Interactions of Soil Nutrient Environment, Pathogenesis and Host Resistance

G. R. DIXON

Department of Bioscience, Royal College, The University of Strathclyde, Glasgow G1 1XQ, United Kingdom

Tel.: +44 1 563 830 251, Fax: +44 1 563 830 251, E-mail: 113541.1364@compuserve.com

Abstract

Host plants and soil borne pathogens that attack them exist within an ecological matrix populated by numerous microbial species that may influence the access of pathogenesis. These events are moderated by physical and chemical components of the soil. The impact of inorganic and organic nutrients on pathogenesis and the development of host resistance are discussed in this review using two host – pathogen combinations as examples. Calcium, boron, nitrogen and pH have been demonstrated to affect the processes of resting spore germination, host invasion and colonisation in the *Plasmodiophora brassicae-Brassica* combination that results in clubroot disease. Organic nutrients that have associated biostimulant properties have been demonstrated to influence the development of *Pythium ultimum-Brassica* combination that results in damping-off disease. This latter combination is affected by the presence of antagonistic microbial flora as demonstrated by increased ATP, extra-cellular enzyme and siderophore production. In both examples there are indications of the manner by which host resistance to pathogenesis may be enhanced by changes to the nutrient status surrounding host plants. These effects are discussed in relation to the development of integrated control strategies that permit disease control with minimal environmental impact.

Keywords: nutrition; host; pathogen; environment; *Plasmodiophora brassicae*; clubroot; *Pythium ultimum*; damping-off; *Brassica*; calcium; boron; nitrogen; pH; biostimulant; liquid seaweed extract; *Pseudomonas* spp.

Recognition of the effects of environmental factors interacting with genotypes to determine the eventual phenotypic characteristics of organisms is central to the Darwinian theory (DARWIN 1859). Plant pathogenic studies raise the complexity of these interactions substantially. Here there are two (or more) genotypes (host and pathogen) interacting with each other and their combined environments over time to determine the eventual outcome in terms of disease development and expression in one (the host) and growth and reproduction in the other (the pathogen). This offers multi-factorial ecological combinations that are complex to elucidate especially where soil borne organisms are the chosen area of study.

Plant breeders and geneticists have tended to work with the interaction between host resistance/susceptibility and pathogen virulence/avirulence. While epidemiologists have restricted themselves to studies of environments and pathogen population growth largely concerned with aerial pathogens. VAN DER PLANK (1982) and ZADOKS and SCHEIN (1979) for example, restricted their mathematical treatments to host and pathogen interactions and included few environmental factors. Epidemiologists led by GARRETT (1956), BAKER (1965) and subsequently GILLIGAN (1987) attempted understandings of population growth of soil borne organisms. But these were restricted largely to the host and pathogen interaction and did not consider the soil biotic and abiotic environmental components.

Understanding soil ecosystems

The few workers who attempted holistic syntheses that included interacting factors inherent in ecological approaches tended to be regarded with suspicion by reductionist scientists. But gradually recognition is developing that it is essential to view the soil as an ecosystem into which the crop host and its associated pathogens are introduced and these disbalance systems in nature where epidemic disease development is the abnormal case. The norm being a condition in which benign (or antagonists of pathogens) organisms are in the majority. One of the few workers who attempted such studies especially including plant nutrients as factors was HUBER (1980).

HUBER (1980) recognised that: The generally adequate availability of most nutrients for plant growth has induced an apathetic recognition of the vital role of minerals in defense (sic) against plant disease. It is doubtful that a satisfactory understanding of most host-pathogen interactions will be obtained until associated nutrient relationships are elucidated. And: The nutrition of a plant determines in large measure its resistance or susceptibility to disease, its histological or morphological structure or properties, the functions of tissues to hasten or slow pathogenesis, and the virulence and ability of pathogens to survive.

Studies in this area require understandings not only of hosts and pathogens but also of their nutrient metabolism. HUBER (1980) went so far as to identify that nutrients are associated with host defence against pathogens on at least three fronts:

- Maximising the inherent resistance of plants
- Facilitating disease escape through increased nutrient availability
- Stimulating plant growth and altering the external environment to influence survival, germination and penetration of pathogens.

