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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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INTRODUCTION

With estimates ranging between 500 and 800 species (and counting!), the oomycetes are a large group of fungus-like micro-organisms with representatives in virtually every terrestrial, marine and freshwater habitat worldwide. Many members of the oomycetes are saprophytic, living on decaying plant and animal remains, but a significant proportion of species are also endophytic and/or parasitic, indeed it is claimed that oomycetes are likely all 'hard wired' for parasitism (Beakes *et al.*, 2012) with many early divergent genera in their phylogenetic tree being marine parasites of a diverse range of organisms.

Some important genera of terrestrial oomycetes are now such specialised pathogens and so strongly co-evolved with their hosts that they have adopted obligate biotrophic lifestyles meaning that they cannot survive and perennate outside living tissues of their specific host species or genera (e.g. the downy mildews and the white rusts). Many other oomycete pathogens are less specialised, being able to attack a wide range of host species, colonising them necrotrophically, whilst a number of *Phytophthora* species (generally more host-specific species) colonise their host hemibiotrophically –starting off like a biotrophe and colonising still-living host tissues but then progressing to a necrotrophic mode of colonisation – killing and digesting the host's tissues.

There is a vast literature on oomycete plant pathogens both in the UK (including a significant number of AHDB Horticulture-funded studies) and worldwide. A simple Google Scholar search will find between 20 and 80 thousand articles just using simple search terms like '*Phytophthora*' or 'oomycete plant pathogens'! Much of this work is focused on key aerial pathogens or pathogen groups on specific economically important crops, such as potato blight (caused by *Phytophthora infestans*), downy mildew of the vines (caused by *Plasmopara viticola*), lettuce downy mildew (caused by *Bremia lactucae*), and brassicas downy mildew (caused by *Hyaloperonospora parasitica*), or where there is an associated quarantine or statutory plant health risk e.g. *Phytophthora ramorum & P. kernoviae* in forestry and ornamental host species. In contrast, comparatively little work has been undertaken on lower stem- and root-infecting oomycete pathogens -predominantly *Pythium*, *Phytophthora* and *Aphanomyces* species -, largely due to the sheer complexity of working in a rhizosphere/soil/growing medium environment.

This is aggravated still further in the horticultural sector where a multitude of host-specific pathogens are problematic on a wide range of minor or specialist crops (grown in many different growing systems including hydroponics and NFT), and where funding is limited relative to broad acre crops such as potatoes & vines. Nevertheless, there is much to be gained from pooling the results of the many often small-scale studies of these pathogens in horticultural crops and considering them together with some of the more in-depth fundamental work that has been achieved in studies of species like *P. infestans*. There are also areas within horticultural practice

that lend themselves to a more generic approach to understanding and tackling oomycete root/collar pathogens, for example the management of substrates and propagation media (including blocks), the logistics and mechanics of movement of plants and materials within the trade and the management and use of water. This review is 'confined' to those oomycete species causing root and stem diseases of horticultural crops, with particular reference to the UK horticulture industry, but many of the statements and broader concepts such as the relative importance of pathogen propagule types and behaviour consider the wider relationships within the oomycetes.

What are oomycetes?

The popularly-used term 'the oomycetes' is a widely accepted name used for a group of organisms that includes the very serious plant pathogen genera *Pythium, Phytophthora* and *Aphanomyces* as well as the downy mildews and the white 'rusts'. These were until quite recently considered as simple but true fungi. However, the use of modern molecular techniques has now shown that the oomycetes are definitely not true fungi and are in fact more closely related to the golden algae (Chrysophyceae), brown algae (Phaeophyceae), yellow-green algae (Xanthophyceae), and diatoms (Bacillariophyceae)(Beakes & Glockling, 2011).

The oomycetes are more correctly referred to as the *Oomycota* and currently with the taxonomy still somewhat 'unsettled', the *Oomycota* can be alternatively considered either as a phylum in the kingdom *Stramenopila* (Alexopoulos *et al*., 1996), or as a class in the kingdom *Chromista* (Kirk *et al.*, 2008)! In addition, an alternative name, the *Peronosporomycetes* has been proposed by Dick (2001). This, although fairly widely used, is considered by many to be possibly an overly strict interpretation of the International Code of Botanical Nomenclature (Lévesque, 2011) and the majority of researchers still use the term 'oomycetes'.

The oomycetes were considered as simple true fungi, despite the early realisation that their reproductive structures show strong similarities to those of the yellow-green alga *Vaucheria* (Pringsheim, 1858), largely because of their mycelial growth habit and their absorptive mode of nutrition. However, the resemblance to fungi is superficial. In the 1960s investigations on cell wall composition (Bartnicki-Garcia, 1966 & 1969; Bartnicki-Garcia & Wang, 1983) and biochemical pathways (Vogel, 1960 & 1961; LéJohn, 1971) indicated closer relationships with certain groups of algae than with the fungi, whilst molecular phylogenic studies from the late 1980s confirmed that the oomycetes are distinctive and more closely related to the algal groups listed above than to the true fungi (Adl *et al.,* 2005; Beakes *et al.,* 2012; Cooke *et al.,* 2000; Lévesque & DeCock, 2004). Nevertheless, it is still common practice to consider oomycetes alongside micro fungi, unfortunately deploying rather confusing terms like 'fungus-like organisms', as they share many basic functional characteristics (Money, 1998), whilst the renamed 'International Code of Nomenclature for algae, fungi, and plants' (McNeill *et al.* 2011) still includes

considerations on their nomenclature under fungi with a small ‘f’ (Schroeder *et al.*, 2013).

Oomycetes as pathogens

Probably the most notorious oomycete plant pathogen species is *Phytophthora infestans,* the causal organism of potato late blight, a crop disease which resulted in the Irish potato famine of the 1840s (Bourke, 1991) and contributed to an estimated 750,000 hunger-associated deaths in continental Europe (Zadoks, 2008). Potato late blight remains a major constraint on potato production (Haverkort *et al*., 2008; Fisher *et al*., 2012; Kamoun *et al*., 2014), and causes worldwide economic losses, in terms of lost yields and revenues combined with the expense of control measures, estimated at $5 billion US per year (Judelson, 2009), with average losses in the USA running at $507 US ha^{-1} (Guenthner *et al*., 2001).

This is really the tip of the iceberg, as other *Phytophthora* species with much broader host ranges also cause both widespread damage to crops as well as extensive ecological damage, for example *P. cinnamomi* which has been given the dubious title ‘the biological bulldozer’ in Australia (Carter, 2004; Scott *et al.,* 2013; Shearer *et al.,* 2004), where it has been responsible for virtually annihilating large areas of biodiverse native forest vegetation (of the 5710 plant species assessed in the South-West Botanical Province, 2284 are susceptible and 800 are highly susceptible to *P. cinnamomi* dieback (Shearer *et al.,* 2004)), as well as causing devastating losses to a range of crops including pineapples and avocados and many important woody and hardy ornamental species worldwide (Zentmyer, 1980, Erwin & Ribeiro, 1996).

Another destructive *Phytophthora* species with a similarly broad host range that has recently caused much concern both in the USA and Europe for the ecological damage it has caused is the ‘sudden oak death’ pathogen *Phytophthora ramorum* (Rizzo *et al*., 2005; Grünwald *et al*., 2008). In addition to *Phytophthora*, species of *Pythium* (Pythiaceae), *Aphanomyces* (Leptolegniaceae) and *Achlya* (Saprolegniaceae) all cause dramatic root and seedling rots of economic importance. For example, *Aphanomyces* root rots of spinach and beets (Larsson & Olofsson, 1994; Williams & Asher, 1996), and of peas and other legumes (Papavizas & Ayers, 1974).

Achlya species are more frequently seen infecting aquatic animals, but one important exception to this is *Achlya klebsiana* which causes seed rot and seedling disease in rice (Webster *et al*., 1970). Many pathogenic species of *Pythium* cause devastating root and lower stem rots as well as damping off in a wide range of vegetable and ornamentals seedlings and young plants (e.g. damping off and seedling rot caused by *Pythium ultimum, P. aphanidermatum, P. disotochum, P. sylvaticum* and *P. irregulare,* Van der Plaats-Niterink, 1981; Rangaswami, 1962; Daughtrey & Chase, 1992).

