Husbandry-the sustainable means of controlling soil borne pathogens-a review

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Husbandry - the sustainable means of controlling soil borne pathogens:- a synoptic review

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Abstract

Soil borne pathogens are especially difficult targets for control by environmentally sustainable methods. The reasons for this include; wide host range providing substantial numbers of alternative hosts including weed species; inoculum distribution varies in depth and intensity across fields and down the soil profile; soil water movement provides means for the redistribution of sexual and asexual propagules; genetic resistance in host species may be limited and variable in effect and the intensity of cropping limits the opportunities for rotational growing. Manipulation of crop husbandry is steadily re-emerging as a key ingredient in disease control strategies. This requires careful strategic planning of crop cycles and the use of resources. Implementing these plans demands continuous monitoring and the introduction of further elements of control in order to maintain crop health. The successful use of husbandry control is critically reviewed with particular reference to Clubroot Disease of Brassica crops (caused *Plasmodiophora brassicae* Wor.). Understanding the impact of nutrient ions and pH on the germination, movement and host colonisation by primary zoospores related to the presence of benign microbes in the soil allows the formulation of coherent husbandry control. Boron, calcium and nitrate salts of nitrogen diminish the germination capacity of resting spores, motility of primary zoospores and their fitness for colonisation. Calcium encourages the expansion of populations of benign microbes. Hence crop fertiliser strategy, soil management and drainage policy can be combined into a sustainable control system for a previously intractable pathogen of horticultural crops.

INTRODUCTION

Soil and air borne plant pathogens interact with the host and surrounding environment over time in a classical manner. Unlike the aerial environment, however, the soil is a complex mixture of organic and inorganic solids, liquids and gases inhabited by a vast array of macro and micro flora and fauna. The scale of this complexity might be judged by the statement that one gram of soil contains numerically more microbes than the accumulated total of the entire human population over the period of man's entire existence. Crop plants are introduced into this environmental maelstrom, provided with optimal resources such as water and nutrients and confronted with numerous pathogenic organisms capable of causing disease. Healthy fruitful crops may be achieved with the aid of synthetic agrochemicals and some natural materials that either prevent pathogen growth and reproduction or minimise their impact on the host plant. This strategy has been used successfully with relatively few soil borne pathogens because of the continuously changing biological, chemical and physical nature of soil environment. The economic, social and statutory constraints now being placed on the use of synthetic agrochemicals reduce still further the use of agrochemicals for the control of soil borne pathogens. It is, for example, a paramount requirement of many fresh vegetable and fruit markets that they should be grown

with minimal or nil use of such materials. This means that either the producer uses pathogen resistant cultivars or deploys highly skilled crop husbandry in the pursuit of disease free crops.
Both options are advantageous in developing robust environmentally sustainable crop production. Breeding cultivars resistant to soil borne pathogens of horticultural crops may not be feasible however, for biological or economic reasons. Consequently, the manipulation of crop husbandry is emerging as the main means of sustainable control for several very destructive pathogens (Dixon, 2007). This is far from being a simple option. It demands the development of well planned crop production strategies based on substantial knowledge of the host – pathogen interactions. Once implemented in practice continuous crop monitoring is essential to ensuring that environmental changes are not shifting the balance away from profitable plant growth and favouring invasion and growth by pathogenic organisms. But successful husbandry control of pathogens provides truly sustainable production. The grower has full control over its use and determines the threshold at which additional controls, for example agrochemicals are deployed where crop damage threatens to become unacceptable. By this means the soil environment is used carefully as part of the disease control strategy. This encourages populations of benign organisms particularly microbes which in turn enhance the growth of healthy crops still further.

Husbandry control is a wide umbrella term covering those aspects of crop production that are directly within the purview and management of producers. It embraces all preparative activities leading up to and including seed sowing or transplanting, through the management of growing crops to harvesting and treatment in preparation for marketing. Factors that may be manipulated in order to reduce the intensity and success of soil borne pathogen infection, invasion, colonisation and subsequent symptom expression are summarised in Table 1. This paper reviews as a specific example, and those factors affecting the progress of *Plasmodiophora brassicae* Wor. the causal agent of Clubroot Disease of brassicas are highlighted in the table.