This list could be supplemented by considerations of the impact of nutrients on populations of benign soil flora and these are of at least as great significance as each of the others. Such holistic ecological studies have lapsed for almost two decades being overshadowed by molecular research. Fortunately they are re-emerging and uniting with molecular biology as identified by LINDOW *et al.* (2002) and LUGTENBERG *et al.* (2002).

The soil nutrient environment

The level of complexity of variables associated with pathological studies in the soil ecosystem is demonstrated in Table 1. Components of the soil nutrient environment identified as having interactions with the host-pathogen combinations are shown in Table 2. These include both inorganic and organic forms. The impact of inorganic nutrients on the host and rhizosphere ecology is well characterised (for example, BOULD *et al.* 1983; WILD (1988); MARSCHNER 1990). The effects of organic materials, especially the developing class of biostimulants is less understood (DIXON & WALSH – in press).

Scope of this review

This review considers two examples in which the nutrient environment influences the course of host-pathogen interactions, the development of populations of benign microbes and with potential implications for mechanisms of host resistance. The first concerns *Plasmodiophora brassicae*, the causal agent of clubroot disease of brassicas. In this case inorganic nutrients affect the course of pathogenesis and significant progress has been made in defining their roles in the interaction of host and pathogen, disease development and possible

Table 1. Variables in the soil ecosystem

Soil	Moisture, organic matter, temperature, aeration, chemical composition, active and reserve (H^+), structure, texture, interactions with the aerial environment [O_2 and CO_2], seasonal and climatic variations
Pathogen	Species, concentration, viability, longevity
Host	Species, cultivar, root morphology, physiology: exudation, absorption of nutrients
Microbial ora	Antagonistic characteristics, production of extra-cellular enzymes, sterols (eg ergosterol), siderophores, mycorrhizal combinations
Husbandry	Addition of fertilisers (types-soluble inorganic and/or insoluble organic), timing of cultivation operations before and during cropping, past cropping histories, application of agrochemicals (herbicides, pesticides, fungicides, adjuvants, wetting agents, biostimulants)

Table 2. Inorganic and organic soil components affecting the host-pathogen interaction

Class	Components
Inorganic	Nitrogen, phosphorus, sulphur, potassium, calcium, strontium, magnesium, boron, iron, manganese, zinc, copper, molybdenum, tungsten, chlorine, bromine, iodine, cobalt, nickel, silicon, sodium, rubidium, vanadium, selenium, chromium, aluminium
Organic-specific compounds	Amino acids, quaternary ammonium compounds (especially betaines such as valeric acid betaine), growth regulators (indoles, gibberellins, cytokinins), fatty acids (for example: triacontanol), vitamins (for example: folic acid), carbohydrates (for example: laminarin, chitin)
Organic-mixtures and natural products	Seaweed extracts, calcified seaweed, humic acid, potassium humate, crab shell meal, fish extracts

effects relating to the expression of host resistance. The second example is *Pythium ultimum*, a cause of damping-off diseases and involves organic compounds and mixtures known as 'biostimulants'. These are of rapidly developing scientific interest because of their effects on the growth and reproduction of host plants and an inter relationship with host-pathogen interactions, the repression of pathogenesis, stimulation of benign soil microbes and potentially effects on the expression of resistance.

Plasmodiphora brassicae Wor. – the causal agent of clubroot disease in *Brassicaceae*

Details of the host symptoms, pathogen life cycle (so far as it is understood), infection processes, host-pathogen physiology, resistance and susceptibility and pathogen distribution are given in DIXON (1999). *Plasmodiophora brassicae*, the cause of clubroot disease, is a pre-eminent example of the interaction between soil nutrient content, host resistance and disease expression. It has long been accepted that adding lime (calcium salts) to soil may reduce clubroot disease, for example, ANDERSON (1855 – 23 years before the classic identification of the causal relationship between *P. brassicae* and clubroot symptoms by WORONIN 1878) recorded that: "Mr Hunter of Haugh limed part of a field ... at the rate of sixty bolls per scotch acre and the turnips afterwards grown on this portion were free of disease". Since then there have been numerous studies of the use of lime and other nutrients for the control of clubroot disease. In the main these are reports of field experiments undertaken on differing soil classes using varying nutrient compositions and wide ranges of climatic and husbandry variables (KARLING 1968). There is limited scientific connection between them and few attempts to understand the underlying effects of nutrients on the host–pathogen interaction.