As well as dramatic disease symptoms, these and other *Pythium* species are responsible for huge losses (sometimes not at all obvious or easily visible) by stunting and slowing of growth in cropping schedules, caused by continuous attrition to root systems by necrotrophic consumption of adventitious roots by moderately aggressive *Pythium* species like those listed above. This is potentially important in protected crops where increased production times can significantly impact on profit margins, for example in cut flowers, where in MAFF-funded trials at HRI Efford, uniformly infected beds of AYR chrysanthemums were not visibly discernible to grower workshop participants but consistently turned out 8-10% yield reductions in comparison with uninfected controls (Pettitt, 2001).

Aside from the crop destruction caused by oomycete phytopathogens, these species are also of enormous ecological importance although our understanding of this is still rudimentary, for example the mechanisms that appear to maintain endemic species in balance when introduced species become invasive 'bulldozers' (e.g. the seemingly minimal impact of *Phytophthora kernoviae* in indigenous forests in North Island, New Zealand, Ramsfield *et al*., 2007, compared to the damage seen in Cornish woodlands and gardens by the same pathogen). Also, several *Pythium* species appear to play an important role in driving the diversity of forest tree flora *via* 'replant-style' disease which prevents seedlings from successfully establishing close to their parent trees (Packer & Clay 2000 & 2003; Van der Putten, 2000). Many other oomycete genera cause important plant diseases including diseases of above ground plant parts caused by the downy mildews, obligate members of the same family as *Phytophthora* (Peronosporaceae), and the white rusts, caused by similarly obligate members of the Albuginaceae family, although these pathogens are not considered in this review.

As briefly mentioned above, there are also a significant number of important oomycete parasites/pathogens of animals, with many aquatic parasites (mostly in the Leptolegniaceae and Saprolegniaceae) of invertebrates, fish and amphibians, including pathogens of huge economic importance to fish farming (e.g. *Saprolegnia parasitica* (Van West, 2006)), second only to bacterial pathogens (Meyer, 1991) and ecological impact (e.g. the *Aphanomyces astaci* Crayfish plague of western Europe, Makkonen, 2013), as well as one oomycete species, *Pythium insidiosum* pathogenic in mammals, and causing 'swamp cancer' in humans (de Cock *et al*., 1987; Gaastra *et al*., 2010).

The parasitism of some oomycetes can be exploited by mankind for example; several species in *Pythium* clade D (Levesque & de Cock, 2004) are mycophagous, readily attacking and feeding upon soil inhabiting fungi and oomycete mycelium (e.g. *P. periplocum, P. acanthicum, P. oligandrum* Clade D, & *P. nunn* Clade J), thus lending themselves for use as BCAs (Martin & Hancock, 1987; Ali-Shtayeh & Saleh, 1999; Paulitz, *et al*., 1990: Vallance *et al*., 2009). Some of the insectivorous species may also be useful in this way for example *Leptolegnia chamanii* and *Lagenidium giganteum* which infect the larvae of the yellow- and dengue-fever bearing mosquito

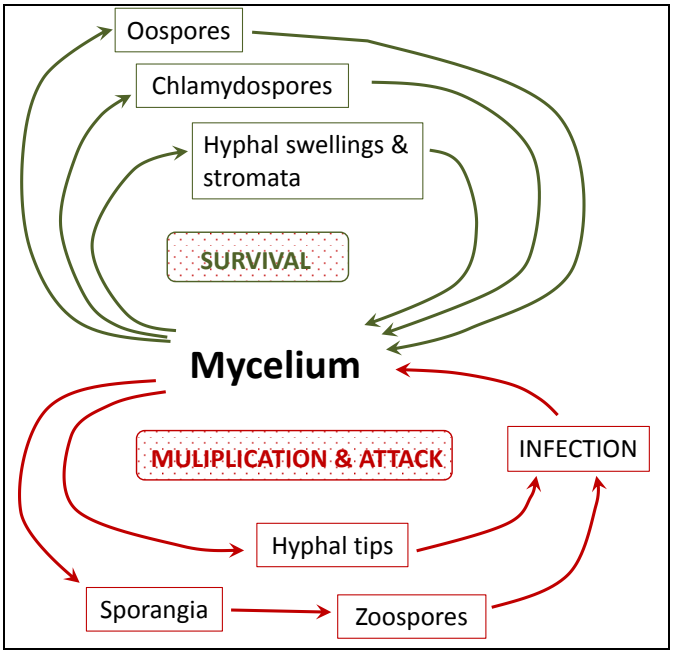
Aedes aegypti as well as other important mosquito species (Golkar *et al.,* 1993; McCray *et al*., 1973; Scholte *et al.,* 2004; Pelizza *et al.*, 2007; De Santo, 2014).

The importance of water

The importance of water in the biology of the oomycetes is indicated by an old name, the 'water moulds', used to describe these and other organisms (mostly fungi) thought to be related to them. Over 20 *Phytophthora* species have been isolated from water around the world (Hüberli *et al.,* 2013), this number is rapidly rising (Cooke, pers. Comm.) and includes important plant pathogens e.g. *P. cactorum, P. cinnamomi, P. citricola, P. cryptogea P. fragariae, P. gonapodyides* and *P. ramorum* (von Broembsen, 1984; Murphy *et al.*, 2009; Palzer, 1980; Pettitt, unpublished; Reeser *et al*., 2011; Smith *et al.*, 2009; Sutton *et al.*, 2009) plus an increasing number of species new to science and of often, as yet, unknown importance to horticulture (Cooke *et al*., 2007; Hong et al., 2008, 2010 & 2012; Lamour, 2013). The precise roles of these new species and their possible pathogenicity have yet to be explored. In addition to *Phytophthora* sp. many other oomycete species are frequently isolated from water samples. Some of these are known pathogen species, many more are of unknown pathogenicity or ecological importance. This has direct relevance to horticultural systems in terms of determining potential disease risks/threats as well as understanding (or otherwise!) the nature of potentially exploitable ecology/biology. For example, helping us to understand why 'endemic' oomycete species remain 'in balance' whereas invasive, introduced species are able to run rampant. It is also important to understand the diversity of these species and their origins to avoid the dangers of a) introducing new and potentially destructive pathogens and/or b) causing the formation of similarly potentially invasive new hybrid species or phylotypes (Érsek & Man in 't Veld, 2013; Parke *et al*., 2014).

Life cycles & sporulation

Figure 1: *Basic oomycete life-cycle*

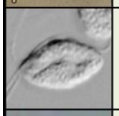
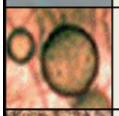
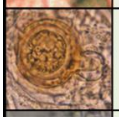
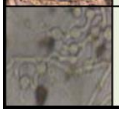


The three main genera under consideration in this review (*Pythium, Phytophthora* and *Aphanomyces*) all share the same basic life cycle around which there are some variations depending on species. For example some species do not produce key spore types, for instance *Pythium violae* and *Pythium sylvaticum* do not produce zoospores (Van der Plaats-Niterink, 1981; Robideau *et al*., 2014). Also there are variations in structure and function of some propagule types as can be seen in the sporangia of *Phytophthora* species; most species are non-caducous and their sporangia remain attached to the hyphae (Erwin & Ribiero, 1996), but many of the 'airborne' species produce caducous (or deciduous) sporangia (Hansen, 2008: Judelson & Blanco, 2005), that readily detach from the mycelium when mature and can disperse by 'wind, splash and trash' in much the same way as fungus conidia. Within the basic life cycle there are three types of asexual spore (chlamydospores, sporangia and zoospores) as well as the tough 'overwintering' oospore which result from sexual recombination (see **Table 1**).

All spores form after a period of vegetative growth by extension of aseptate tubular hyphae or mycelium. This forms the actively-growing 'body' of the organism and is responsible for the actual act of infection (infection hyphae), the colonisation of substrates including host plants, and the secretion of enzymes and the absorptive uptake of nutrients. On culture media, with continued sub-culturing, oomycetes can keep growing vegetatively as hyphal mycelium, but in nature where the nutrient supply is intermittent, spores are required to enable survival and movement to new hosts or food sources. **Figure 1** shows the basic pattern of an oomycete life-cycle. This essentially can be broken down into (a) survival and (b) rapid expansion/colonisation ('attack') phases. Survival is achieved by the formation of tough structures; probably the toughest are the double-walled, sexual oospores, closely followed by asexual chlamydospores and mycelial/hyphal swellings, both of

which exist with a range of wall thicknesses (Ribeiro, 1983) and probably therefore different levels of resilience/resistance, although this is by no means fully understood (McCarren *et al.*, 2005). The 'attack' phase is typified by structures evolved for rapid growth and dissemination; mycelium, sporangia and zoospores, whilst aggressive, are also highly vulnerable to environmental conditions and aggression from other micro-organisms as a consequence of their fine-tuning for active growth and colonisation.

