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Plasmodiophora brassicae in its Environment

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Abstract *Plasmodiophora brassicae* Wor. is viewed in this article from the standpoint of 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 colonization 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, and nitrogen content; and the presence of active microbial antagonists. 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 physiologic 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 opportunities to destroy this organism. The approach adopted here is borne of several decades spent studying *P. brassicae* and the respect that has been 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 colonization are included because they form a continuum. Interactions with the molecular and biochemical cellular environment are considered in other sections in this special edition.

Keywords *Plasmodiophora brassicae* · Clubroot · Environment · Inoculum potential · Biological, chemical and physical interactions

> "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" (Anderson 1855). This early observation presages the confusion in our understanding of the interaction of this microbe[1] and its environment which has continued for 150 years.

Introduction

Plasmodiophora brassicae Wor., the microbe that causes clubroot disease[2] of the Brassicaceae, is very well fitted for successful life on three counts. First, 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

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

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

arrives. Second, 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. Third, 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 Ludwig-Müller and others this issue).

For only short times and across minute distances in the soil the primary zoospores are 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; these have been gathered mostly 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 toward explaining why effective control of clubroot disease has proved so difficult to achieve. This article 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 article; elsewhere, for example, in Donald and Porter (this issue), others have considered manipulating such understanding to the disadvantage of *P. brassicae*.

Resting Spores

The resting spore of *P. brassicae* is an obvious point to commence considering environmental interactions. The structure of the resting spores is described in Kageyama and Asano (this issue). 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 (Wallenhamar 1999). 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[3] could become rife with clubroot disease after 1 or 2 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 (Fedorintschik 1935; Monteith 1924). 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 (Hata and others 2002; Ohi and others 2003) 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, Humphrey (1892), Chupp (1917), Naumov (1925), Honig (1931), and Colhoun (1958). In general, they concluded that bacteria and other organisms disintegrate the diseased host tissues and "condition" spores for more effective germination. 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 because 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 highly acidic 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 physiologic races completed by Toxopeus and others (1986) indicated a predominance of virulences for *B. napus* in these regions. At the more microgeographical

[3] Ley is a English agricultural term for grassland pasture providing grazing over an extended period of time.

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 *italica*) (P. Corfield, personal communication). Dirty machinery, wheels, boxes, and stillages all provide potential means for the spread of *P. brassicae*. Wild and weedy members of the Brassicaceae and infested crop transplants harbor, spread, and perpetuate the pathogen (see Faggian and Strelkov this issue). Once established in a soil profile, subsequent distribution is related to the soil's 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 (Kim and others 2000). Because 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 2002a, b) unique to that particular site.

At germination the resting-spore volume increases as vacuoles enlarge and the walls thicken, becoming more transparent (Chupp 1917; Favorski 1910; Woronin 1878). A single swarm zoospore is liberated from each resting spore leaving behind residual cytoplasm. Germination is characterized by a loss of refractile globules, characteristic of stored reserves in dormant spores and probably indicating the enzymic mobilization of these resources. Immersion of spores in water may encourage germination (Bawden 1948; Chupp 1917). Ayers (1944) obtained germination in 1–10 days using tap water and the 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°C in the absence of seedling roots. Optimal temperature for resting-spore germination was established as 24°C and pH 6.0–6.7, with an upper lethal temperature of 45°C and visible light inhibiting germination. Spores may be stored as dense suspensions at 3–4°C for 3 years without loss of viability (Macfarlane 1958), apparently withstanding anaerobic conditions and are not killed by exposure to −20°C for 3 days. It is standard practice to store galls at −20°C 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 and 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 be known, standardized, and reproducible. All too often 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 and Last (1957), Bochow (1963, 1965), and Macfarlane (1970). Substantial studies by Kowalski and Bochow (1996) concluded that the stimulant effect for germination is nonspecific and could come from exudates emanating from 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 (green broccoli) and perennial ryegrass stimulated spore germination. Some specific stimulant effects were suggested by Ohi and others (2003) and Hata and others (2002). Greatest germination (75%) was found to be induced by 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 and 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; for example, drought encourages a release of amino acids. Page (2001) implicated calcium as a factor in generating soil suppressiveness to *P. brassicae* and hence adversely affecting germination but recognized 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).