Table 3. Interaction of calcium with *Plasmodiophora brassicae* and *Brassica* hosts

- Concentrations of 55–75 per mel calcium reduce symptom expression, the extent of this reduction is dependent on inoculum concentration
- Compounds which raise calcium content and pH such as calcium carbonate and calcium hydroxide reduce symptom expression
- Combinations of high calcium (55–75 per mel) and alkaline pH reduce symptoms more effectively than either factor operating in isolation
- Applications of calcium at 30–75 per mel reduced root hair infection and multiplication within 0–3 days of inoculation and symptom expression with 0–7 days of inoculation
- Continuation of increased calcium beyond 7 days after inoculation failed to affect root hair colonisation
- Increased calcium (30–75 per mel) at pH = 6.2 or 7.2 reduced the numbers of root hair infections and the maturation rate from plasmodia to secondary sporangia. These effects were moderated by inoculum density

Controlled experimentation by such workers as COLHOUN (1953, 1961), PALM (1963) and MYERS and CAMPBELL (1985) identified aspects of the interaction between the inoculum potential of *P. brassicae*, nutrient, temperature and moisture environments and host symptom expression.

Extended and detailed research has now indicated the roles of calcium, boron, nitrogen and pH in the development of *P. brassicae*, infection and colonisation of the hosts and interaction with resistance to the pathogen, DIXON (1996), DIXON and WEBSTER (1988), DIXON and PAGE (1998), PAGE (2001), WEB-

Table 4. Interaction of pH with *Plasmodiophora brassicae* and *Brassica* hosts

- Alkaline pH values reduced total root hair infection numbers and retarded the maturation of the pathogen in the absence of increased calcium concentration
- These pH effects were most potent within three days of inoculation, beyond three days the effects declined
- Disease index could be reduced by increasingly alkaline pH values applied 3–7 days after inoculation
- Alkaline pH reduces disease expression and root hair infection more effectively when combined with increased concentrations of calcium, boron and nitrogen

Table 5. Interaction of boron with *Plasmodiophora brassicae* and *Brassica* hosts

- Increasing boron concentrations beyond 5 ppm reduces symptom expression and inhibits the maturation of sporangia in the root hair
- Boron is more effective when associated with lower inoculum pressures or when used in combination with pH = 7.2 and beyond
- The inhibition of sporangial maturation and diminished symptom expression correlated strongly with increased boron concentrations
- Increased boron used at any stage in the disease cycle diminished symptom expression
- Suppression of both root hair infection and galling appears to be related to host exposure to a given concentration of boron, thus long exposure to lower boron concentrations or shorter exposures to higher boron concentrations were equally effective in reducing symptom expression

Table 6. Interaction of nitrogen with *Plasmodiophora brassicae* and *Brassica* hosts – nitrogen effects

- Symptom expression is reduced by application of nitrate - nitrogen concentrations in excess of 10 per mel
- Calcium nitrate was most effective in reducing disease expression and numbers of root hair infections
- Similar but less marked effects were obtained with ammonium nitrate
- Applications of nitrate - nitrogen and ammonium – nitrogen compounds reduced the rate of maturation of root hair infections but to a lesser extent than calcium and boron
- Nitrate-nitrogen and ammonium-nitrogen effects were more marked at lower inoculum concentrations
- The viability of resting spores and the ability of primary zoospores to infect root hairs are reduced by the presence of calcium nitrate
- The presence of calcium nitrate in the rhizosphere was associated with changes to the predominance of particular pathotypes of *P. brassicae* as determined by use of the European Clubroot Differential Series (ECD) (TOXOPEUS *et al*. 1986)
- Soil suppressiveness (*sensu* Alabouvette) to *P. brassicae* could be related to the presence of calcium and microbes in certain fields

STER (1986), WEBSTER and DIXON (1991a,b). Results from these studies are summarised in Tables 3–6.