ASPECTS OF THE BIOLOGY OF *Plasmodiophora brassicae*

This organism cannot be cultured axenically and most of the life cycle takes place inside the host plant. There is only a short period between resting spore germination and the penetration and colonisation of root hairs during which *P. brassicae* is exposed outside the host and in this time it is obscured from direct observation by the soil. Once inside the host this pathogen passes through a stage of primary reproduction and thereafter colonises more deeply into the root tissues where secondary reproduction occurs and host symptoms are expressed. Ultimately this microbe forms resting spores within heavily distorted root parenchyma tissues and these are returned into the soil as a result of host root disintegration incited by secondary bacterial action. Some authorities suggest that *P. brassicae* is exposed to the soil environment in an interval between primary and secondary reproduction. This seems to be an unnecessary exposure to antagonistic microbes and an evolutionarily redundant step. In any event scientifically robust studies of the relationship between this organism and the host and soil environments are sparse (Dixon, 2006).

NUTRIENTS AND THE GROWTH AND REPRODUCTION OF *Plasmodiophora brassicae*

Interactions between host nutrition pathogen growth and disease incidence are well recognised. Soils of acidic pH are traditionally associated with the occurrence of clubroot disease. This was noticed even before the causal organism, *Plasmodiophora brassicae* was identified by Woronin in 1878. Scientifically robust studies of the relationship between this organism and the host and soil environments are sparse. There are ample field based studies demonstrating that the application of various types of lime is associated with reductions in disease development and intensity. Few researchers have attempted to define why calcium or other salts should have this effect, identify the chemical and physical properties required, to determine whether an alkaline pH is an essential feature of this reaction or other substances have similar effects.
Recent detailed studies have investigated the impact of soil environments supplemented with calcium oxide, calcium cyanamide, calcium nitrate, sodium borate, the manner by which pH is affected and its separated impact on *P. brassicae*, and subsequent development of suppressiveness in soils regulated by calcium content interacting with fluorescent pseudomonas bacteria. The impact of calcium on pH results from both its chemical form and physical characteristics. There is an inverse relationship between particle size and the speed with which soil pH reacts to applications of calcium salts. Use of small particle sizes leads to more rapid changes in pH but the longevity of the effects is reduced. Thus where finely divided forms of lime especially those which are most chemically active such as calcium oxide or hydroxide are applied soil pH changes over a relatively short period of time (days). But this effect is lost more quickly. Use of larger particle sizes such as ground calcium carbonate requires greater periods of time (months) for pH changes to take effect and larger quantities are needed. Calcium carbonate is less reactive than the oxide and hydroxide molecules taking longer to influence pH but once an effect has occurred it is more stable. Calcium sulphate is required in even bigger amounts in order to induce pH shifts towards alkaline values, partially this reflects the counterbalancing acidifying role of the sulphate anion. Where equivalent amounts of calcium from the carbonate, hydroxide and

sulphate sources are introduced into soil or compost infested with *P. brassicae* then similar reductions in disease development are achieved.

Studies of the growth and reproduction of *P. brassicae* in root hairs identified increasing the calcium content or providing an alkaline pH environment affected the viability of the pathogen. Where these factors were increased then concomitantly the speed of change by *P. brassicae* from primary plasmodia into sporangia diminished. Although the quantity of calcium or magnitude of the shift in pH governed the rate and extent of growth and maturation by *P. brassicae* the population size interacted in this process. Where larger spore populations were used to generate root hair infection then larger quantities of calcium or bigger shifts in pH were required compared with the impact of lesser numbers of pathogen propagules (Dixon & Webster, 1988; Webster & Dixon 1991 a&b).

