements were minimised (Watson, 1967). Mathematical modeling by Yang and others (2004) demonstrated a relationship between soil moisture and host invasion.

Temperature

Temperature has been regarded as a factor of lesser importance than soil moisture affecting the successful movement and invasion of *P. brassicae*. Its study has produced conflicting results in a similar manner to soil moisture and for comparable reasons. Severe infection developed in acidic soils at air temperatures of 16.6 ^{0}C, when alkaline soils are used disease expression was less severe. Disease development was more favoured when working with alkaline soils by air temperature of 23 ^{0}C and fluctuations around the mean (Colhoun, 1953). As with soil moisture Colhoun's results showed that providing conditions where a cardinal environmental factor greatly favoured the pathogen allowed disease development despite other apparently disadvantageous factors. Previously, temperatures below 20 ^{o}C were thought to present a barrier to

clubroot disease development (Chupp, 1917; Gibbs, 1931a). But, Monteith (1924) showed that symptoms developed throughout the range 9 to 30 ^{0}C and was supported in this contention by Wellman (1930). In New Zealand Ayers, (1944) identified the minimum temperature for root hair infection of swede at 12-14 ^{0}C. Studies by Johnson in the 1960s at the Welsh Plant Breeding Station [now The University of Wales, Aberystwyth] (Johnson, *personal* communication) showed that the early stages of root hair infection immediately following inoculation required temperatures > 22.5 ^{o}C. Once root-hair invasion was completed then he believed that lower and fluctuating temperatures were sufficient to support symptom formation. Growth analysis studies by Buczacki and others (1978) suggested that temperature is most significant as a regulatory factor in the second week after inoculation, this is when root hair colonisation reaches its peak and zoosporangia are forming.

It is possible that the predominance of individual environmental factor alters according to the stage in the life cycle under consideration. Webster (1986) postuled: "that when one factor limits disease expression (such as pH) another may significantly modulate their levels (such as temperature)"; this implied that one factor sets an actual limit while another interacts establishing a frequency or intensity. This argument is consistent with Colhoun's previous contentions. Wallenhammar (1999) citing C. Williamson suggested that galling could develop at 7 ^{o}C with diurnal fluctuations in temperature and increases of 8 to 12 hours in day length. Certainly rising spring temperatures were associated with infection and disease expression in Swedish oil seed rape (*B. napus*) crops Wallenhammar claimed as daylength increases and host growth accelerates. Interactions may go further and relate to effects on the constitution of the host since Robak & Gabrielson (1988) found that temperature influenced the expression of host resistance, a finding not uncommon with foliar invading microbes but not frequently suggested for soil borne organisms. He suggested that cauliflower cvs resistant at 15 ^{o}C could show susceptibility at 20 ^{o}C with high inoculum potentials.

Light Intensity

Since this is a soil borne microbe it would not be expected that light has any significant impact on growth. But Colhoun (1961) showed that light intensity had marked effects on the relationship between spore load and the number of plants that develop clubroot disease. Consequently, Grainger (1962) was able to relate clubroot development with his C_P/R_S ratio system where C_P = the weight of total carbohydrate in the whole plant and R_S = the residual dry weight of the shoot. Webster (1986) commented that "light may influence disease expression via an effect on host photosynthetic efficiency and hence on energy reserves available to fuel clubbing". Light may also regulate the balance between shoot and root growth. Rausch and others (1981) showed that low light intensities gave a greater reduction in root growth in infected compared with control plants.

Soil Texture and Structure

Some early workers associated clubroot disease expression with light soils (Colhoun, 1958), others expressed a contrary view (Milburn, 1855; Russell, 1859; Eriksson, 1930). In controlled experiments Palm & McNew (1956) demonstrated infection happened more readily in mixtures of soil and sand or of clay and sand or in undiluted soil as compared with pure sand cultures. Soil compaction and consequent water logging caused by animals or machinery is associated with increased disease severity around headlands and gateways (Anderson, 1855, Russell, 1859, Somerville, 1895). By contrast Anderson (1855) associated the disease with loose open soils and Somerville (1895) associated the disease on turnips (*B. rapa*) with soil aeration caused by hoeing during winter and Larson & Walker (1934) contended that aerating alkaline soil favoured infection. Light, sandy, humus–rich and clayey soils are thought most disease prone. As with the interaction of clubroot and acidity or alkalinity there is a dearth of rigorously tested science–based evidence concerning disease development and soil characteristics. Soil type was shown by Tinggal (1980) to influence the disease causing abilities of physiological races of *P. brassicae* (as defined by the European Clubroot Differential Series, ECD). Clay and loam soils are prone to compaction and may also be calcareous resulting in interactions between conducive and suppressive effects moderated by factors such as inoculum potential thereby determining disease expression. Soils of high water holding capacity such as silts are likely to encourage this pathogen. Soil type interacting with calcium and pH are considered by Campbell & Geathead (1996) as factors determining disease intensity.