The effects of boron, calcium, nitrate–nitrogen and pH appear so far to relate to: the germination of the resting spore of *P. brassicae*, motility and/or viability of the primary free-swimming zoospore, colonisation and maturation in the root hair, expression of symptoms in the second phase of the life cycle, predominance of particular virulence combinations in the pathogen population and potentially the development of forms of soil suppressiveness. Endogenous calcium fluxes have been associated with forms of increased host resistance in aerial pathogens (KIM *et al.* 2002) and this may be relevant to *P. brassicae*.

Pythium ultimum, a causal agent of damping-off in *Brassicaceae* and other hosts

For information describing the pathology and mycology of *Pythium ultimum* and attendant damping-off diseases (see DIXON 1981). This includes a discussion of the effects of nitrate nitrogen, potassium and organic soil amendments in diminishing disease development and possible enhancement of host resistance. The opportunities offered by organic soil amendments are the subject of increasing attention especially in relation to the class of materials known as biostimulants. These are defined as "natural or synthetic products of either mineral or organic composition that by their mode of action positively contribute to crop nutrition and the development of healthy plants" (S. D. Hankins – pers. commun.). The biostimulant formulations that have received the most scientific investigation are the liquid seaweed extracts (LSE), and their properties are highly characterised. They are known to contain specific active ingredients such as: amino acids, quaternary ammonium compounds (betaines – WHAPHAM *et al.* 1994), and carbohydrates (laminarin) (PATTISON 1994). Soils and other growing media substrates treated with liquid seaweed based biostimulants show substantially increased microbial activity (WALSH 1997).

In vitro research (DIXON &WALSH – unpublished) demonstrated that adding liquid extracts of the seaweed *Ascophyllum nodosum* to peat-based potting compost resulted in a reduction of *Pythium ultimum* induced damping-off disease. Experiments indicated, however, that application of the liquid seaweed extract (LSE) after compost is infested with *P. ultimum* is not effective. It was therefore, postulated that the seaweed extract did not affect the pathogen directly but stimulated beneficial microbial antagonists, possibly due to the laminarin content in the seaweed extract. Further research confirmed the ability of LSE to diminish damping-off disease of *Brassica oleracea* seedlings when applied prior to pathogen arrival (Table 7).

Effective biological control of damping-off disease can be achieved using single, specific bacterial antagonists, most notably species of the genus *Pseudomonas* (WALSH *et al.* 2001). The use of bacterial antagonists, however, may not always be effective under commercial conditions due to variations in soil-type and biological composition that may be unfavourable for antagonist survival and activity. The stimulation of resident bacteria and fungi that are naturally antagonistic to plant pathogens offers a fruitful approach to the development of biological control as part of integrated crop management strategies.

Biostimulants provide a means by which populations of antagonists in the rhizosphere can be mobilised to form a barrier against soil-borne pathogens. These compounds have natural origins. Biostimulants encourage natural systems of fungistasis and antibiosis in a manner analogous to the properties of soils that naturally suppress pathogens. Consequently, they are unlikely to pose threats to the environment. Additionally, they appear to affect several components of metabolic pathways

Table 7. Interaction of liquid seaweed extract biostimulant with *Pythium ultimum* and *Brassica* hosts

Water control plants grown without *Pythium ultimum* resulted in almost complete seedling germination and survival
Adding the pathogen in the absence of liquid seaweed extract reduced seedling survival by 75 to 80%
Applying the pathogen to compost previously treated with LSE resulted in significantly increased seedling survival in all treatments
Delaying seed sowing for 2 days resulted in a significant increase in seedling survival compared with seed sown at the same time as inoculum was applied
The effectiveness of LSE increased with concentration in the majority of cases and the 1.0% treatment had the most significant effect especially when *P. ultimum* was applied at the 10% level

producing differing effects in pathogens and antagonists. It is unlikely, therefore, that pathogen tolerance will develop to erode their efficacy.