The number of germinated resting spores in soil correlates with levels of root hair invasion. When soil calcium

declined so did the number of germinated spores and the level of root hair invasion. Therefore, it seems that not only do host exudates affect germinability, but the numbers of spores available for germination in the first place 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 from 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 colonization is retarded by the presence of calcium and alkaline pH values. Niwa and others (2007) found that the addition over 15 years of large amounts of organic matter raised soil calcium concentration and 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 a 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-spore numbers have been estimated by the methods of Shinoda and others (2003) and this topic is further explored in Faggian and Strelkov (this issue). 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 1977, 1984b; Dixon and Doodson 1970, 1971). 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 the resting-spore load in the soil reaches even modest concentrations, disease severity increases (Murakami and others 2002a, b).

Zoospore Motility and the Processes of Invasion

Direct knowledge of the movement of zoospores is very limited, relating as it does to behavior 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) because *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 labors. 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 helpful treatment for some other flagellate organisms in Amos and Duckett (1982), whereas de Weger and others (1987) confirmed that removal of flagellae from the bacterium *Pseudomonas fluorescens* impaired subsequent colonization 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 compared with secondary stages (Dixon 1984b).

Soil Moisture

From the earliest studies of *P. brassicae* soil moisture was viewed empirically as the medium by which the host is reached. In practice, of course, the impact of seasonal water supply varies; thus, although 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 moisture deficit. Clubroot disease, however, is considered to be associated with low-lying, poorly drained soils and it is most severe after wet weather. For this reason soil moisture is classified as a dominant environmental factor in interactions with *P. brassicae*. This contention is supported by only limited serious scientific experimentation.

Colhoun, at variance with 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 are vulnerable to clubroot disease when other environmental factors are weighted heavily in favor of the pathogen. In support of this contention, infection developed in 10–18 h 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 and Garrett (1945) because 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 and Crête 1978). Where soil moisture rises from 50% of maximum water-holding capacity up to saturation (Dixon 1981, 1984b) disease severity escalates. Lange and Olson (1983) emphasized 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 traveled by soil-borne zoospores are relatively short, probably between 10 and 20 mm, judging by information about *Olpidium brassicae* or *Synchitrium endobioticum*, both relatives of *P. brassicae.* Invasion of root hairs occurred up to 75 mm from the source of *P. brassicae* infection in soils where water mass movements were minimized (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, like the results for soil moisture and for comparable reasons. Severe infection developed in acidic soils at air temperatures of 16.6°C; when alkaline soils were used, disease expression was less severe. Conditions for disease development were more favorable when working with alkaline soils and air temperature of 23°C and fluctuations around the mean (Colhoun 1953). As with soil moisture, Colhoun's results showed that providing conditions in which a cardinal environmental factor greatly favored the pathogen allowed disease development despite other factors being apparently disadvantageous. Previously, temperatures below 20°C were thought to present a barrier to clubroot disease development (Chupp 1917; Gibbs 1931a). Monteith (1924) showed however, that symptoms developed throughout the range of 9–30°C; this contention was supported by Wellman (1930). In New Zealand, Ayers (1944) identified the minimum temperature for root hair infection of swede to be 12–14°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 above 22.5°C. Once root hair invasion was completed, 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 which is when root hair colonization reaches its peak and zoosporangia are forming.

It is possible that the predominance of an individual environmental factor changes according to the stage in the life cycle under consideration. Webster (1986) postulated "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, whereas another interacts to establish a frequency or intensity. This argument is consistent with Colhoun's previous contentions. Wallenhamar (1999), citing C. Williamson, suggested that galling could develop at 7°C with diurnal fluctuations in temperature and increases of 8–12 h 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 increased and host growth accelerated. Interactions may go further and relate to effects on the constitution of the host because Roback and 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 cultivars resistant at 15°C could show susceptibility at 20°C with high inoculum potentials.