Spore Load

A relationship between root hair infections and cortical clubbing could be expected from epidemiological theory (van der Plank, 1975) and was investigated by Naiki and others (1984) using the ECD series of genotypes and 54 common Japanese crucifers. Despite detailed recordings of root hair infection in susceptible and resistant types, disease frequency could not be related to subsequent clubbing reinforcing the results of much earlier studies such as those by Naumov (1925). Nonetheless, spore load is accepted as seminally important in determining the intensity of subsequent disease especially at low concentrations. Key studies by Samuel & Garrett (1945) where number of infected root-hairs increased concomitantly with spore density and Macfarlane (1952) who related rising spore load and percentage of clubbed plants laid early foundations to knowledge in this area. But importantly Macfarlane's (1952) association broke down at very high inoculum levels when he postulated that an early supply of nutrients was available. There are interactions with spore maturity such that older spores appeared more capable of causing infection compared with younger ones in what Colhoun (1958) termed 'infective power'. Under less than optimal environments spore load related to the number of diseased plants thus with alkaline soils he found a direct relationship between spore load and number of diseased plants.

The conditions under which resting spores are stored affected their viability (Macfarlane & Last, 1957) while germination was encouraged and the proportion of germinated spores was increased by the presence of host root exudates. Interacting factors such as:- moisture, temperature, pH, light intensity, and internal factors including spore size, age and nutritional status affected the overall outcome of the interaction between host and parasite. "Inoculum pressure is modified by environment" noted Webster (1986), she concluded that under environmentally unlimiting conditions, and below the threshold level of infection needed for maximum disease expression, severity of clubbing is proportional to increasing inoculum concentration and total root hair infection. Above this threshold level, however, increasing spore concentration may generate higher root-hair infection levels but not relate to increased disease severity. Webster's 'threshold' is effectively a saturation point beyond which the physiological and biochemical processes (see chapter 5) governing symptom development within the host cannot be affected by inoculum load. Saturation itself is not a fixed and immutable value since Webster's work supported the view that only a low percentage of spores in an inoculum are capable of causing successful infection or invasion at any one time. Saturation of the root hair space within an observed section of root, distribution of spores around susceptible root-hairs and distances over which they can travel are all factors influencing the chances of an additional spore being able to establish an infection and proceeding to cause clubroot disease. The two stage life cycle of *P. brassicae* inevitably means that only a limited number of invading zoospores can ultimately proceed to incite symptoms. Not least there may be competition for root-hair space by different physiological races of *P. brassicae* as suggested by Jones (1980). Both antagonistic and synergistic relationships between races of *P. brassicae* may affect relationships between physiological forms (Dixon, 1979, 1980, Jones and others, 1981, Dixon and others, 1981). Within any population of *P. brassicae* spores there may be a range of vigour or infective capacities such that some on establishing infection proceed more rapidly through the life cycle than others. Jones (1980) reported that more than one physiological race of *P. brassicae* may occur within a population or within a spore suspension prepared from a single gall. Jones and others (1981) and Dixon and others (1981) further demonstrated competition between physiological races in experiments using inocula composed of mixed races. Races varied in 'vigour' or 'aggressiveness as indicated by the intensity of differential reactions between races and hosts using the ECD series. For example, inoculum from *B napus* cv Marian clubs has been shown to generate a higher disease index on cv. Wilhelmsburger than on cv Nevin, but the reverse was the case for incoculum from cv Acme clubs (Dixon and others 1981). Recently, Tanaka and others (2006b) suggested that there is a suppression of plasmodial development during secondary colonisation in *B. rapa* ssp *pekinensis* cv Kubai 70 which occurs differentially when using a range of isolates of *P. brassicae*. How differences in vigour, aggressiveness or infective capacity are derived and when they come into action are questions yet to be answered.