Table 1: *Typical oomycete spore types and growth structures, their functions and estimated survival in various environments*.

Structure	Function	Estimated survival/longevity		
		In soil	In water	Dry
 **Mycelium**	Main 'body' of the organism. Mycelial threads colonise substrates, infect new hosts and absorb nutrients	Hours-Days	Hours-Days	-
 **Sporangia** 	Asexual zoospore-producing structures that can act as spores in their own right by 'direct germination'	Hours-Days	Hours-Days	-
 **Zoospores**	Motile (swimming), asexual spores	Hours-Days	Hours-Days	-
 **Zoospore cysts**	Non-motile asexual structures formed by zoospores (a) as precursor to infection or (b) as survival capsules	Days-weeks	>3 months	-
 **Chlamydospores**	Tough, asexual survival spores	Years	?	+
 **Oospores** 	Tough, sexual survival spores	Years	?	+
 **Hyphal swellings**	Tough, survival? structures formed by mycelium of some oomycete species	Days-months	?	?

Oospores:

As indicated above, not all oomycete species produce the complete complement of spore types and different species vary greatly in the spore types they favour and their abundance of sporulation. Oospores are thick-walled and very durable sexual spores and are very important as long-term survival structures for many oomycete species. They are formed by the fusion of male and female gametangia. When male and female gametangia can be produced by the same body of mycelium and are compatible and able to form oospores, the strain or species is known as homothallic. For many homothallic species, the oospore is of key importance,

allowing long-term survival in soils, infected plant debris, dirt and even dust, for example in *Phytophthora cactorum* where oospores have been recorded to persist for as long as 15 years in ploughland previously under orchards (Waterhouse & Waterston, 1966), and can survive freezing and drying (Sneh & McIntosh, 1974) or in the important 'damping-off' species *Pythium aphanidermatum* and *P. ultimum* that are regularly isolated from soil dust and detritus (Lin *et al*. 2002; Pettitt *et al*., 2001).

Many oomycetes are heterothallic, needing two separate mating types (A1 and A2) to combine to produce oospores; about half of all *Phytophthora* species and at least seven species of *Pythium* are heterothallic (Van der Plaats-Niterink, 1981; Drenth & Goodwin, 1999; Judelson & Blanco, 2005). Sometimes invasive pathogen species introduced to new habitats only exist in these areas as one mating type. This used to be the case with *Phytophthora infestans* potato blight in the UK and, linked with the fact that this species does not readily form tough chlamydospores either, meant that the pathogen was not as tenacious as other oosporic/chlamydosporic oomycete species. This situation has changed since the late 1980s when both mating types were introduced in many parts of the world including the UK, making control of this important pathogen more difficult. Other heterothallic species *do* readily produce chlamydospores, for example the A1 strain of *Phytophthora ramorum* present in Northern Europe and the UK which although possibly incapable of reproducing sexually in nature (Brasier, 2003; Cooke, 2007; Grünwald & Goss, 2011) nonetheless produces chlamydospores in abundance and seems as a result able to effectively survive and 'overwinter' in infected soil and debris for long periods in the absence of susceptible hosts.

Asexual spores:

In general the majority of spores produced by oomycete pathogens are asexual types; chlamydospores are thick-walled 'resting' spores and are important in longer-term survival, whilst zoospores, produced in sporangia, are important in dispersal and infection. Chlamydospores are especially important in several *Phytophthora* species including the much-studied species *P. cinnamomi*, *P. ramorum* and *P.palmivora,* where they often appear to be the predominant 'survival' spore in the absence or relatively sparse production of oospores (Hardham, 2005; Shaffer & Parke, 2013; Ko, 1982).

Nevertheless, surprisingly little is known generally about their biology and epidemiological importance, despite their widespread occurrence in both Phytophthora and Pythium and closely related genera (Ribeiro, 1978; van der Plaats-Niterink, 1981), and they're often more or less overlooked in general studies and reviews (e.g. Martin's 1994 review of phytopathogenic *Pythium*). Germinating chlamydospores produce one or more (often several) germ tubes (Hemmes & Wong, 1975; Shaffer & Parke, 2013), these can penetrate host tissues directly and cause

infections (Basu, 1980) or when in open soil away from host tissues, will often produce sporangia at their growing tips (Tsao, 1969; Hwang & Ko, 1978), enabling the initiation of infection(s) *via* zoospores.

As survival spores, Chlamydospores are often formed within host tissues in the phases of infection, sometimes alongside hyphal swellings or stromata and even oospores (Crone *et al*., 2013). These authors used fluorescent in-situ hybridisation (FISH) to confirm *P. cinnamomi* structures in a range of hosts and they found varying mixtures with host and with co-colonising oomycete species, for example *P. cinnamomi* oospores were produced in much reduced numbers in mixed infections with *Pythium* spp. compared to pure *P. cinnamomi* infections. Chlamydospores are most often constitutively dormant, that is dormant from the time of initiation (McCarren, 2006), and can survive in soils for considerable periods. Basu (1980) recorded soil-survival of *Phytophthora megasperma* chlamydospores of at least 7 months and Kuhlman (1964) found that bare *P. cinnamomi* spores survived up to 18 months, whilst they remained for up to 6 years in infected dead feeder roots of avocado trees (Mircetich & Zentmeyer 1966; Zentmeyer & Erwin, 1970). In recent years more interest has been shown in these spores as they appear to be more resistant than other Phytophthora structures to phosphite fungicidal treatments previously considered to be fully effective (McCarren *et al*., 2005; Hardy *et al*., 2001).

In some species, especially in the genus *Phytophthora,* sporangia can germinate directly as an alternative to producing zoospores and in the caducous species mentioned above, sporangia are also important in dispersal. This evolution of two different germination pathways is extraordinary (Judelson & Blanco, 2005) and it greatly increases the opportunities to *Phytophthora* species for infection. With the two germination pathways there are also a number of permutations that can occur that give great adaptability to this ‘spore system’ (**Figure 2**).

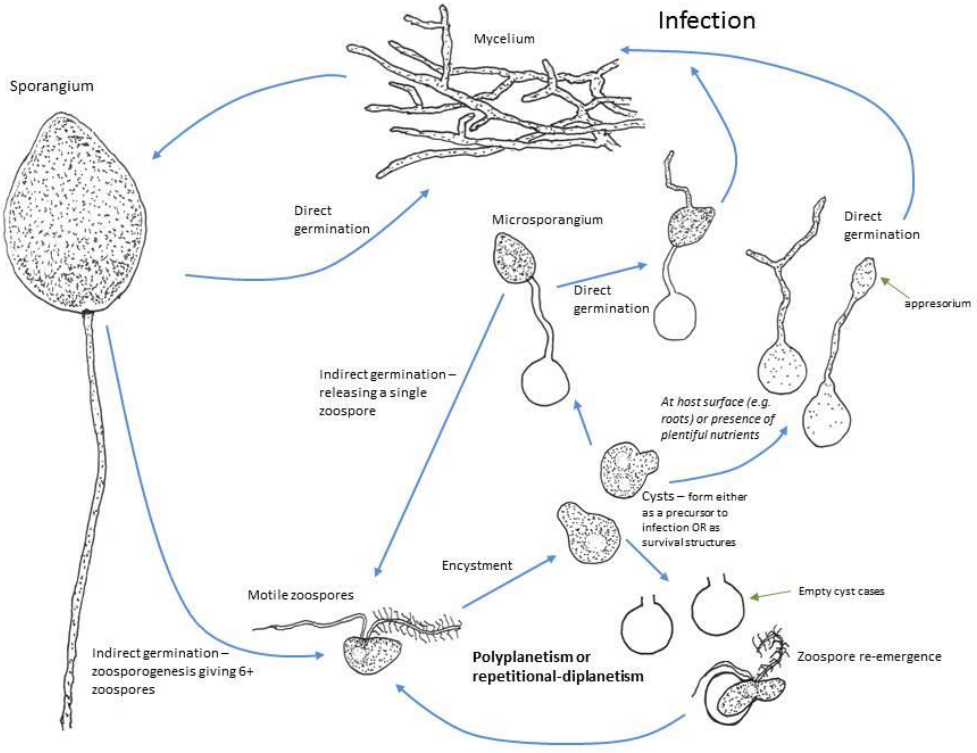
Firstly sporangia can germinate ‘directly’ to produce one or more germ tubes which are capable of either initiating plant infections by forming an infection structure called an appressorium, or of giving rise to fresh sporangia by a process called proliferation. Alternatively, six or more zoospores can differentiate within the sporangium, they then are normally released in water (either (a) thin films on leaf surfaces or in soils, or (b) free water) to swim, although sometimes they remain within the sporangium and germinate *in situ* providing multiple infective germ tubes. Released zoospores are negatively geotactic (i.e. they swim upwards away from gravitational force) and positively chemo- and electro-tactic (see zoospore taxis below) and effectively swim towards potential infection sites where they encyst and germinate.