Early research showed that clubroot disease in the field is reduced where applications of boron salts are applied, notably boric acid or sodium tetraborate. Laboratory studies identified that boron has effects that are closely comparable with those of calcium. Raising boron concentrations in the rhizosphere represses the rate and scale of change by *P. brassicae* from plasmodia to sporangia in the root hair. Lesser quantities of boron are required in order to achieve similar levels of effect compared with calcium. Boron also has the capacity to affect the progress of *P. brassicae* in the secondary phase when it is more deeply seated in the parenchymatous cells of the root cortex. Symptom expression diminished where the concentration of boron increased (Dixon, 1996). Clubroot symptom development results from the derangement of the host growth hormone metabolism engineered by the presence of the pathogen but not as a direct effect of microbial activity. The effects of boron could reflect involvement in host metabolism since it is accepted as having roles in the formation and activity of indole auxins.

Soil borne pathogens may be effectively suppressed in some soils and inhibited from causing disease whereas in adjacent fields severe infections develop. This well characterised effect is defined as 'suppressive' and 'conducive' soils. Much work particularly in France demonstrated this effect particularly with the organism causing wilt of musk melon, *Fusarium oxysporum* f sp *melonis*. Studies with *P. brassicae* in Brittany identified a similar effect related to the incidence of Clubroot Disease. An analysis of Clubroot suppressive soils in Scotland associated the effect with the presence of elevated calcium content and the presence of bacteria in a manner comparable with results obtained in Germany. The presence of benign microbes and elevated calcium concentrations has been associated with reduced germination capacities for the resting spores of *P. brassicae*. Consequently it appears that suppressivity is related to soil calcium content in a manner not yet defined and this relates to the presence of bacteria that encourage biological control (Niwa *et al.,* 2007).

Calcium cyanamide, a nitrogenous fertiliser with significant liming ability, has been long associated with the suppression of soil borne pathogens (Dixon & Wilson, 1983; Naiki & Dixon, 1987). Key to this effect is the need for repeated applications over several seasons in order to achieve lasting reductions in disease intensity. Research associates the use of calcium cyanamide with reduced incidence of Clubroot Disease. The most likely mode of action is by the enhancement of the growth and reproduction of soil microbial populations antagonistic to *P. brassicae*. There is also the possibility that some of the breakdown products of calcium cyanamide possess fungistatic properties that affect the motile primary zoospores of *P. brassicae* as they move from the resting spore towards the surface of the root hair. Calcium nitrate an important specialist fertiliser used for leafy vegetables, has been associated with diminished impact of Clubroot Disease for many decades. This is one of the most soluble forms of calcium and when introduced into the rhizosphere of growing plants is rapidly taken up by the plant. It encourages benign microbes that are antagonistic to *P. brassicae* in common with calcium cyanamide. The forms of nitrogen made available to the host plant by calcium cyanamide and calcium nitrate (urea and nitrate, respectively) encourage tolerance to disease as compared with the effects of ammonium ions (Dixon & Page, 1998).

PATHOGEN INOCULUM POTENTIAL

Inoculum potential is a concept which associates the concentration of soil borne resting spores of a pathogen with the resultant capacity to cause disease (Garrett, 1956). Normally it is estimated as a direct function of the concentration of spores in soil. This is in contrast with its application for aerial pathogens where a range of measurable meteorological factors may be used in order to achieve greater accuracy in the prediction of the potential scale of an epidemic. In the vast majority of instances such helpful data are unavailable for use with soil borne pathogens consequently the prediction of the likelihood that an epidemic will occur rests solely on knowledge of the spore load present in soil. There are however, several studies of *P. brassicae* that link spore concentration with subsequent disease symptom intensity on susceptible hosts. These are almost wholly laboratory based controlled environment studies. Making assertions from field inoculum concentrations is much more difficult, not least because of the empirical nature of the test methods available to quantify spore concentrations in soil taken from putatively infested land. In the main these rely solely on a seedling bio-assay method whereby soil samples taken from the field are sown or planted with a highly susceptible host. Most often

this is a cultivar of Chinese cabbage (*Brassica rapa*). After 4-6 weeks exposure to the soil sample the resultant plants are uprooted and the presence or absence of clubroot symptoms recorded using a standard disease assessment key.