Host resistance may be considered as an environmental component affecting the success of *P. brassicae.* In that perspective it becomes an additional sink for the energy expended by invading spores. The process of breaching host resistance may be a function of the biological fitness of successive waves of invasions by zoospores both primary and secondary and diminishing general resistance in the host. Ultimately, successful infections are established in the root-hairs of resistant cultivars. It appears that specific resistance is expressed against the secondary phase of the *P. brassicae* life cycle. Hence during that phase more energy may be expended by *P. brassicae*, fruitlessly where robust resistance is present but more successfully when this is not the case. In view of the highly polygenic nature of some forms of resistance to *P. brassicae* especially in *B. oleracea* these events might go some way towards explaining the lags in time between invasions which result in less advanced states of infections on assessment days where plants are subjected to lower inoculm concentrations. As a result infection numbers fall below an observable threshold and in practise are not counted in assays used to determine the value of resistant genotypes. These phenomena have generated much discussion of what constitutes observable or phenotypic resistance to *P. brassicae* (Toxopeus and others, 1975).

Calcium

Possibly the most vexed issue relating to clubroot disease is soil calcium content and the associated hydrogen–ion content (pH) of soil. Calcium emerges as a fundamental factor in the life cycles of both *P. brassicae* and its hosts. Datnoff and others (2007) summarised the involvement of calcium in host metabolism, physiology and signalling of many host-pathogen interactions indicating a relationship with expression of resistance. From the earliest studies of *P. brassicae* and clubroot onwards the disease was associated with acidic soils and claims that it was alleviated by the use of various forms of agricultural lime. Much of the work is, however, contradictory in terms of the forms of lime used, their sources, rates applied, date of application, recipient soil types and the measurement of efficacy. It is now possible to conclude that clubroot disease incidence is not limited at pH 7.0 as is still claimed especially in much farm advisory and home gardening literature. As commented by Colhoun (1958):- "results obtained by field experimentation show the difficulty encountered in determining the *exact upper limit* (my italics) of the soil pH at which infection can (*still*) occur". This begs the question as to whether there is an *exact upper limit.* Colhoun goes on to argue that "observations have been made without due attention to the variety of other factors which also influence infection" are of little if any value. He advocated the use of potted seedling tests which could be completed in 'controlled' conditions. As he also indicates pot tests have been undertaken at high soil moisture content but have failed to control spore load for example and they are much affected by seasonality. Glasshouse experiments running through a winter are far less acceptable because of the weaker host growth compared with those made in spring or early autumn, while summer time experiments are likely to suffer from excessive lifts in air temperature. The chemical

and physical forms and quantities of calcium used also affect the results and add further levels of variables to each experiment. Here again Colhoun (1958) reinforces, as with moisture and temperature, lessons from the classical studies of Samuel & Garrett (1945) related to the impact of spore load, inoculum potential and intensity. Theirs was one of the earliest scientific validations that the effects of pH and of calcium could be separated and quantified individually as factors influenceing the environmental success of *P. brassicae*.

Subsequent to Colhoun (1958) practical studies indicated that the impact of the balance of nutrients in the soil is significant while the actual content of individual ions is still important. For example, Myers & Campbell (1985) suggested that clubroot disease expression depends on the balance between pH and the amounts of calcium and magnesium in the soil. While Dobson and others (1983) concluded from their work using roughly and thoroughly mixed limed soils that if roots and spores occur within small pockets of low calcium and / or low pH, invasion is possible despite high overall soil calcium and pH estimations. Fletcher and others (1982) achieved greatest effects of clubroot disease with field applications of calcium carbonate and calcium nitrate which increased pH to 7.9 and 8.3 respectively. They also concluded that although pH was a major factor in reducing disease expression, some other factor than pH possibly the Ca^{++} (calcium) ion itself was involved. Using controlled conditions Hamilton & Crête (1978) formed similar conclusions. These results still however, beg the question of "where and when is *P. brassicae* influenced by the presence of calcium and by pH value?" There is a tendency to assume that these factors affect the microbe while in the soil but since *P. brassicae* spends most of its life cycle within the host it could be fair to suggest that calcium and pH also affect these environments. A role for calcium in the post-infection development of *P. brassicae* is supported by the demonstration that incorporation into roots is pH-dependent (Myers & Campbell, 1985) also Campbell & Greathead (1996) contended that *P. brassicae* is affected at more than one point in the life cycle between spore germination and the completion of resting spore formation in the cortical cells by pH and calcium concentration. Detailed long term experimentation of:- Webster (1986), Dixon & Webster (1988), Webster & Dixon (1991), Dixon and Page (1998) and Page (2001) has confirmed this. It is evident that the greatest impact of calcium is when it is present in the period between spore germination to post-penetration of root-hairs. The latter appears to be when root-hair infection has the biggest impact on subsequent gall formation. There may apparently be separate mechanisms since the periods 0-3 and 0-7 days post-penetration seem to be separated in the extent of their influence on subsequent disease development. The expression of effect seems to be cumulative since it took longer when a 30 mel^{-1} Ca^{++} solution was used as compared with one containing 55 mel^{-1} Ca^{++} in order to achieve similar final results. The host-pathogen response varies also with pH however, that is a separate factor. But it is worth recording here that calcium at pH 7.2 needed to be present by day 14 in order to suppress root-hair infection or alter the progress of galling. The pathogen may be affected by the calcium environment in the root-hair and this alters subsequent