If conditions are favourable at a potential infection site, encysted zoospores normally germinate ‘directly’, producing a germ tube which then forms a swelling called an appressorium which adheres to the plant surface and from which penetration hyphae emerge and penetrate the host tissues initiating plant infection. However, zoospore

cysts are often capable of two different types of 'indirect' germination in addition to direct germination (**Figure 2**). An emergent hypha can form an apical microsporangium instead of an appressorium, and this can in turn either germinate directly by hyphal tip, or indirectly to release a single zoospore (Drechsler, 1930 & 1931).

The second and probably more important form of indirect germination for a cyst is zoospore re-emergence. In this case, a new revitalised zoospore emerges directly from the old cyst and is able to swim for a further 10-20 h if needs be, leaving the empty cyst case behind looking rather like a transparent 'cartoon bomb'. In addition to being induced at potential infection sites, encystment can occur as a protective response to hostile environments; either those lacking in nutrients or chemical/electrostatic cues for the initiation of taxis (Deacon & Donaldson, 1993) or situations of physical or chemical shock (e.g. Tokunaga & Bartnicki-Garcia, 1971; Pegg & Holderness, 1984; Donaldson & Deacon, 1992; Pettitt & Wainwright, 1997; van de Mortel *et al*., 2009; Ahonsi *et al*., 2010). Cycles of encystment followed by re-

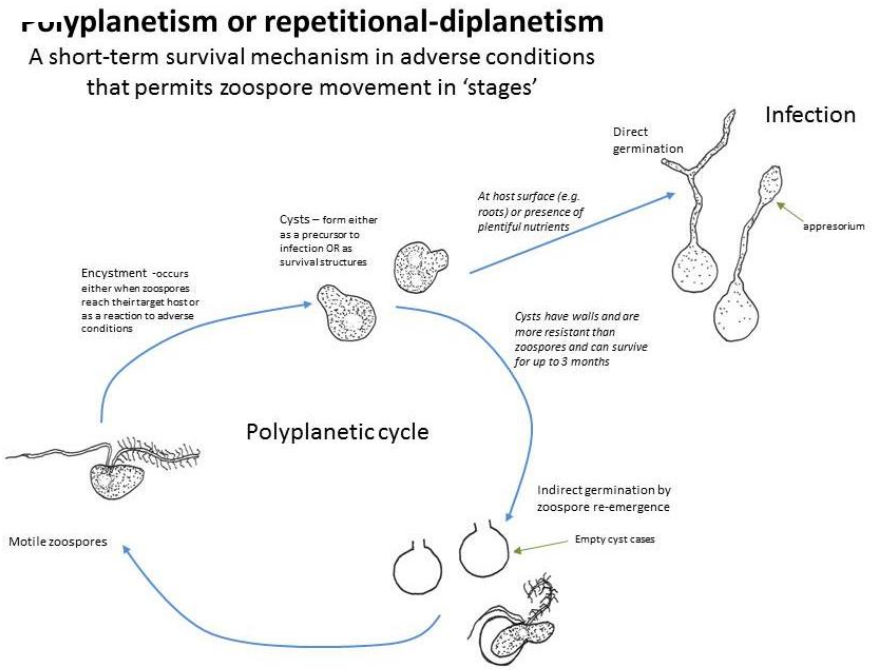
Figure 2: *Illustration of part of the life cycle of Phytophthora cryptogea demonstrating the various germination permutations generally possible for oomycete sporangia and zoospores.*



emergence followed by encystment and so on, are quite common. Known as repetitional diplanetism (Drechsler, 1930 & 1931) or polyplanetism, this process is generally neglected by the scientific literature and yet is probably a key survival mechanism and an additional subtlety to the pattern of inoculum spread and survival

and it should be incorporated in text-book illustrations of the oomycete life cycle (**Figure 3**). At HRI Efford repetitional diplanetism was successfully maintained through >5 successive cycles and ungerminated cysts of *Phytophthora cryptogea* maintained between cycles in 9cm Petri dishes containing irrigation water could remain viable for up to 3 months, re-emerging or germinating by germ tube when given the right stimulus (either placement of several 5 mm rhododendron leaf disks or the addition of 10 mM glucose Pettitt & Wainwright, 1995-97 unpublished…yet!). This cycle is of some epidemiological importance as cysts do appear to be more resistant than highly chemo-sensitive and therefore vulnerable zoopores. For example, zoospore cysts are resistant to CO_2 injections into water whereas zoospores are rapidly killed (Ahonsi *et al.*, 2010), and in *P. infestans* are more resistant than zoospores and mycelium to the cyclic lipopeptide massetolide A (van de Mortel *et al*., 2009) whilst in P. palmivora cysts required 3-8 mg l^{-1} Cu^{++} compared to 1 mg l^{-1} for inactivation of zoospores (Pettitt *et al*., 1991).

Figure 3



The resilience of zoospore cysts and Polyplanetic zoospore re-emergence are probably also often responsible for the survival of oomycete inoculum in moist/wet soils. For example, Vannini et al. (2012) demonstrated that zoospore inoculum of *Phytophthora cambivora,* applied to non-sterile peat-based substrate survived well for 45 days in the absence of any host tissues, despite the fact that this species does not produce chlamydospores and is heterothallic with a single mating type prevalent nature. These authors found that spore counts and rDNA determined by qPCR (see diagnostics section below), showed peaks and declines with numbers reviving during periods of flooding treatment and suggested that encysted zoospores and

microsporangia were the main inocula dispersed in the flood water (Thomson, 1972; MacDonald et al., 1994; von Broembsen & Charlton, 2001; Moralejo & Descals, 2011) and proposed microsporangia as survival structures (Thomson, 1972; Thomson & Allen, 1976).

However, throughout their study, even when microsporangia and hyphae were practically absent, Vannini et al. (2012) detected live zoospores, strongly indicating polyplanetism by re-emergence from zoospore cysts. Cysts of a number of *Pythium* species have been demonstrated to survive in wet soils, although air drying can rapidly eliminate them; *P. aphanidermatum* survived for at least 7 days (Stanghellini & Burr, 1973), considerably longer times were recorded for *P. aquatile* and *P. intermedium* (Hardman *et al*., 1989), and periods up to 80 days for *P. oligandrum* (Madsen *et al*., 1995). These studies all indicate the high potential danger of cryptic spread of oomycete pathogen inoculum in uncovered growing substrates (Schrader & Unger, 2003; Brasier, 2008; Desprez-Loustau, 2009).

Zoospore taxis:

Oomycete zoospores exhibit three kinds of tactic response that enable them to actively swim in numbers towards target host tissues or potential food sources: chemotaxis or swimming up a concentration gradient towards a chemical attractant, autotaxis (auto-aggregation) or 'swarming' and electrotaxis or swimming towards an electrical field (Walker & van West,2007).

Chemotaxis: The chemotactic attraction of zoospores can be highly specific, for example zoospores of *Aphanomyces cochlioides* are strongly attracted to cochliophilin A, a host-specific flavone exuded by roots of sugar beet, spinach and some other members of the Chenopodiaceae and Amaranthaceae (Sakihama et al., 2004; Islam, 2010). Based on observations of the highly-specific chemo-attraction of zoospores of *Phytophthora sojae* to the isoflavones daidzein and genistein from soybean roots (Morris & Ward, 1992; Tyler *et al*., 1996), and its close genetic links to encystment, cyst germination and infection *via* gene PsGPA1 (Hua *et al*., 2008), Oßwald *et al*. (2014) suggested that chemotaxis of *Phytophthora* zoospores to root exudates plays a key role in host recognition and infection and may have an influence on host range.