Obviously such tests are time consuming and the results provide a simple statement regarding the presence or absence of *P. brassicae*. The tests can easily provide falsely negative results because symptoms fail to develop for environmental reasons. Little is learnt from such tests other than the presence of the pathogen and the likelihood that severe or moderately severe symptoms may develop in a susceptible crop. Techniques have been developed using fluorescent dyes allowing the identification of spores of *P. brassicae* in soil samples and in some instances the determination of the viability of the spores. These methods demand intensive laboratory studies and are really only suited for use in research programmes.

Alternatively molecular methods applying the polymerase chain reaction (PCR) have for at least the past decade been seen as offering a potential means for evaluating soil borne spore loads of *P. brassicae*. Significant difficulties have been encountered in their application particularly in finding means whereby pathogen DNA may be prevented from adhering to humic molecules derived from the soil. Most recently it appears that the problem may be resolved by using forms of ELISA test employing antibodies raised against *P. brassicae* which do not appear to suffer from this defect. This will allow the components of husbandry control to be matched with the level of inocula concentrations in soil or other substrates.

ASSEMBLING HUSBANDRY CONTROL RELATIVE TO DEMAND

When the need for control of *P. brassicae* is relatively low then basic husbandry techniques may be sufficient to allow profitable and sustainable cropping (Donald *et al*., 2006). In this situation the use of rational crop planning may be sufficient. Crop rotation that allows the population of pathogenic resting spores to decay, knowing that the half life of *P. brassicae* is 4.5 years may be sufficient. This could well be linked with the use of deep primary cultivation improving soil structure and aeration. Reducing the opportunities for primary zoospores of *P. brassicae* to swim towards the roots of weed hosts by increasing drainage and percolation are useful results of chisel ploughing. Additionally, increased aeration improves opportunities for the multiplication of benign microbes that can be directly antagonistic to propagules of this pathogen. Including particular crops in rotations for example cereals most notably maize and alliums such as leeks has been shown to repress the viability of *P. brassicae*. It is most likely that the mode of action here is via the production and release of molecules which are themselves deleterious to the pathogen. Raising the benign microbial content of soils to achieve forms of biological control may be further accelerated by improving the humus content by adding organic manures. Initial husbandry techniques may be insufficient to cope with more severe infestations of *P. brassicae*. At this point further measures must be incorporated. It may well be that pH requires adjustment to levels beyond 7.0. This might simply represent good husbandry practice or alternatively becomes part of a standardised protocol for coping with an increasing concentration of *P. brassicae* spores in the soil. Using 'slow release' liming materials such as ground limestone (calcium carbonate) are the first phase in this process. Added into the strategy at this point might also be the use of bed systems and ridges for cropping. These reduce opportunities for water logging, add aeration into the soil making it more difficult for primary zoospores to move from the germinated resting spore towards the host plant root hairs. At this point more active measures might be appropriate such as adding non – ionic wetters and additional boron (at 15 to 20 ppm) into starter fertiliser provided at the point of transplanting. Movement of the primary zoospore is achieved by sinusoid motion of its two flagellae this is made more difficult if the surface tension of the soil moisture films is reduced. Maintenance of crop hygiene becomes increasingly important as the inoculum potential rises. Destruction of alternative weed hosts especially other members of the *Brassicaceae* such as ground keeping oil seed rape is essential.