behaviour in the cortical cells.The work of Webster (1986), Dixon & Webster (1988), Webster & Dixon (1991), Dixon and Page (1998) and Page (2001) is supported by results of Donald and others (2004), Donald (2005) in Australia (see Chapter 9). Of major significance is the finding that high concentrations of calcium at pH 6.2 or 7.2 reduce total numbers of root-hair infections and the rate of maturation through plasmodial, sporangial and zoosporangial stages as compared with the controls. Raised concentrations of calcium completely inhibit the later stages of *P. brassicae* development in the root-hair even where high inoculums doses are applied. The calcium effect commences in the soil since Dixon & Page (1998) showed that the germination of resting spores, motility of zoospores and the composition of benign microbial flora around roots are altered. High concentrations of calcium could possibly reduce flagellar action as Satir (1982) and Sleigh & Barlow (1982) reported that changes in calcium of the order of 10^{-6} to 10^{-4} M affected the action of demembranated flagellae, whether this would hold for the flagellae of *P. brassicae* requires to be determined.

Acidity and Alkalinity

Recently, Wallenhammar (1999) pointed to the uneven distribution of acidic and alkaline areas of soil in individual fields with pH ranging from 5.73-8.45 in localised patches. Mattsson (1995) identified that pH values of the subsoil are frequently more alkaline that the upper horizons in Sweden especially in the calcareous glacier clay region near Uppsala in eastern central Sweden. This modernises aspects of *Colhoun's Dilemma* related to pH. Earlier Palm (1958, 1963) had concluded that the effect of pH is not restricted solely to the establishment of *P. brassicae* as a parasite because the rate of gall proliferation was markedly suppressed by an alkaline condition of the medium after infection in the host tissues. It was suggested that changes in the soil reaction may have more drastic effects on gall development than on the number of infections by zoospores. Using organic buffers Myers & Campbell (1985) adjusted pH and calcium content separately from each other and showed that at 10 mel^{-1} Ca^{2+} and a pH of above 7.1 reduced the numbers of primary zoosporangia in root-hairs thus inhibiting galling. Webster & Dixon (1991) demonstrated that the effects of pH are independent of calcium concentration and found that alkaline pH reduced total root-hair infection number and retarded the maturation of plasmodia, sporangia and zoosporangia. The pH effect on the maturation of root-hair infections is activated by exposure to alkaline pH within 3 days of penetration. Prolonged exposure beyond 3 days gives no additional effect.

There may be a dual effect in that alkaline pH increases sensitivity of the host and / or *P. brassicae* to calcium effects as well as increasing the efficiency of calcium uptake. The effects of pH and calcium are remarkably similar but this does not necessarily mean they are one and the same as has been suggested by some workers. They may regulate the pathogenic potential of an inoculum quite separately. Since pH regulates the response to calcium intracellular function may be modified in addition. A high concentration of H^+ ions in plant tissues is potentially antagonistic to calcium. Membrane permeability is lowered by both alkaline pH and by high calcium. This

environment could affect the growth and reproduction of *P. brassicae* as it proliferates within the host root-hair and epidermal cells or within the cortical cells. Alkaline environments could affect primary and secondary invasions, cortical migration and cell hypertrophy. An involvement of Ca^{2+} ions in the growth and reproduction of *P. brassicae* ultimately leading to induced cell death or hypersensitivity is suggested by Takahashi and others (2006). At the agronomic level promoting high alkalinity linked with continuous cropping is suggested by Shinoda and others (2005) as a means of reducing the soil inoculum load.