Light Intensity

Because this is a soil-borne microbe, it would not be expected that light has any significant impact on growth. Colhoun (1961) showed however, 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 is the weight of total carbohydrate in the whole plant and R_S is 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 (Eriksson 1930; Milburn 1855; Russell 1859). In controlled experiments, Palm and McNew (1956) demonstrated that infection happened more readily in mixtures of soil and sand or of clay and sand or in undiluted soil 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, Somerville 1895) associated the disease in turnips (*B. rapa*) with soil aeration caused by hoeing during winter, and Larson and Walker (1934) contended that aerating alkaline soil favored infection. Light, sandy, humus-rich, and clayey soils are thought most disease-prone. As with the interaction of clubroot with 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 the physiologic 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 disease-conducive and -suppressive effects moderated by factors such as inoculum potential thereby determining disease expression. Soils with high water-holding capacity, such as silts, are likely to encourage this pathogen. Soil type interactions with calcium and pH are considered by Campbell and Greathead (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 and Garrett (1945), who found that the number of infected root hairs increased concomitantly with spore density and by Macfarlane (1952), who related rising spore load with percentage of clubbed plants, laid the early foundations of knowledge in this area. 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; Colhoun (1958) termed this "infective power." Under less than optimal conditions spore load was related with the number of diseased plants; thus, with alkaline soils Colhoun found a direct relationship between spore load and number of diseased plants.

The conditions under which resting spores are stored affects their viability (Macfarlane and Last 1957), whereas germination is encouraged and the proportion of germinated spores increases with 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, affect 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 Siemens and others this issue) governing symptom development within the host cannot be affected by inoculum load. Saturation itself is not a fixed and immutable value because 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 that influence the chances of an additional spore being able to establish infection and proceed 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. Also importantly, there may be competition for root hair space by different physiologic races of *P. brassicae* as suggested by Jones (1980). Both antagonistic and synergistic relationships between races of *P. brassicae* may affect relationships between physiologic forms (Dixon 1979, 1980; Dixon and others 1981; Jones and others 1981). Within any population of *P. brassicae* spores there may be a range of vigor or infective capacity such that on establishing infection some proceed more rapidly through the life cycle than others. Jones (1980) reported that more than one physiologic 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 physiologic races in experiments using inocula composed of mixed races. Races varied in "vigor" or "aggressiveness" as indicated by the intensity of differential reactions between races and hosts defined by 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 inoculum from cv Acme clubs (Dixon and others 1981). Recently, Tanaka and others (2006b) suggested that there is a suppression of plasmodial development during secondary colonization in *B. rapa* ssp. *pekinensis* cv Kubai 70 which occurs differentially when using a range of isolates of *P. brassicae*. How differences in vigor, aggressiveness, or infective capacity are derived and when they come into action are questions yet to be answered.

Host resistance may be considered an environmental component that affects 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, diminishing the general resistance of 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 toward explaining the lags in time between invasions which result in less advanced states of infections on assessment days where plants are subjected to lower inoculum 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 vexing issue relating to *P. brassicae* and its interaction with the environment is calcium content and the associated hydrogen ion content (pH) of soil. Calcium emerges as a fundamental factor in the life cycle of both *P. brassicae* and its hosts. Datnoff and others (2007) summarized the involvement of calcium in host metabolism, physiology, and signaling of many host-pathogen interactions, indicating a relationship of calcium with expression of resistance. From the earliest studies of *P. brassicae* and clubroot, 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 however, is contradictory with respect to 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 the incidence of clubroot disease is not limited at pH 7.0 as is still claimed, especially in much farm advisory and home gardening literature. As Colhoun (1958) stated, "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 because he also indicated that 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 during the winter are far less acceptable because of the weaker host growth compared with those made in spring or early autumn, whereas summer-time experiments are likely to suffer from excessive increases in air temperature. The chemical and physical forms and quantities of calcium used also affect the results and add additional variables to each experiment. As with moisture and temperature, Colhoun (1958) reinforces lessons from the classical studies of Samuel and Garrett (1945) that are 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 influencing the environmental success of *P. brassicae*.