For those soils where the inoculum potential is rising to severe then more positive measures still are demanded. This includes the addition of a fertiliser policy directed towards improving the robustness of crop health and deliberately increasing the presence of benign microbes as soil suppressiveness is increased. As a consequence liming policy may change deliberately to make use of the properties of finely divided calcium oxide or hydroxide which can effect pH changes at a more accelerated rate. Fertiliser use moves towards using calcium cyanamide and calcium nitrate, the later possibly with additional boron ingredient. The imperative shifts towards releasing more calcium into the rhizosphere environment such that it is moved into the root hair by mass flow. Once there, it apparently affects the growth and reproduction of plasmodia and sporangia of *P. brassicae*. This brings about reductions in the impact of Clubroot Disease on the host plant. In some crops there may also be opportunities for the use of some of the cultivars resistant to *P. brassicae* that are now appearing. Where these are available it is important that husbandry techniques are retained in order to reduce the pressure on resistant cultivars and diminish the rate at which the 'boom and bust' cycle will erode their usefulness. European growers of brassicas have no opportunity to make use of agrochemicals as a last line of action against this pathogen, although some very effective molecules are permitted for use in Japan, Australasia and southern Africa.

Results shown in Table 2 demonstrate the manner by which combining fertilisers with calcium oxide treatments were associated with diminished impact of Clubroot Disease in a commercial trial. These data are derived from an observation trial undertaken using crops of calabrese (*B. oleracea* var. *italic*) and combining applications of calcium oxide with calcium cyanamide, calcium nitrate and boron.

Pyramiding fertilisers with calcium oxide had a substantial benefit in terms of reducing the severity of disease symptom development and this was most prominent with calcium cyanamide. The crops were top dressed with either ammonium nitrate or calcium nitrate. Where the latter was used even larger reductions in clubroot symptoms were apparent. Encouraging soil suppressivity to *P. brassicae* through the addition of elevated soil calcium and boron reduced the incidence of root galling as measured by a standard disease assessment key.

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Table 1 Crop husbandry factors that influence the progress of soil borne pathogen infection, invasion, colonisation and disease symptom expression

Field and preparation	Growing season	Post harvest and market preparation
Site selection; altitude, aspect and slope$^{\phi}$, cropping history$^{\phi}$ and previous rotations$^{\phi}$; restricting the frequency of vulnerable crops$^{\phi}$; Soil; structure$^{\phi}$, texture$^{\phi}$, drainage$^{\phi}$, depth$^{\phi}$, organic matter$^{\phi}$ and nutrient content$^{\phi}$, pH$^{\phi}$ and pC$^{\phi}$; microbial content$^{\phi}$, Cultivation; primary$^{\phi}$ and secondary$^{\phi}$; addition of lime$^{\phi}$, oxides$^{\phi}$, sulphates$^{\phi}$ and carbonates$^{\phi}$, particle size$^{\phi}$, organic$^{\phi}$ and inorganic fertilisers$^{\phi}$ and other added resources; Trap cropping$^{\phi}$, monitoring and measuring disease development$^{\phi}$ and plant destruction$^{\phi}$; application of high glucosinolate plants$^{\phi}$ Seed sowing and / or transplanting$^{\phi}$; propagator hygiene$^{\phi}$, crop geometry – intra$^{\phi}$ and inter row spacing$^{\phi}$, use of beds and ridges$^{\phi}$, starter fertilisers$^{\phi}$, crop covers$^{\phi}$, undersowing$^{\phi}$, companion planting$^{\phi}$;	Nutrition: top dressing fertilisers$^{\phi}$ in solid and liquid formulations$^{\phi}$; Irrigation: frequency to restore field capacity$^{\phi}$, rate of application and droplet size; Cropping with alternative hosts, weed removal before$^{\phi}$ and after seeding$^{\phi}$ and transplanting$^{\phi}$; Machinery & labour movements$^{\phi}$; use of tramlines$^{\phi}$; movements between areas of high and low inoculum content$^{\phi}$; Monitoring; measuring and controlling in coming inoculum load and type$^{\phi}$; Field harvesting; trimming and the usefulness of residual waste$^{\phi}$; machinery trafficking$^{\phi}$ and damage to soil structure$^{\phi}$ and drainage$^{\phi}$; Training staff and contractors in disease control$^{\phi}$;	Packhouse: trimming and grading; modified atmosphere packaging; cool – humidity controlled storage; use of senescence / ethylene inhibitors; Monitoring: quality and shelf life longevity; retention / loss of taste, flavour, succulence, health well – being promoting substances (functional foods), development of fibrousness; Post harvest 'cleaning – up' with animals$^{\phi}$;

ϕ = husbandry factors affecting the incidence and severity of Clubroot Disease of Brassicas caused by *Plasmodiophora brassicae* Wor.