Boron

This element has associated with affecting the activities of *P. brassicae* from the 1930s (O'Brian and Dennis, 1936) onwards. One of the first controlled studies was that of Palm (1963) who investigated the effect of boron on *P. brassicae* in sand cultures and recorded maximum root-hair infection at 0.3 mel^{-1} or less. He further demonstrated that in the absence of boron the inhibitory effect of calcium on root-hair infection is suppressed, he suggested that lime may fail to diminish clubroot disease in boron deficient soils. Dixon and Wilson (1983), Dixon (1983), Dixon & Wilson (1984 ab), Dixon 1984 (ab), Dixon & Wilson (1985) Dixon (1985) have achieved significant reductions in disease index with sodium tetraborate applied to acidic granitic soils in three successive years of field studies. More recent studies showed that environments with elevated boron concentration there are significant effects both in the root-hair and cortical phases of *P. brassicae*. Throughout the *in planta* stages of the life cycle of *P. brassicae* boron has an impact on the microbe. There appears also to be a relationship with the quantity of boron in the plant which is moderated by uptake over time and space as determined by the size of the plant root system and its capacity to absorb boron. It is likely that there are interactions with other ions. For example, lime applications in the forms of calcium carbonate or oxide may alter the nutrient environment in soil to the detriment of *P. brassicae* and therefore, make the host–parasite association more affected by other factors such as boron. Alternatively, boron may have a primary effect because Webster & Dixon (1991) found that the effects of boron interact with both the primary and secondary stages of development of *P. brassicae* ultimately affecting the intensity of symptom expression. The environment induced by boron in cells where membrane permeability and wall structure are altered may be to the detriment of *P. brassicae*. It could also make for conditions less conducive for nuclear division by the microbe. Quite possibly boron effects are distinct from those of calcium and pH. Dixon (1991) identified that boron affects the progress of *P.brassicae* by retarding the rate of sporangial maturation. The correlation of diminished intensity of disease expression and boron suppression of root-hair infection and gall formation appears related to host exposure. Long exposures to low concentrations seem to equate with the effects of shorter exposures to higher concentrations. Field and controlled laboratory studies (Craig & Dixon 1993 ab) identified that boron has a substantial effect on the ability of *P. brassicae* to invade root-hairs and establish colonisation in the field. Raising the

boron content of the rhizosphere prior to the availability of a susceptible host to infested soil limited the subsequent ability of *P. brassicae* zoospores to penetrate, colonise root hairs and cause symptoms.

Nitrogen

Nitrogen is reported as influencing host parasite associations (see recent general summary by Datnoff and others, 2007), yet there have been few investigations into its effect on clubroot disease. High concentrations of nitrate (4–6 times standard) consistently suppress disease symptoms and Webster (1986) postulated a two phase response with low concentrations enhancing and high concentrations retarding, as found with other biostimulants (Dixon, 1991). Adding nitrate nitrogen results in the stimulation of cellular free amino acid pools. If this stimulated arginine or lysine rich histones then this could possibly lead to repression of RNA polymerases in the microbe preventing it from making access to the gene products needed for pathogenesis. Webster (1986) postulated that as nitrate concentration increased enzyme sites became saturated with consequent substrate and / or product inhibition and the amino acid moieties being diverted towards forming an environment inhibitory to *P. brassicae*. Nitrate metabolism is regulated by the availability of reduced co-factors NAD(P)H (nicotinamide adenine dinucleotide (phosphate)–reduced) for conversion to the ammonium form (Hewitt, 1970). If under conditions of high nitrate supply all available NAD(P)H were used up, then a shortage of such co-factors could influence the activity of *P. brassicae in planta*. Raising nitrate levels above 20 mel^{-1} reduced symptom expression and numbers of infected plants. Results obtained in controlled conditions using split root techniques (Dixon & Khatan, unpublished, 1997) demonstrated the effects of nitrate ions in influencing the rhizosphere environment to the detriment of *P. brassicae*. These results were then supported by glasshouse and subsequent field experiments (Dixon, 2009a). In experiments in India, Bhattacharya & Mandal (2006) found that calcium ammonium nitrate and calcium nitrate significantly reduced the intensity of clubroot disease and supported the results of Page (2001). She showed by detailed laboratory and field studies that calcium nitrate is associated with decreases in *P. brassicae* infection with a subsequent reduction in the severity of symptom expression. During the soil phase of *P. brassicae* the viability of resting spores and the ability of primary zoospores to invade the host are reduced by the presence of calcium nitrate. There may be changes to the fitness of *P. brassicae* as a result of the presence of calcium nitrate. In this compound, calcium is available in a highly soluble form linked to the nitrate ion. Page (2001), also concluded that the presence of calcium nitrate in the rhizosphere may also have been associated with changes to the dominant physiological race of *P. brassicae*.