Subsequent to the work of 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 and Campbell (1985) suggested that clubroot disease expression depends on the balance between pH and the amounts of calcium and magnesium in the soil. Although Dobson and others (1983) concluded from their work, in which roughly and thoroughly mixed limed soils were used, 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 the greatest effects on 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 factor other than pH, possibly calcium itself, was involved. Using controlled conditions, Hamilton and Crête (1978) formed similar conclusions. These results, however, still 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 because *P. brassicae* spends most of its life cycle within the host, it could be fair to suggest that calcium and pH also affect that environment. A role for calcium in the postinfection development of *P. brassicae* is supported by the demonstration that incorporation into roots is pH-dependent (Myers and Campbell 1985). Also, Campbell and Greathead (1996) contended that *P. brassicae* is affected by pH and calcium concentration at more than one point in the life cycle between spore germination and the completion of resting-spore formation in the cortical cells. Detailed long-term experimentation by Webster (1986), Dixon and Webster (1988), Webster and Dixon (1991a), Dixon and Page (1998), and Page (2001) has confirmed this. It is evident that calcium has the greatest impact when it is present in the period between spore germination and postpenetration of root hairs. The latter appears to be when root hair infection has the biggest impact on subsequent gall formation. Apparently, there may be separate mechanisms involved because the periods of 0–3 and 0–7 days postpenetration seem to be different with respect to the extent of their influence on subsequent disease development. The expression of effect seems to be cumulative because it took longer when a 30-mel^{-1} Ca^{2+} solution was used compared with a 55-mel^{-1} Ca^{2+} solution to achieve similar final results. The host-pathogen response varies also with pH; however, that is a separate factor. It is worth stating here that calcium at pH 7.2 needed to be present by day 14 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 behavior in the cortical cells. The work of Webster (1986), Dixon and Webster (1988), Webster and Dixon (1991a), Dixon and Page (1998), and Page (2001) is supported by results of Donald and others (2004) and Donald (2005) in Australia (see Donald and Porter this issue). 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 compared with the controls. Higher concentrations of calcium completely inhibit the later stages of *P. brassicae* development in the root hair, even when high inoculum doses are applied. The calcium effect commences in the soil because as Dixon and Page (1998) showed, the germination of resting spores, the motility of zoospores, and the composition of benign microbial flora around roots are altered. High concentrations of calcium could possibly reduce flagellar action; Satir (1982) and Sleigh and Barlow (1982) reported that changes in calcium on 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* has to be determined.

Acidity and Alkalinity

Recently, Wallenhamar (1999) pointed to the uneven distribution of acidic and alkaline areas of soil in individual fields with pH ranging from 5.73 to 8.45 in localized patches. Mattsson (1995) showed 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 modernizes 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 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 and Campbell (1985) adjusted pH and calcium content separately and showed that at 10 mel^{-1} Ca^{2+} and pH above 7.1 there were reduced numbers of primary zoosporangia in root hairs thus inhibiting galling. Webster and Dixon (1991a) 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 has 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 and increases 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. Because pH regulates the response to calcium, intracellular function also may be modified. A high concentration of H^+ ions in plant tissues is potentially antagonistic to calcium. Membrane permeability is decreased by both alkaline pH and high calcium. This environment could affect the growth and reproduction of *P. brassicae* as it proliferates within the host's root hair and epidermal cells or within the cortical cells. Alkaline environments could affect primary

and secondary invasions, cortical migration, and cell hypertrophy. Involvement of calcium in the growth and reproduction of *P. brassicae*, ultimately leading to induced cell death or hypersensitivity, is suggested by Takahashi and others (2002, 2006). At the agronomic level, promotion of high alkalinity linked with continuous cropping is suggested by Shinoda and others (2005) as a means of reducing the soil inoculum load.