Research suggests that biostimulants in liquid seaweed extracts must be present in advance of the pathogen. It is postulated that populations of antagonistic organisms build up and colonise the potting compost in sufficient numbers resulting in the adequate control of the pathogen *Pythium ultimum*, once introduced into the compost. Substantial microbial activity develops in composts treated with liquid seaweed extracts. This was quantified by PATTISON (1994) as increased ATP present in the compost and further verified by WALSH (1997) and DIXON and WALSH (1998) who determined that there were substantial increases in the production of extra-cellular enzymes resulting from the addition of liquid seaweed extracts. Populations of fluorescent *Pseudomonas* spp. increased rapidly in composts treated with liquid seaweed extracts and these are known to be very efficient antagonists of plant pathogens such as *Pythium ultimum*.

Biostimulants appear to be active in modifying the environment in which infection by pathogens takes place. The environment changes due to the alteration by biostimulants of the balance between the pathogen and organisms that are antagonistic to it. But it is entirely feasible that biostimulants may also affect the virulence and/or aggressiveness of pathogens and the resistance expressed by the host. In the case of liquid seaweed extracts, the presence of the carbohydrate laminarin (β-1, 3-glucan, structurally similar to fungal cell-wall components) may result in both the stimulation of fungal antagonists and elicit a plant defence response.

Underlying principles in HUBER's original propositions (1980) of maximising the inherent resistance of plants: facilitating disease escape through increased nutrient availability; stimulating plant growth and altering the external environment to influence survival, germination and penetration of pathogens though knowledge of the soil nutrient environment can now be extended. Alabouvette and his co-workers (ALABOUVETTE *et al.* 1985, 1996) has provided extensive information on the manner by which soil suppressiveness to disease develops. He indicated this results from the encouragement of benign microbes that are antagonists of plant pathogens. Such events are likely to occur when soils are supplemented with inorganic and organic nutrients. CHEN *et al.* (2002) for example has shown that boron stimulates quorum sensing in bacteria. This is also likely to result from the addition of calcium to soils (PAGE – unpublished). Biostimulants provide an organic stimulus that encourages soil fungistasis and antibiosis. In each case this can result in the indirect stimulation of host plant growth and reproduction which is additional to the direct effects derived from adding extra nutrients for the host plants. Adding nutrients that encourage plant growth and reproduction results in more robust growth which is resistant (or tolerant) to invasion by plant pathogens. Some fragments of scientific knowledge are beginning to emerge that demonstrate that forms of generalised host resistance (or tolerance) are increased when inorganic and organic nutrients are available. Distinguishing whether this is a direct result of the presence of nutrients acting on host metabolism or indirect effect derived from the enhanced presence of benign microbes will require substantial research effort especially where soil-borne pathogens and microbes are concerned. Nonetheless such studies are likely to yield improvements to the understanding of the manner by which hosts, pathogens and benign microbes interact within the soil environment. In turn this offers more ecologically sound philosophies for the control of plant pathogens that avoid damage to the environment since they do not require the use of site specific, artificial, toxic molecules for pathogen control. Integration of the manipulation of crop nutrition with plant pathology adds substantial dynamism to minimising losses caused by diseases. Seeking to understand the basic mechanisms is of major importance since this will make the processes of pathogen control more focused and enable them to be refined and improved while still sustaining the environment, increasing world food security, diminishing pollution and encouraging the preservation of biodiversity.

References

ALABOUVETTE C., COUTEAUDIER Y., LOUVET J. (1985): Soils suppressive to *Fusarium* wilt: mechanisms and management of suppressiveness. In: PARKER C.A., ROVIRA A.D., MOORE K.J., WONG P.T.W., KOLLMORGEN J.F. (eds): Ecology and Management of Soilborne Plant Pathogens. Am. Phytopathol. Soc., St. Paul, Minnesota: 101–106.