Interactions between the fertiliser calcium cyanamide, *P. brassicae* and other soil microbes have been studied for well over 70 years. A substantial body of information has been built up (Dixon & Wilson (1983); Dixon & Williamson (1985); Naiki & Dixon (1987); Coulshed & Dixon (1990); Humpherson-Jones and others (1992) and Dixon

(2009b) demonstrating that calcium cyanamide and the products of its degradation, calcium and nitrate nitrogen, are associated with the reduced viability of *P. brassicae*. Much further research is required to elucidate the means by which this effect is achieved, but it is now considered likely that calcium cyanamide alters the balance of biological components in the soil environment surrounding *P. brassicae*. Thereby, the growth and reproduction of soil-borne microbes antagonistic to *P. brassicae* are encouraged. Detailed research worldwide by for example:- Klasse (1999) in Germany; Donald and others (2002 and 2004), Donald (2005) in Australia; Porth and others (2003) in the USA; McDonald and others (2004), Belec and others (2004) Tremblay and others (2005), Manolii and others (2005) in Canada and Murakami and others (2002) in Japan, supports the contention that adding calcium cyanamide to soil infested with *P. brassicae* results ultimately in the reduction in the intensity of clubroot disease (see also chapter 8).

Other calcareous substances such as calcified seaweed (a form of coral), or true extracts of algal seaweed which contain inorganic nutrient ions and organic compounds including plant growth regulators and extracts of composts (Tilston and others, 2002) have been associated with changes to the biological environment of soil to the detriment of growth and reproduction of *P. brassicae*. Recently, particular interest has focused on phosphonate and phosphite formulations. These apparently interact with the secondary disease expression phase but no indication has yet been offered for their role in the soil environment (Abbasi & Lazarovits, 2006 a & b). Sen (2005) comments on effects of molybdenum along with calcium and boron in the root environment in relation to pathogenesis of *P. brassicae* on rapeseed mustard in West Bengal India. Interaction of the nutrient environment, pathogenesis and resistance is discussed by Dixon & Walsh (1998), Huber & Graham (1999), Dixon (2002).

Biological Soil Constituents

Little is known of the relationships between *P. brassicae* and the macro- and micro-flora and fauna in soil. The free swimming zoospores of *P. brassicae* are undoubtedly at risk from the predatory habits of soil inhabitants. Instances of 'disease suppression' may well relate to the presence of such organisms which could increase in unquantified amounts either naturally or following husbandry activities. Soil suppressiveness to pathogenic organisms resulting from the activities of saprophytic microflora is a well accepted phenomenon (Alabouvette and others, 1996) validated by extensive research. Adding organic or inorganic soil amendments that stimulate the microflora has a significant effect the survival of *P. brassicae*. Bacteria such as *Bacillus* spp. and fluorescent *Pseudomonas* spp. are recognised as affecting the growth of *P. brassicae* (Einhorn and others, 1991). Biotic suppressive soils were identified in Taiwan with pH of above 7.4 and a calcium content of 1210 ppm (Hseih and Wang, 1986). Since the resting spore walls contain chitin it is likely that chitinolytic bacteria could be major antagonists of *P. brassicae* reducing the inoculum potential (Anon 2008). Antibiosis resulting from microbial sources has usually been approached as a means for the

biological control of *P. brassicae* as opposed to developing an understanding of the ecological relationships between organisms.