Boron

Boron has been associated with affecting the activities of *P. brassicae* from the 1930s (O'Brian and Dennis 1936). 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 (1983, 1984a, b, 1985) and Dixon and Wilson (1983, 1984a, b, 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 (Webster and Dixon 1991b) have shown that in environments with elevated boron concentration there are significant effects in both the root hair phase and the cortical phase of *P. brassicae*. Throughout the *in planta* stages of the life cycle of *P. brassicae*, boron has an impact on the microbe. There also appears to be a relationship between 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 form of calcium carbonate or oxide may alter the nutrient environment in soil to the detriment of *P. brassicae* and, therefore, cause the host–parasite association to be more affected by other factors such as boron. Alternatively, boron may have a primary effect because as was found by Webster and Dixon (1991b), 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 in which membrane permeability and wall structure are altered may be to the detriment of *P. brassicae*. It could also make conditions less conducive for nuclear division by the microbe. Quite possibly boron effects are distinct from those of calcium and pH. Dixon (1991) found that boron affects the progress of *P.brassicae* by retarding the rate of sporangial maturation. The correlation of the 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 be equated with the effects of shorter exposures to higher concentrations. Field and controlled laboratory studies (Craig and Dixon 1993a, b) identified that boron has a substantial effect on the ability of *P. brassicae* to invade root hairs and establish colonization in the field. Raising the boron content of the rhizosphere before the availability of a susceptible host to infested soil limited the subsequent ability of *P. brassicae* zoospores to penetrate and colonize root hairs and cause symptoms.

Nitrogen

Nitrogen has been reported as influencing many 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. Webster (1986) postulated a two-phase response with low concentrations enhancing and high concentrations retarding symptoms, as found with other biostimulants (Dixon 1991). Adding nitrate nitrogen results in the stimulation of cellular free amino acid pools. If this stimulates arginine- or lysine-rich histones, then this could possibly lead to repression of RNA polymerases in the microbe, preventing its access to the gene products needed for pathogenesis. Webster (1986) postulated that as nitrate concentration increases, enzyme sites become saturated with consequent substrate and/or product inhibition and the amino acid moieties are diverted toward forming an environment inhibitory to *P. brassicae*. Nitrate metabolism is regulated by the availability of reduced cofactors 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 cofactors could influence the activity of *P. brassicae in planta*. Raising nitrate levels to above 20 mel^{-1} reduced symptom expression and the number of infected plants. Results obtained in controlled conditions using split-root techniques (Dixon and Khatan unpublished 1997) demonstrated the effects of nitrate ions on 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 and Mandal (2006) found that calcium ammonium nitrate and calcium nitrate significantly reduced the intensity of clubroot disease and supported the results of Page (2001). Page showed by detailed laboratory and field studies that calcium nitrate is associated with a decrease 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 physiologic race of *P. brassicae*.

Interactions between the fertilizer calcium cyanamide, *P. brassicae*, and other soil microbes have been studied for well over 70 years. A substantial body of information has been accumulated (Coulshed and Dixon 1990; Dixon 2009b; Dixon and Williamson 1985; Dixon and Wilson 1983; Humpherson-Jones and others 1992; Naiki and Dixon 1987) that demonstrates that calcium cyanamide and the products of its degradation, calcium and nitrate nitrogen, are associated with the reduced viability of *P. brassicae*. Additional 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 soilborne microbes antagonistic to *P. brassicae* are encouraged. Detailed research worldwide by, for example, Klasse (1999) in Germany; Donald and others (2002), Donald and Porter (2004) and Donald (2005) in Australia; Porth and others (2003) in the U.S.; McDonald and others (2004), Belec and others (2004), Tremblay and others (2005), and Manolii and others (2005) in Canada; and Murakami and others (2002a, b) 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 Faggian and Strelkov this issue).

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 the 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 and Lazarovits 2006a, b). Sen (2005) commented on the effects of molybdenum along with calcium and boron in the root environment on the pathogenesis of *P. brassicae* on rapeseed mustard in West Bengal India. Interaction between the nutrient environment, pathogenesis, and resistance is discussed by Dixon and Walsh (1998), Huber and Graham (1999), and Dixon (2002).