The latter suggestion relating to host range is only likely to strictly apply when species are exclusively attracted to specific molecules or groups of molecules (e.g. isolflavones as above). Hyphal germ tubes of *P. sojae* have also been demonstrated to respond to isolflavones (Morris et al., 1998), and since cysts may readily form on non-host roots anyway (van West *et al*., 2003, Raftoyannis & Dick, 2006), possibly to re-emerge by diplanetism, such compounds could still have an influence on host selection *via* this route too. Zoospores of *Aphanomyces euteiches* are also strongly attracted to isoflavones from legume roots, whereas *Pythium* species appear not to be (Heungens & Parke, 2000). Similar observations of narrow host ranges relating

to specific chemo-attraction have been made with *Phytophthora palmivora* zoospores which are strongly attracted to isovaleraldehyde and valeraldehyde exuded from *Theobroma cacao* roots (Cameron & Carlile, 1978 & 1981), and such a phenomenon may be important in the case of *Phytophthora rubi* in *Rubus* and this will now come under consideration in the new AHDB Horticulture project SF158.

Auto-aggregation or swarming of zoospores: This appears to be the result of a combination of chemotaxis by as yet unknown but possibly species specific molecules (as zoospores of different species are generally considered to be unable to form aggregates together; Reid *et al*., 1995; Tyler, 2002; Walker & van West, 2007, although quorum signal molecules triggering oomycete zoospore infect appear less specific, Kong *et al*., 2010), and of bioconvection (Savory *et al*., 2014). Bioconvection is a process that is the result of the natural up-swimming of zoospores (and other motile micro-organisms). The spores, being slightly denser than the water, form a dense layer at the top of the water and this becomes unstable resulting in the microbes sinking and setting up convection patterns which vary according to the depth of the water layer (Platt, 1961; Pedley & Kessler, 1990; Kitsunezaki *et al*., 2007). In *Phytophthora parasitica* aggregates of zoospore cysts on Nicotiana tabacum host plant surfaces have been observed to form into microcolonies with a fully organised biofilm structure (Galiana *et al*., 2008). It is thought that these structures increase both the resilience of the pathogen biomass at the host plant surface and improve its pathogenicity (or inoculum potential?), although this remains to be fully tested.

Electrotaxis: Plant roots generate weak electric fields in the rhizosphere that vary in charge and magnitude depending on their condition and stage of growth (e.g. root tips or wound sites Mitchel *et al*., 2002), and the resistivity and therefore the salt content of the water films around the roots (Miller *et al*., 1988; Morris *et al*., 1992). Oomycete zoospores are attracted to these electric fields to varying extents, for example zoospores of *Pythium aphanidermatum* exhibited cathodic electrotaxis (cathodotactic - drawn towards a negative charge) whereas zoospores of *Pythium dissotocum* and of *Phytophthora palmivora* showed anodic electrotaxis (anodotactic - drawn to positively charged fields, Morris & Gow, 1993). The nature of the electrotaxis appeared to be governed by the surface charge on their flagellae, for example the cathodotactic zoospore of *P. aphanidermatum* bore a negatively-charged posterior- and a positively-charged anterior flagellum, whilst in the anodotactic species *P. palimora*, the opposite was the case (Morris & Gow, 1993).

This effect of electric field is thought to act in addition to chemotaxis, often refining the location on a root where infections are likely to be initiated for example the cathodotactic zoospores of *Pythium aphanidermatum* were strongly attracted to typically cathodic wound sites and regions behind the root apices (van West *et al*., 2002). These authors also demonstrated that it is possible to use microelectrodes to apply localised electric fields that recruited zoospores to sites on roots not normally attractive, thus overriding normal endogenous zoospore recruitment signals and

indicating that electrotaxis is a very specific and important short-range guidance mechanism that steers zoospore aggregation patterns on roots (van West *et al*., 2003). Interestingly, Islam & Tahara (2001) found that taxis of zoospores of *Aphanomyces cochliodes* could be disrupted by non-hosts by the exudation of as yet unidentified 'non-host metabolites', the identification and use of these might become an important feature of future biorational control programs.

Some metal ions are important in regulating zoospore and zoospore cyst activity, especially potassium which plays an important role in regulating swimming patterns and speed of zoospores (Appiah et al., 2005) and calcium which plays key roles in encystment, and determining whether cyst germination is direct or by release of a secondary zoospore (von Broembsen & Deacon, 1996; Xu & Morris, 1998). Calcium influxes are also important in the early stages of infection (Warburton *et al*., 1998; Islam & Tahara, 2001), and von Broembsen & Deacon (1997) investigated the potential of manipulating exogenous calcium levels in nutrient solutions to induce premature encystment and inhibit cyst germination by zoospore re-emergence, although Pettitt & Wainwright (1997-2000, unpublished) found that this latter inhibition could sometimes be overridden by the addition of low concentrations (5-10 mM) of D-glucose.

Dispersal:

This is an area where a large proportion of our knowledge is based on generic observations drawn from 'major' pathogens like *P. cinnamomi* (Hardham, 2005). The range of spore types and growth strategies give oomycetes broad options for dispersal. Generally, inoculum produced in the 'attack' phase of oomycete life cycles is vulnerable to desiccation and requires water for dispersal. The one exception to this are the potentially wind-blown caducous (detachable) sporangia of some *Phytophthora* species, although even these still require wet weather conditions to survive and anyway are a group not under consideration in this review.

In some species of *Pythium* (e.g. *P. sylvaticum* and *P. aphanidermatum*), the rate of mycelial extension growth under optimum conditions (normally warm temperatures of 20-30^{o}C, depending on species, in substrates with a high moisture content) can be measured in mm per hour and distances of several cm can be travelled over 24 h. This phenomenon seems to be assisted by *Pythium* species' capacity for saprophytic growth (Martin & Loper, 1999) and can be seen by the rapid rates of recolonization of small areas of steam sterilised soil when surrounded by still-contaminated ground even when not occupied by susceptible plant hosts (Pettitt, 2001), and does not appear to be important to the same degree in *Phytophthora* species where the capacity for saprophytic behaviour in soil is considered limited (McCarren, 2006) and zoospores the most important primary colonisation propagule (e.g. Vannini *et al*., 2012)

All oomycete propagule types can be dispersed in water. Generally in the literature there is a strong accent on dispersal by zoospores in water and yet there are virtually no direct attempts to dissect the spectrum of inocula/propagules actually found in water samples published (Pettitt *et al*., 2002). This is largely a consequence of the methods generally used to determine 'inoculum concentration' in water samples. Baiting and dipstick assay systems (Cahill & Hardham, 1994a; Hardham, 2005; Werres *et al*., 2014), apply a strong selection in favour of zoospores since they both rely largely on taxis to draw out or bait their targets. Membrane filtration-colony plating and zoospore trapping immunoassay (Wakeham *et al*., 1997; Pettitt *et al*., 2002) can reveal propagule types present although there is still no possibility of discerning between motile zoospores and zoospore cysts.

Currently widely-used molecular approaches (e.g. qPCR, see 'Diagnostics' section below) also do not give any indication of propagule types. Despite this, zoospores do appear to be the predominant if *not the only* stem and root rot inoculum type dispersed in water, and the polyplanetic cycle is likely to be of key importance in expanding their range by extending their potential period of viability. Zoospores are dispersed exclusively in water and are able to swim and move passively in currents in surface films of water on plant parts and in soils without necessarily encysting (Newhook *et al*., 1981), and are particularly favoured by water-logged conditions. They are often present in run-off (tailwater in USA) and in puddles (White et al., 1998; Pettitt et al., 2001) and can readily contaminate irrigation water (Moorman *et al*., 2014). Zoospores swim in an α-helical pattern brought about by their two flagellae. The anterior 'tinsel' flagellum, which is covered in short (straw-like, hence the name 'stramenopiles' from Latin, *stramineus* = of straw, Patterson (1999)) bristles or mastigonemes generates thrust, whilst the smooth posterior flagellum steers, enabling fast turning while swimming (Carlile, 1983; Cahill *et al*, 1996; Walker & van West, 2007).

Swimming speeds vary with temperature (Ho & Hickman, 1967; Allen & Newhook, 1973), presumably due to metabolic factors (Carlile, 1983), and are quite similar for a number of *Pythium* and *Phytophthora* species – ranging from 50-250 μm S^{-1} (Carlile, 1983; Appiah *et al*., 2005). Swimming times vary according to environmental conditions, but on average zoospores remain motile for 8-24 h and would therefore be able to swim a distance of almost 13 m in 24 h, assuming an average speed of 159 μm S^{-1} and no obstacles or conflicting stimuli. Greater distances can of course be covered once spores enter water flows and again, zoospores' tendency to encyst when experiencing physical agitation (see above), would be an advantage here, enabling them to 'batten down the hatches' and 'go with the flow'!