Extensive studies of soil suppressiveness relating to *P. brassicae* have come from researchers in the Fukushima area of northern Honshū, Japan. Haplic andosol soils were found to be more conducive to *P. brassicae* than low-humic andosols even when high spore concentrations were present in the latter. It was suggested that the suppressiveness of low-humic andosols relates to the presence of biological antagonists (Murakami and others, 2000). Biotic supression of *P. brassicae* in presence of Chinese cabbage (*B. raopa*) host plants reportedly resulted from the presence of the soil endophytic fungus *Heteroconium chaetospira* (Narisawa and others, 2005). Soil moisture content, pH and spores density significantly affected the level of repression of *P. brassicae*. Crop rotations particularly those containing maize (*Zea mays*) depressed the activities of *P. brassicae* (Yamada and others, 2003). This may be expressed as ecological interaction and biological control as described by Dixon (2003 ab).

Primary plasmodia were found in the root cultures of both susceptible and resistant cultivars by Takahashi and others (2006) but secondary plasmodia proliferated only in cultures of susceptible hosts. These authors concluded that the alkalisation of the root culture of resistant cultivars was responsible for this difference. In the rhizosphere (Nicholas, 1965) saprophytic species thrive supported by nutrients in plant root exudates. This is the environment in which the primary zoospores of *P. brassicae* are actively attempting penetration and colonisation of the host root-hairs. This is a dynamic situation in constant flux as the microbial flora changes under the influence of substantial alterations to root activity, for example root-hairs themselves last for only a few hours, and the range of microbial species alters in some instances almost hourly. This situation must have a major impact on the inoculum potential of *P. brassicae*. Ultimately, this potential includes the supply of biological energy needed for penetration and colonisation of a host. It is a function of inoculum density or intensity (mass or units of inoculm per unit of soil), available nutrient (both internal and external to the propagule), environmental factors and genetic capacity of *P. brassicae* itself (extended from Martinson, 1963). For a particular host species, the levels of genetic resistance varying as they may do during its life cycle and interactions with the environment are key factors determining the outcome of encounters with *P. brassicae*. Combining all these factors of host and microbe interacting with their respective environments offers a predictive index for the success of growth and reproduction by *P. brassicae*.

Despite well over a century of study there is little information concerning the life and death of *P. brassicae* in soil. That is now in very marked contrast to our understanding of interactions within the host (see chapters 4, 5, 6, and 7). Dixon & Walsh (1998) showed that while *P. brassicae* only interacts with the soil environment for a short period both in time and space, this period is critical for the success of *P. brassicae* in establishing subsequent growth and reproduction within the host. These authors emphasise that the rhizosphere is not a rigid entity either in its parameters of shape,

content or time. The propagules of *P. brassicae* experience an environment in which there is an irregular distribution of nutrients, water and oxygen. Elements such as calcium which move towards the root suface by mass flow may accumulate in the rhizosphere in quantities larger than required by the plant roots for uptake. As a result surplus ions accumulate in the immediate environs of the rhizosphere and could have immense impact on *P. brassicae* for example; while the rhizosphere around the root apex is acidic that further back can by alkaline with obvious effects on the colonisation and invasion of those areas by this microbe. Important aspects for the survival of soil borne microbes include the conditions prevailing at the time of arrival at the root surface and in the surrounding rhizosphere affecting establishment and penetration (Bowen & Rovira, 1999). This aspect of the infection court has been rarely examined for even a few pathogenic microbes and not at all for *P. brassicae*. Only work such as that of Page (2001) showing that under some circumstances the presence of calcium and nitrate nitrogen can be associated with changes in the virulence spectrum of the pathogen population begins to indicate the powerful forces present in the rhizosphere which impinge on the primary zoospores of *P. brassicae*. It appears that signals could pass out from the host root to the resting spores of *P. brassicae* triggering germination and then the primary zoospore proceed with location finding and flagella motion towards the root surface (see Figure 1). There are suggestions that sugars and / or carbohydrates in root exudates are involved in triggering the germination of *P. brassicae* and subsequent processes. If this is accurate then quite possibly these compounds also offer an external source of energy for the microbe. Location and direction finding by the zoospore is most likely a result of some form of general or specific chemotaxis such as a gradient of compounds such as carbon dioxide, oxygen or a redox gradient. Alternatively, the zoospores may simply be passively swept through the soil water films by physical flow. That might explain why high levels of soil moisture are prerequisiste for successful colonisation. Since temperature is also an important factor possibly it regulates the rates of energy release within the zoospore and chemical interactions with soil components.