Biological Soil Constituents

Little is known of the relationships between *P. brassicae* and the macro- and microflora 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. Organic or inorganic soil amendments that stimulate the microflora have a significant effect on the survival of *P. brassicae*. Bacteria such as *Bacillus* spp. and fluorescent *Pseudomonas* spp. are recognized as affecting the growth of *P. brassicae* (Einhorn and others 1991). Biotic suppressive soils with pH above 7.4 and a calcium content of 1210 ppm were identified in Taiwan (Hseith and Wang 1986). Because 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 used 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 been conducted by 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 suppression of *P. brassicae* in the presence of Chinese cabbage (*B. rapa*) host plants reportedly resulted from the presence of the soil endophytic fungus *Heteroconium chaetospira* (Narisawa and others 2005). Soil moisture content, pH, and spore 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 (2003a, b).

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 alkalization 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 colonization of the host root hairs. This is a dynamic situation in constant flux because the microbial flora changes under the influence of substantial alterations in 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 colonization of a host. It is a function of inoculum density or intensity (mass or units of inoculum per unit of soil), available nutrients (both internal and external to the propagule), environmental factors, and the genetic capacity of *P. brassicae* itself (extended from Martinson 1963). For a particular host species, the levels of genetic resistance, which vary during its life cycle, and interactions with the environment are key factors determining the outcome of encounters with *P. brassicae*. The combination of all these factors of host and microbe interactions 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 about the life and death of *P. brassicae* in soil. That is in very marked contrast to our understanding of the interactions within the host (see Diederichsen and others this issue; Ludwig-Müller and others this issue; Piao and others this issue; Siemens and others this issue). Dixon and Walsh (1998) showed that although *P. brassicae* interacts with the soil environment only for a short period of time and space, this period is critical for the success of *P. brassicae* in establishing subsequent growth and reproduction within the host. These authors emphasize 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 toward the root surface by mass flow may accumulate in the rhizosphere in quantities larger than required for uptake by the plant roots. As a result, surplus ions accumulate in the immediate environs of the rhizosphere and could have an immense impact on *P. brassicae*. Although the rhizosphere around the root apex is acidic that further back can by alkaline with obvious effects on the colonization and invasion of those areas by this microbe. Important to the survival of soil-borne microbes are the conditions prevailing at the time of arrival at the root surface and in the surrounding rhizosphere which affect establishment and penetration (Bowen and Rovira 1999). This aspect of infection 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), that shows 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 that 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 zoospores proceed with location finding and flagella motion toward the root surface (summarized in Fig. 1). It has been suggested 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 like 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 a prerequisite for successful colonization. Because temperature is also an important factor, it possibly 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 colonization but without successful secondary-stage colonization and subsequent symptom expression (Murakami and others 2000). Presumably such hosts offer sources of energy for germination and colonization but do

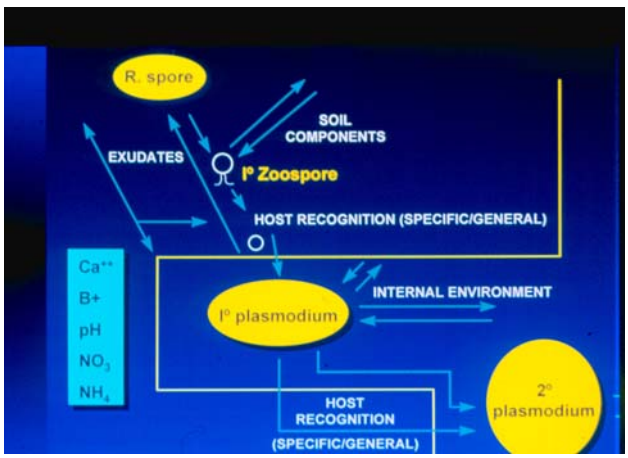

Fig. 1 Summary diagram of factors in the soil environment that affect the germination of resting spores, movement of primary zoospores, penetration of root hairs, and root hair conditions for *Plasmodiophora brassicae* Woronin (the cause of clubroot disease in the Brassicaceae) (devised by Dixon and Page unpublished)

not have 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 also could indirectly influence these processes.