ALABOUVETTE C., LEMANCEAU P., STEINBERG C. (1996): Biological control of *Fusarium* wilts: opportunities for development of a commercial product. In: HALL R. (ed.): Principles and Practice of Managing Soilborne Plant Pathogens. Am. Phytopathol. Soc., St. Paul, Minnesota: 192–212.

ANDERSON A. (1855): Report on the disease of finger and toe in turnips. Trans. Highland Agric. Soc. Scotland, Ser. 3, 6: 118–140.

BAKER R. (1965): The dynamics of inoculum. In: BAKER K.F., SNYDER W.C. (eds): Ecology of Soil-Borne Plant Pathogens. Univ. California Press, Berkeley: 395–403 and 415–419.

BOULD C., HEWITT E.J., NEEDHAM P., ROBINSON J.B.D. (1983): Diagnosis of Mineral Disorders in Plants: Principles. Vol. 1. Her Majesty's Stationery Office, London.

CHEN X., SCHAUDER S., POTIER N., DORSSELAR VAN A., PELCZER I., BASSIER B.L., HUGHSON F.M. (2002): Structural identification of a bacterial quorum – sensing signal containing boron. Nature, **415**: 545–549.

COLHOUN J. (1953): A study of the epidemiology of clubroot disease of crucifers. Ann. Appl. Biology, **40**: 263–283.

COLHOUN J. (1961): Spore load, light intensity and plant nutrition as factors in uencing the incidence of clubroot of *Brassicae*. Mycolog. Res., **44**: 593– 600.

DARWIN C. (1859): The Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life. Oldham Press, London.

DIXON G.R. (1981): Vegetable Crop Diseases. MacMillan Publ., London.

DIXON G.R. (1996): Repression of the morphogenesis of *Plasmodiophora brassicae* Wor. by boron – a review. Acta Hortic., **407**: 393–402.

DIXON G.R. (1999): *Plasmodiphora brassicae* – the cause of clubroot disease in *Brassicaceae*. CAB Int. Compedium, Wallingford, Oxon.

DIXON G.R., PAGE L.V. (1998): Calcium and nitrogen elicting alterations to growth and reproduction of *Plasmodiophora brassicae* (clubroot). Acta Hortic., **459**: 343–349.

DIXON G.R., WALSH U.F. (1998): Suppression of plant pathogens by organic extracts – a review. In: Proc. Int. Symp. Composting and Use of Composted Materials for Horticulture. (SZMIDT R.A.K. – ed.). Acta Hortic., **469**: 383–390.

DIXON G.R., WALSH U.F. (2002): Suppressing *Pythium ultimum* induced damping-off in cabbage seedlings by biostimulation with proprietory liquid seaweed extracts. Acta Hortic. (in press).

DIXON G.R., WEBSTER M.A. (1988): Antagonistic effects of boron, calcium and pH on pathogenesis caused by *Plasmodiphora brassicae* (clubroot) – A review of recent work. Crop Res. (Hortic. Res.), **28**: 83–95.

GARRETT S.D. (1956): Biology of Root – Infecting Fungi. Cambridge Univ. Press, London.

GILLIGAN C.A. (1987): Epidemiology of soil-borne plant pathogens. Chapter 10. In: WOLFE M.S., CATEN C.E. (eds): Populations of Plant Pathogens: Their Dynamics and Genetics. Blackwell Sci. Publ., Oxford: 119–133

HUBER D.M. (1980): The role of mineral nutrition in defense. In: HORSFALL J.G., COWLING E.B.(eds): Plant Disease – An Advanced Treatise, Vol. V. How Plants Defend Themselves, Acad. Press, London: 381–406.

KARLING J.S. (1968): The Plasmodiophorales. Hafner Publishing Company, New York.

KIM M.C., PANSTRUGA R., ELLIOTT C., MÜLLER J., DEVOTO A., YOON H.W., PARK H.C., CHO M.J., SCHULZE-LEFERT P. (2002): Calmodulin interacts with MLO protein to regulate defence against mildew in barley. Nature, **416**: 447–450.