Splash is an important mode of dispersal for all propagule types. In studies of *Phytophthora cactorum* in strawberries Grove et al. (1985) found that a mixture of zoospores/cysts, sporangia and mycelial fragments spread up to 120 cm from a point source during a single simulated rain event. Interestingly, this rate of spread could be greatly reduced by appropriate use of mulches. Unfortunately plastic

mulches were the worst, reducing spread by 20%, followed by soil/sand at 53-64%, with straw giving the best performance, reducing spread by 85% (Madden & Ellis, 1990). For measurements of longer-term spread by splash, Ivors & Moorman (2014) cite Garbelotto (unpublished), who found that *Phytophthora ramorum* inoculum spread 100-200 m in one season in the absence of wind, alternatively Shearer *et al.* (2014) report movement of *P. cinnamomi* disease fronts in Australian soils as between 0.1 and 1.46m year $^{-1}$.

The survival propagules, oospores, chlamydospores, mycelial swellings and stromata, are all formed in infected host tissues and are generally released from decaying plant matter and soil organic matter fractions. Structures with thick cell walls and high oil contents, especially oospores, are able to survive desiccation, and viable oospores are frequently found in dust and dirt around contaminated areas on nurseries (White et al. 1998; Pettitt, 2003). This material can be readily dispersed around nurseries on dirty equipment, tools, boot, tyres, trays and containers, and Danish trolleys, whilst wind-blown dust and debris can contaminate gutters, open water tanks and reservoirs, lying water and puddles, and exposed/opened packs of growing media and containers.

Disease

Table 2, showing oomycete stem and root rot pathogens isolated from diseased horticultural crop species grown in the UK is by no means comprehensive, but does give a clear indication of not only the numbers of possible pathogen species, but the relative differences in susceptibility of some crop species to this group of pathogens. Some species (e.g. Rhododendrons and Tomatoes) appear highly susceptible to many oomycete species, whereas others, especially monocotyledonous species (e.g. Iris and Narcissus) are quite resistant to most oomycete pathogens.

On the face of it, the diseases caused by root and stem rot oomycetes in UK horticulture seem very diverse. Over 90 potential pathogen species have been recorded world-wide on the top 150 or so (in themselves highly diverse) crops grown in the UK (Table 2). Of these, 28 pathogen species are already important in a wide range of crops with another 26 recorded in mainland Europe. Many of the latter are currently prevalent in warmer areas of the continent, but with the strong general pole-wards migration of oomycete pathogens recorded with climate change (Bebber et al., 2013); it is likely that some of these will become an increasing threat. Despite the diversity, the symptom types and aetiologies can be broken down into three main categories: root rots; crown and collar rots (‘stem rots’); and damping-off (seedling rots), and diseases within these groups share many similarities.

Root infections by oomycete species are generally initiated on the adventitious roots in the juvenile tissues around the growing tips (Martin & Loper, 1999; Oßwald *et al*., 2014), in fact infective zoospores of *Phytophthora* and many *Pythium* species are

attracted to the root elongation zone, often avoiding both the cap and the root hair zones (Hardham, 2007). After gaining entrance to the root tissues, the pathogen may cause a rapid black rot or a light brown - brown, water soaked rot that can, in more aggressive infections, affect entire primary roots and even progress into the lower stem and crown tissues, although often root rots remain confined to the adventitious roots.

Symptoms can manifest as wilting foliage and main shoots, plant collapse, stunting and yellowing of shoots. Sometimes, low-level infections can be so widely distributed that an entire crop can be 'slowed down' without any obvious symptoms. This last phenomenon was observed in DEFRA-funded trials on hydroponic AYR chrysanthemum crops at HRI Efford (MAFF HH1505 SPC; Pettitt, 2001) where entire beds were evenly infected by *Pythium sylvaticum* and yet symptoms were not discernible to visiting grower experts and only measurable in terms of consistent 7-10% reductions flower stem heights, fresh weights and numbers of class 1 stems at 'grade out'.

Table 2: *List of key UK horticultural crops with species of oomycete rot and/or stem rot pathogens known worldwide to cause disease in them, indicating where possible those species already recorded in the UK (marked in green) and in mainland Europe (marked in blue).*

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
Abutilon	*Phytophthora capsici* *Phytophthora citricola*		*Globisporangium intermedium*		
Acer spp	*Phytophthora acerina* *Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora citricola* III *Phytophthora plurivora* *Phytophthora ramorum*	*Pythium sp.*			
Acer palmatum	*Phytophthora. Cactorum*	*Pythium sp.*			
Almond	*Phytophthora cactorum* *Phytophthora citrophthora* *Phytophthora cryptogea* *Phytophthora niederhauserii* *Phytophthora parsiana*				
Anemone	*Phytophthora cryptogea*				
Antirrhinum	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora cryptogea* *Phytophthora nicotianae* *Phytophthora pini*	*Pythium hydnosporum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium megalacanthum* *Globisporangium spinosum* *Globisporangium ultimum*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
Apple	*Phytophthora cactorum* *Phytophthora cambivora* *Phytophthora cinnamomi* *Phytophthora citrophthora* *Phytophthora cryptogea* *Phytophthora drechsleri* *Phytophthora gonapodyides* *Phytophthora megasperma* *Phytophthora nicotianae* *Phytophthora parsiana* *Phytophthora rosacearum* *Phytophthora syringae*		*Globisporangium sylvaticum* *Globisporangium ultimum*	*Phytopythium vexans*	
Apricot	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora rosacearum*				
Arabis			*Globisporangium intermedium*		
Arbutus	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora ramorum*				
Artichoke					
Arum					
Asparagus	*Phytophthora asparagi* *Phytophthora cryptogea* *Phytophthora richardiae*		*Globisporangium irregulare* *Globisporangium ultimum*		
Aster	*Phytophthora cactorum* *Phytophthora cryptogea*		*Globisporangium irregulare* *Globisporangium debaryanum* *Globisporangium ultimum*		
Azalea	*Phytophthora cactorum*	*Pythium acanthicum*		*Phytopythium helicoides*	

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	Phytophthora cinnamomi (*Phytophthora foliorum*) *Phytophthora hydropathica* *Phytophthora irrigata*				
Beet	*Phytophthora cryptogea* *Phytophthora iranica*	*Pythium afertile* *Pythium aphanidermatum* *Pythium coloratum* *Pythium dissotocum* *Pythium salpingophorum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium paroecandrum* *Globisporangium recalcitrans* *Globisporangium spinosum* *Globisporangium ultimum*	*Phytopythium vexans*	*Aphanomyces cochlioides* *Aphanomyces laevis*
Begonia	*Phytophthora cactorum* *Phytophthora niederhauserii*		*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium intermedium* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium ultimum*	*Phytopythium vexans*	
Belladonna	*Phytophthora erythroseptica*	*Pythium perniciosum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium ultimum*		
Blackberry	*Phytophthora bishii*				
Black current/Red current	*Phytophthora cactorum*				
Box	*Phytophthora citricola* *Phytophthora cinnamomi* *Phytophthora citrophthora* *Phytophthora nicotianae*				