Table 2 Results from a commercial observation trial that combined several nutrient treatments on the intensity of clubroot disease symptoms in field crops of calabrese (green broccoli) (*Brassica oleracea* var. *italic* cv. Marathon)

Additional fertiliser treatment ($kgha^{-1}$)	Disease Intensity[1] (maximum value = 90)		
	*Kirkmay + ammonium nitrate	*Barnsmuir + ammonium nitrate	*Barnsmuir + calcium nitrate
Calcium cyanamide 400	Nt	49	34
Calcium cyanamide 400 + active lime	30	37	33
Calcium cyanamide 200	Nt	47	35
Calcium cyanamide 200 + active lime	32	40	31
Calcium cyanamide 100	Nt	45	44
Calcium cyanamide 100 + active lime	41	50	38
Active lime (calcium oxide)	77	69	62
Calcium nitrate 120 + boron	Nt	45	46
Calcium nitrate 120 + boron + active lime	65	43	38
Control	71	74	51

*Location:- East Fife, Scotland, site 1 = Kirkmay (Ordinance Survey ref: NO 604 073); site 2 = Barnsmuir (Ordinance Survey ref: NO 596 062);
Active lime (calcium oxide) applied at 2,500 $kgha^{-1}$ with neutralising value of 105% calculated to increase pH value by 0.5 units;
Both crops received 730 $kgha^{-1}$ basal dressing of fertiliser 5:24:24 = 36N:175P:175K
plus 64 $kgha^{-1}$ nitrogen applied as ammonium nitrate;
Crops planted at Kirkmay and Barnsmuir (ammonium nitrate) were top dressed 5 weeks after planting with 125 $kgha^{-1}$ Nitrogen as ammonium nitrate and Barnsmuir (calcium nitrate) received 125 $kgha^{-1}$ Nitrogen as calcium nitrate;
Calcium cyanamide applied as 400, 200 or 100 $kgha^{-1}$ product = Perlka ™;
Boron applied at 15 ppm as Solubor ™;
Control plots received solely the pre-planting fertiliser applications;
These were studies made in commercial crops and unreplicated;
[1] = Disease intensity = treatments assessed as 30 root samples per plot as cumulative values on the scale: 0 – 3 where: 0 = nil symptoms; 1 = slight symptoms (galls on up to 10% of lateral roots); 2 = moderated galling (galls on 11 – 50% of lateral roots); 3 = severe galling (galls on >51% of lateral roots typically with severe 'finger and toe' deformations); 30 plants per plot assessed hence maximum disease value = 90.
Basal fertiliser treatments were applied between 11th and 14th June 2003, planting was completed on 14th June and disease assessments were made on 28th August 2003.
Nt = no treatment.

Figure 1 Scheme for pyramiding husbandry techniques to avoid Clubroot Disease

INOCULUM POTENTIAL LOW ⟶ ⟶ HIGH	
ROTATION ANTAGONISTIC CROPS	
DRAINAGE SOIL STRUCTURE SOIL AERATION SOIL ORGANIC MATTER BENIGN MICROBES SLOW pH CHANGE – COARSE PARTICLES (CALCIUM CARBONATE)	CALCIUM CYANAMIDE CALCIUM NITRATE BORONATED FORMULATIONS SUPPRESSIVE SOIL RAPID pH CHANGE – FINE PARTICLES (CALCIUM OXIDE)
MONITORING WEED & GROUNDKEEPER CONTROL CONTROL MACHINERY MOVEMENTS AVOID AMMONIUM SULPHATE	BED & RIDGE PLANTING ANIONIC WETTER + BORON SEASONALITY CULTIVAR RESISTANCE