LINDOW S., MONIER J.-M., LEVEAU J.H.J. (2002): Characterising the Microhabitats of Bacteria on Leaves. In: LEONG S.A., ALLEN C., TRIPLETT E.W. (eds): Biology of Plant Microbe Interactions. Vol. 3. Int. Soc. Molecular Plant-Microbe Interactions, St. Paul, Minnesota: 241–249.

LUGTENBERG B., BLOEMBERG G., BOLWERK A., CAMACHO M., CHIN-A-WOENG T., DEKKERS L., KRAVCHENKO L., KUIPER I., LAGENDIJK E., LAGOPODI A., MULDERS I., RADJKOEMAR-BANSRAJ M., TUINMAN S., WEERT DE S., WIJFJES A. (2002): Improving Root Colonisation by *Pseudomonas* Inoculants. In: LEONG S.A., ALLEN C., TRIPLETT E.W. (eds): Biology of Plant Microbe Interactions. Vol. 3. Int. Soc. Molecular Plant-Microbe Interactions, St. Paul, Minnesota: 274–284.

MARSCHNER H. (1990): Mineral Nutrition of Higher Plants. Acad. Press, London.

MYERS D.F., CAMPBELL R.N. (1985): Lime and the control of clubroot of crucifer: effects of pH, calcium, magnesium and their interactions. Phytopathology, **75**: 670–673.

PAGE L.V. (2001): Studies pf components for a potential integrated control system for *Plasmodiphora brassicae*. [Ph.D. Thesis.] University of Strathclyde, Glasgow.

PALM E.T. (1963): Effects of mineral nutrition on the invasion and response of turnip tissue to *Plasmodiophora brassicae* Wor. Contributions of the Boyce Thompson Institute, 22: 91–112.

PATTISON D. (1994): Morphological changes induced in *Phytophthora cinnamomi* Rands. by Extracts of *Ascophyllum nodosum* and Effects on Soilborne Microbial Populations. [Ph.D. Thesis.] University of Strathclyde, Glasgow.

PLANK VAN DER J.E. (1982): Host-Pathogen Interactions in Plant Disease. Acad. Press, London.

TOXOPEUS H., DIXON G.R., MATTUSCH P. (1986): Physiological specialisation in *Plasmodiophora brassicae* – an analysis by international experimentation. Mycolog. Res., **87**: 279–287.

WALSH U.F. (1997): The Effects of *Ascophyllum nodosum* Liquid Seaweed Extract on Microbial Growth, Activity and Pathogenicity. [Ph.D. Thesis.] University of Strathclyde, Glasgow.

WALSH U.F., MORRISEY J.P., O'GARA F. (2001): *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Curr. Opin. Biotechnol., **12**: 289–295.

WEBSTER M.A. (1986): pH and nutritional effects on infection by *Plasmodiophora brassicae* Wor., and on clubroot symptoms. [Ph.D. Thesis.] University of Aberdeen.

WEBSTER M.A., DIXON G.R. (1991a): Calcium, pH and inoculum concentration as factors limiting root hair colonization by *Plasmodiophora brassicae* Wor. Mycolog. Res., **95**: 64–73.

WEBSTER M.A., DIXON G.R. (1991b): Boron, pH and inoculum concentration as factors limiting root hair colonization by *Plasmodiophora brassicae* Wor. Mycolog. Res., **95**: 74–79.

WHAPHAM C.A., BLUNDEN G., JENKINS T., HANKINS S.D. (1994): Significance of betaines in the increase of chlorophyll content of plants treated with seaweed extract. J. Appl. Phycology, **5**: 231–234.

WILD A. (ed.) (1988): Russell's Soil Conditions and Plant Growth. Longman Scientific & Technical, Harlow, Essex.

WORONIN M.S. (1878): *Plasmodiophora brassicae*. Urheber der Kohlp anzen – Hernie. Jb. Wiss. Bot., **11**: 548–574; translated as: CHUPP C. (1934): *Plasmodiophora brassicae*, the cause of cabbage hernia. Phytopathological Classics, No. 4. Am. Phytopathol. Soc., St. Paul, Minnesota.

ZADOCKS J.C., SCHEIN R.D. (1979): Epidemiology and Plant Disease Management. Oxford Univ. Press, Oxford.