While *P. brassicae* is germinating and in motion the primary zoospores are subject to attack by other soil inhabitants such as *Bacillus* species. Decoy crops such as the Japanese leafy daikon (*Raphanus sativus*) appear to operate by encouraging resting spore germination and root-hair colonisation but without successful secondary stage colonisation and subsequent symptom expression (Murakami and others, 2000). Presumably such hosts offer sources of energy for germination and colonisation but without an internal environment conducive to the growth and reproduction of the secondary stages of *P. brassicae*. The degree of decomposition of organic matter apparently influences its suppressive effects in soil and could indirectly also influence these processes.

The fungus *Heteroconium chaetospira* inhibited the activities of *P. brassicae* even where soil physical conditions of moisture and pH would have otherwise have been conducive

(Narisawa and others 2005). Chinese cabbage (*B. rapa*) roots became colonised by hyphae of *Heteroconium chaetospira,* but there is no visible evidence of host cell wall degradation, host reactions or invagination of the host plasma memberane around the hypahe (Yonezawa and others 2004). Earlier work by these authors linked the suppressive and electrical properties of soil. Those with negative charges matching similar potential on spores of *P. brassicae* were conducive to development of the microbe while those with positive charges were suppressive (Murakami and others, 2004). Other members of the soil microflora such as *Bacillus* spp., *Pseudomonas* spp. and *Trichoderma* spp. are recorded as reducing the activity of *P. brassicae* (Yeoung and others, 2003) and *Streptomyces* (Cheah and others, 2001; Joo and others, 2004).

The biotic basis for soil suppressiveness to *P. brassicae* was earlier shown to be supported by abiotic factors (Murakami and others, 2000; Murakami and others 2007) reflecting earlier research by Bochow and colleagues (Bochow, 1961, 1963, 1965; Einhorn and others, 1991). The effect of rhizosphere components on the success of *P. brassicae* was emphasised by Belec and others (2004).

Host and Non-Host Plants

Soil environments created by non-host and host plants such as:- leek (*Allium porrum*), winter rye (*Secale cereal)* and perennial ryegrass (*Lolium perenne*) tended in glasshouse studies to reduce the growth of *P. brassicae* but such effects have been less dramatic in the field. There was no species–specific interaction between *P. brassicaee* and non–host types (Friberg and others, 2006). Root exudates from *Lolium perenne* stimulated more spore germination than was obtained from other plants (Friberg and others 2005). These differences could not be explained by variantions in the composition of the exudates or differences in root activity. But alternatively such an environment could mitigate against *P. brassicae* such that the microbe fails to invade otherwise susceptible hosts such as *Cardamine flexuosa* as reported by Tanaka and others (2006a). Break or rotational crops may alter the soil environment in a manner suppressive to *P. brassicae* (Cheah and others 2006). Studies of the association of *P. brassicae* and hosts other than cruciferous types have attempted to construct rotations which are antagonistic to *P. brassicae.*

The existence of pathotypes of *P. brassicae* is well established. These exist in complex mixtures within galls, fields and more widely as determined by Toxopeus and others (1986). The pathotypes interact and are influenced in these interactions by the surrounding environments (Tinggal, 1980; Jones, 1980). The extent to which such interactions are influenced by host plants remains to be determined.

The success of *P. brassicae* is dependent on the density of resting spores, soil type, soil pH and host susceptibility. Dose response curves vary even where soils are of similar pedological type (Murakami and others, 2002). The impact of edaphic chemistry

including boron, calcium, nitrogen concentrations and pH on the growth and reproduction of *P. brassicae* within host cells are described by Dixon (2002), Dixon & Page (1998), Webster & Dixon (1991 a & b). Their results describe implications for the manner by which resting spores germinate, the motility of primary zoospores, growth and reproductive efficiency of *P. brassicae in planta* and for the expression of forms of host resistance. These topics and their wider implications have been reviewed by Dixon (2009a).

Considerable focus has been placed on factors affecting the life of *P. brassicae* in this Chapter and elsewhere in this volume are discussions of its life within the hosts. But knowledge of the adversities causing the death of *P. brassicae* can only be surmised at by inversion of those factors apparently aiding the microbe. The death of *Plasmodiophora brassicae* apparently occurs logarithmically in soil so that a few propagules persist for a very long time (Macfarlane, 1952). This conforms with Wallenhammar's findings. Possibly even the lowest level of inoculum can be significant for the survival of *P. brassicae* since Ayers (1944) described infection as commencing from a single zoospore. That being so then *Plasmodiophora brassicae* is indeed superbly well evolved and fitted for survival in hostile soil environments.

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