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	Phytophthora niederhauserii				
Brassica rappa	*Phytophthora cryptogea* *Phytophthora erythroseptica* *Phytophthora nicotianae*		*Globisporangium irregulare* *Globisporangium spinosum*		
Brussels Sprouts	*Phytophthora cryptogea*	*Pythium afertile* *Pythium coloratum* *Pythium torulosum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium ultimum*		*Aphanomyces brassicae*
Cabbage	*Phytophthora brassicae* *Phytophthora cactorum* *Phytophthora cryptogea* *Phytophthora megasperma*	*Pythium afertile* *Pythium aphanidermatum* *Pythium dissotocum* *Pythium myriotylum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium megalacanthum* *Globisporangium proliferum* *Globisporangium polymastum* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium ultimum*	*Phytopythium vexans*	*Aphanomyces brassicae*
Cactus	*Phytophthora cactorum* *Phytophthora capsici* *Phytophthora nicotianae*		*Globisporangium irregulare* *Globisporangium paroecandrum*		
Callistephus (China aster)	*Phytophthora cactorum* *Phytophthora cryptogea*		*Globisporangium megalacanthum* *Globisporangium spinosum* *Globisporangium ultimum*		
Calluna	*Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora cryptogea* *Phytophthora ramorum*	*Pythium afertile*	*Globisporangium irregulare*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
Camellia	*Phytophthora cinnamomi* *Phytophthora cryptogea* *Phytophthora ramorum*	*Pythium acanthicum* *Pythium perniciosum*	*Globisporangium irregulare* *Globisporangium spinosum*	*Phytopythium vexans*	
Campanula	*Phytophthora cryptogea* *Phytophthora megasperma* *Phytophthora porri*		*Globisporangium spinosum*		
Carnation/Pink	*Phytophthora cactorum* *Phytophthora nicotianae*	*Pythium myriotylum*	*Globisporangium debaryanum* *Globisporangium spinosum* *Globisporangium ultimum*	*Phytopythium vexans*	
Carrot	*Phytophthora brassicae* *Phytophthora cactorum* *Phytophthora capsici* *Phytophthora drechsleri* *Phytophthora megasperma* *Phytophthora nicotianae* *Phytophthora porri* *Phytophthora richardiae*	*Pythium aphanidermatum* *Pythium coloratum* *Pythium diclinum* *Pythium myriotylum* *Pythium salpingophorum* *Pythium sulcatum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium paroecandrum* *Globisporangium polymastum* *Globisporangium spinosum* *Globisporangium sylvaticum* *Globisporangium ultimum* *Globisporangium violae*	*Phytopythium vexans*	
Cauliflower	*Phytophthora cryptogea*	*Pythium afertile* *Pythium aphanidermatum* *Pythium tracheiphilum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium ultimum*		*Aphanomyces brassicae*
Ceanothus	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora citrophthora* *Phytophthora cryptogea* *Phytophthora*	*Pythium salpingophorum*	*Globisporangium spinosum*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	niederhauserii *Phytophthora ramorum*				
Celery	*Phytophthora cryptogea*	*Pythium afertile* *Pythium hydnosporum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium splendens* *Globisporangium ultimum*		
Chamaecyparis	*Phytophthora cryptogea* *Phytophthora cinnamomi* *Phytophthora lateralis* *Phytophthora niederhauserii*	*Pythium afertile* *Pythium aquatile*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium intermedium* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium ultimum*		
Cherry	*Phytophthora cactorum* *Phytophthora cambivora* *Phytophthora cinnamomi* *Phytophthora citrophthora* *Phytophthora rosacearum* *Phytophthora syringae*	*Pythium afertile* *Pythium monospermum*			
Cherry laurel	*Phytophthora cambivora* *Phytophthora cinnamomi* *Phytophthora cryptogea*		*Globisporangium attrantheridium* *Globisporangium heterothallicum* *Globisporangium intermedium* *Globisporangium sylvaticum*		
Chicory	*Phytophthora cryptogea*				
Choisya	*Phytophthora cryptogea* *Phytophthora ramorum*				
Chrysanthemum	*Phytophthora chrysanthemi* *Phytophthora cryptogea* *Phytophthora tentaculata*	*Pythium aphanidermatum* *Pythium dissotocum*	*Globisporangium irregulare* *Globisporangium intermedium* *Globisporangium*	*Phytopythium helicoides* *Phytopythium oedochilum*	

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
			megalacanthum *Globisporangium paroecandrum* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium sylvaticum* *Globisporangium ultimum*	*Phytopythium polytylum*	
Cineraria	*Phytophthora cinnamomi* *Phytophthora cryptogea*		*Globisporangium megalacanthum* *Globisporangium ultimum*		
Cistus	*Phytophthora cryptogea* *Phytophthora niederhauserii*				
Clematis	*Phytophthora cactorum*	*Pythium sp.*	*Globisporangium ultimum*		
Cordyline	*Phytophthora nicotianae*				
Coriander	*Phytophthora nicotianae*	*Pythium sulcatum*	*Globisporangium spinosum*		
Cotoneaster	*Phytophthora cactorum* *Phytophthora cryptogea*		*Globisporangium intermedium*		
Courgette/Marrow/Squash	*Phytophthora capsici* *Phytophthora cryptogea*	*Pythium aphanidermatum* *Pythium myriotylum*	*Globisporangium mamillatum* *Globisporangium ultimum*		
Crategus	*Phytophthora cactorum* *Phytophthora syringae*				
Cress (*Lepidium sativum*)	*Phytophthora cactorum*		*Globisporangium irregulare* *Globisporangium megalacanthum* *Globisporangium paroecandrum* *Globisporangium sylvaticum* *Globisporangium ultimum*		
Crocus			*Globisporangium ultimum*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
			Globisporangium irregulare		
Cucumber	*Phytophthora cactorum* *Phytophthora cryptogea* *Phytophthora hydropathica* *Phytophthora melonis* *Phytophthora niederhauserii*	*Pythium afertile* *Pythium anandrum* *Pythium aphanidermatum* *Pythium butleri* *Pythium myriotylum* *Pythium tracheiphilum*	*Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium paroecandrum* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium sylvaticum* *Globisporangium ultimum*		
Cupressus	*Phytophthora cinnamomi*		*Globisporangium debaryanum* *Globisporangium ultimum*		
Cyclamen			*Globisporangium debaryanum*		
Dahlia	*Phytophthora cactorum* *Phytophthora cryptogea* *Phytophthora verrucosa*	*Pythium acanthicum*	*Globisporangium debaryanum* *Globisporangium ultimum*	*Phytopythium helicoides*	
Daphne	*Phytophthora cactorum* *Phytophthora cinnamomi*		*Globisporangium debaryanum*		
Dogwood	*Phytophthora cactorum* *Phytophthora cinnamomi*				
Elaeagnus	*Phytophthora cactorum*				
Erica	*Phytophthora cactorum* *Phytophthora cambivora* *Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora cryptogea*	*Pythium hydnosporum*	*Globisporangium irregulare* *Globisporangium intermedium* *Globisporangium megalacanthum* *Globisporangium spinosum* *Globisporangium splendens*		
Euonymus	*Phytophthora ramorum*				
Euphorbia		*Pythium aphanidermatum*	*Globisporangium irregulare* *Globisporangium megalacanthum*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
Eustoma	*Phytophthora acerina*				
Ferns		*Pythium perniciosum*	*Globisporangium intermedium*		
Fig	*Phytophthora parsiana* *Phytophthora niederhauserii*	*Pythium aphanidermatum*			
Fuchsia			*Globisporangium debaryanum* *Globisporangium spinosum*		
Garlic		*Pythium graminicola* *Pythium coloratum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium paroecandrum* *Globisporangium spinosum* *Globisporangium sylvaticum* *Globisporangium ultimum*	*Phytopythium vexans*	
Gerbera	*Phytophthora cryptogea* *Phytophthora drechsleri*		*Globisporangium irregulare*		
Gladiolus	*Phytophthora cactorum* *Phytophthora cryptogea*				
Gloxinia	*Phytophthora niederhauserii* *Phytophthora nicotianae*				
Godetia	*Phytophthora cactorum* *Phytophthora nicotianae*		*Globisporangium debaryanum* *Globisporangium intermedium*		
Gooseberry	*Phytophthora cactorum*				
Grape vine	*Phytophthora cinnamomi* *Phytophthora cryptogea* *Phytophthora inundata* *Phytophthora niederhauserii*		*Globisporangium irregulare* *Globisporangium splendens* *Globisporangium ultimum*	*Phytopythium vexans*	
Hazel	*Phytophthora ramorum*		*Globisporangium intermedium*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
Hebe	*Phytophthora hibernalis* *Phytophthora cinnamomi*	*Pythium acanthicum* *Pythium afertile* *Pythium aquatile* *Pythium middletonii*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium intermedium* *Globisporangium recalcitrans* *Globisporangium spinosum*		
Holly	*Phytophthora cryptogea* *Phytophthora ilicis* *Phytophthora psychrophila* *Phytophthora ramorum*		*Globisporangium spinosum*	*Phytopythium helicoides*	
Hollyhock	*Phytophthora megasperma*		*Globisporangium debaryanum*		
Hop	*Phytophthora cactorum* *Phytophthora citricola*		*Globisporangium intermedium*		
Hyacinth		*Pythium dissotocum*	*Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium ultimum* *Globisporangium violae*		
Hydrangea			*Globisporangium irregulare*	*Phytopythium vexans*	
Iris	*Phytophthora cactorum* *Phytophthora cryptogea* *Phytophthora niederhauserii*	*Pythium dissotocum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium macrosporum*		*Aphanomyces iridis*
Ivy	*Phytophthora megasperma* *Phytophthora niederhauserii*		*Globisporangium splendens*		
Jasmine	*Phytophthora cactorum*				
Juniper	*Phytophthora austrocedrae** *Phytophthora cinnamomi* *Phytophthora cryptogea*	*Pythium acanthicum* *Pythium monospermum*			