The fungus *Heteroconium chaetospira* inhibited the activities of *P. brassicae*, even when the soil's physical conditions of moisture and pH would have otherwise have been conducive (Narisawa and others 2005). Chinese cabbage (*B. rapa*) roots became colonized by hyphae of *Heteroconium chaetospira,* but there was no visible evidence of host cell wall degradation, host reactions, or invagination of the host plasma membrane around the hyphae (Yonezawa and others 2004). Earlier work by these authors linked the suppressive and electrical properties of soil. Those soils with negative charges matching a similar potential on spores of *P. brassicae* were conducive to development of the microbe, whereas those soils with positive charges were suppressive (Murakami and others 2004a, b). Other members of the soil microflora such as *Bacillus* spp., *Pseudomonas* spp., and *Trichoderma* spp. have been found to reduce 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 shown to be supported by abiotic factors (Murakami and others 2000, 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 emphasized by Belec and others (2004).

Host and Nonhost Plants

Soil environments created by nonhost and host plants such as leek (*Allium porrum*), winter rye (*Secale cereale*), and perennial ryegrass (*Lolium perenne*) in glasshouse studies tended 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. brassicae* and nonhost types (Friberg and others 2006). Root exudates from *Lolium perenne* stimulated more spore germination than that obtained from other plants (Friberg and others 2005). These differences could not be explained by variations in the composition of the exudates or differences in root activity. 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 crucifers have attempted to construct rotations that are antagonistic to *P. brassicae*.

The existence of pathotypes of *P. brassicae* is well established. These exist in complex mixtures within galls and fields and more extensively, as determined by Toxopeus and others (1986). The pathotypes interact and are influenced in these interactions by the surrounding environments (Jones 1980; Tinggal 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 2002a, b). The impact of edaphic chemistry, including boron, calcium, nitrogen concentrations, and pH, on the growth and reproduction of *P. brassicae* within host cells is described by Dixon (2002), Dixon and Page (1998), and Webster and Dixon (1991). Their results describe the implications of the manner by which resting spores germinate, the motility of primary zoospores, growth and reproductive efficiency of *P. brassicae in planta*, and the expression of forms of host resistance (Fig. 2). These topics and their wider implications have been reviewed by Dixon (2009a).

In this article, considerable focus has been placed on factors that affect the life of *P. brassicae*. Elsewhere in this issue are discussions about its life within the hosts. Knowledge of the adversities causing the death of *P. brassicae* can only be surmised by inversion of those factors that apparently aid 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* because, as Ayers (1944) described, infection commences from a

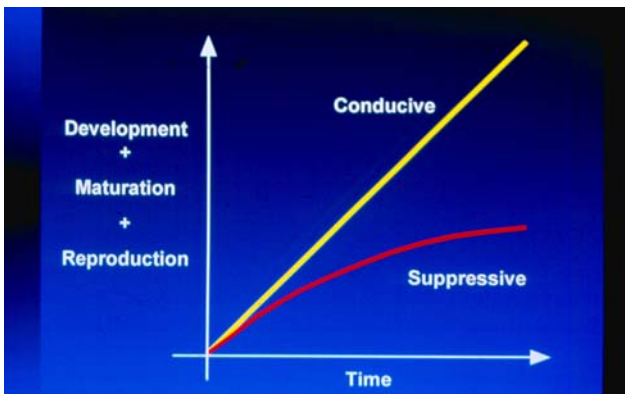

Fig. 2 Generalized curves demonstrating the effects of conducive and suppressive environments on the rate of development, maturation, and reproduction of *Plasmodiophora brassicae* (the cause of clubroot disease) over time

single zoospore. That being so, *Plasmodiophora brassicae* is indeed superbly well evolved and suited for survival in hostile soil environments.

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