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	Phytophthora niederhauserii				
Kalanchoë	*Phytophthora cactorum* *Phytophthora niederhauserii*				
Kale/Seakale					
Lavender	*Phytophthora cinnamomi* *Phytophthora citricola*				
Leek	*Phytophthora porri*				
Lettuce	*Phytophthora cryptogea*	*Pythium afertile* *Pythium aphanidermatum* *Pythium coloratum* *Pythium dissotocum* *Pythium myriotylum* *Pythium tracheiphilum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium megalacanthum* *Globisporangium polymastum* *Globisporangium spinosum* *Globisporangium sylvaticum* *Globisporangium ultimum*	*Phytopythium vexans*	
Leyland Cypress	*Phytophthora cinnamomi*		*Globisporangium intermedium*		
Lilac	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora ramorum* *Phytophthora syringae*				
Lily	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora nicotianae*	*Pythium sp*	*Globisporangium debaryanum* *Globisporangium ultimum*		
Lobelia		*Pythium sp*	*Globisporangium irregular*		
Loganberry	(*Phytophthora fragariae)*		*Globisporangium debaryanum*		
Lupin	*Phytophthora cinnamomi*	*Pythium dissotocum*	*Globisporangium debaryanum*	*Phytopythium vexans*	

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	Phytophthora cryptogea *Phytophthora nicotianae* *Phytophthora sojae*	*Pythium hydnosporum* *Pythium salpingophorum*	*Globisporangium intermedium* *Globisporangium irregular* *Globisporangium ultimum*		
Magnolia	*Phytophthora kernoviae* *Phytophthora ramorum*				
Mahonia	*Phytophthora ramorum*		*Globisporangium debaryanum*		
Marigold	*Phytophthora cryptogea*				
Meconopsis	*Phytophthora cactorum* *Phytophthora nicotianae* *Phytophthora verrucosa*				
Medlar	*Phytophthora cactorum*				
Melon	*Phytophthora cactorum* *Phytophthora cryptogea*	*Pythium acanthicum* *Pythium aphanidermatum* *Pythium volutum*	*Globisporangium megalacanthum* *Globisporangium ultimum*		
Morning Glory		*Pythium aphanidermatum* *Pythium volutum*		*Phytopythium vexans*	
Mulberry	*Phytophthora cinnamomi*				
Mint		*Pythium aphanidermatum*			
Mustard: ***Brassica*** juncea			*Globisporangium ultimum*		*Aphanomyces brassicae*
Narcissus		*Pythium sp.*			
Nasturtium			*Globisporangium intermedium* *Globisporangium ultimum*		
Neanthe (palm)			*Globisporangium debaryanum*		
Onion	*Phytophthora cinnamomi* *Phytophthora porri*	*Pythium graminicola* *Pythium coloratum*	*Globisporangium debaryanum* *Globisporangium irregulare*	*Phytopythium vexans*	

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	What was/is *Pythium*			Other genera
		Pythium	*Globisporangium*	*Phytopythium*	
			Globisporangium mamillatum *Globisporangium paroecandrum* *Globisporangium spinosum* *Globisporangium sylvaticum* *Globisporangium ultimum*		
Allium			*Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium paroecandrum*		
Osteospermum	*Phytophthora cryptogea*				
Parsley		*Pythium sulcatum*	*Globisporangium debaryanum* *Globisporangium megalacanthum?* *Globisporangium paroecandrum* *Globisporangium ultimum*		
Parsnip		*Pythium afertile* *Pythium sulcatum*	*Globisporangium violae*		
Pea	*Phytophthora cactorum* *Phytophthora cryptogea* *Phytophthora drechsleri* *Phytophthora pisi*	*Pythium acanthicum;* *Pythium aphanidermatum;* *Pythium coloratum* *Pythium graminicola* *Pythium hydnosporum* *Pythium salpingophorum* *Pythium tracheiphilum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium sylvaticum* *Globisporangium ultimum*	*Phytopythium helicoides*	*Aphanomyces euteiches*
Pear	*Phytophthora cactorum* *Phytophthora cinnamomi*		*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium megalacanthum*	*Phytopythium vexans*	

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
			Globisporangium splendens *Globisporangium ultimum*		
Pelargonium	*Phytophthora cactorum* *Phytophthora cinnamomi*		*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium mamillatum* *Globisporangium megalacanthum* *Globisporangium paroecandrum* *Globisporangium splendens* *Globisporangium ultimum*	*Phytopythium vexans*	
Pepper	*Phytophthora cactorum* *Phytophthora capsici* *Phytophthora cryptogea* *Phytophthora hydropathica* *Phytophthora irrigata*	*Pythium aphanidermatum* *Pythium dissotocum* *Pythium myriotylum*	*Globisporangium carolinianum* *Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium ultimum*		
Petunia	*Phytophthora cryptogea* *Phytophthora infestans* *Phytophthora nicotianae*				
Phlox	*Phytophthora nicotianae*				
Photinia	*Phytophthora cactorum* *Phytophthora ramorum*			*Phytopythium helicoides*	
Pieris	*Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora cryptogea* *Phytophthora kernoviae* *Phytophthora plurivora* (*Phytophthora obscura)* *Phytophthora ramorum*				

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
Plum	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora citrophthora*				
Poinsettia	*Phytophthora nicotianae* *Phytophthora drechsleri*	*Pythium aphanidermatum* *Pythium ultimum* *Pythium perniciosum* *Pythium myriotylum*	*Globisporangium ultimum*	*Phytopythium helicoides*	
Poppy			*Globisporangium megalacanthum*		
Primula	*Phytophthora cactorum* *Phytophthora citricola* *Phytophthora nicotianae* *Phytophthora primulae* *Phytophthora verrucosa*	*Pythium diclinum*	*Globisporangium irregulare* *Globisporangium megalacanthum* *Globisporangium spinosum* *Globisporangium ultimum*		
Privet	*Phytophthora cactorum*				
Protea	*Phytophthora cinnamomi* (*Phytophthora niederhauserii* on *Grevillea olivacea*) (*Phytophthora niederhauserii* on *Banksia* spp.)				
Pyracantha	*Phytophthora cactorum* *Phytophthora ramorum* *Phytophthora syringae*			*Phytopythium helicoides*	
Quince	*Phytophthora cactorum*				
Radish		*Pythium aphanidermatum*	*Globisporangium debaryanum* *Globisporangium irregulare*		*Aphanomyces raphani*

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
		Pythium hydnosporum *Pythium myriotylum*	*Globisporangium megalacanthum?* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium sylvaticum* *Globisporangium ultimum*		
Raspberry	*Phytophthora bisheria* *Phytophthora cinnamomi* *Phytophthora citricola* E *Phytophthora cryptogea* *Phytophthora idaei* *Phytophthora rubi*	*Pythium middletonii*			
Rhododendron	*Phytophthora aquimorbida* *Phytophthora cactorum* *Phytophthora cambivora* *Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora citricola* II *Phytophthora cryptogea* *Phytophthora drechsleri* *Phytophthora foliorum* *Phytophthora hedraiandra* *Phytophthora heveae* *Phytophthora hibernalis* *Phytophthora hydropathica* *Phytophthora inflata* *Phytophthora insolita* *Phytophthora kernoviae* Phytophthora nicotianae	*Pythium anandrum* *Pythium dimorphum* *Pythium helicandrum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium sylvaticum* *Globisporangium ultimum*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	(*Phytophthora obscura*) *Phytophthora plurivora* *Phytophthora ramorum* *Phytophthora syringae*				
Rhubarb	*Phytophthora cactorum* *Phytophthora nicotianae*	*Pythium anandrum*	*Globisporangium irregulare* *Globisporangium splendens* *Globisporangium ultimum*		
Ribes	*Phytophthora cryptogea*		*Globisporangium irregular*		
Rose	*Phytophthora bisheria* *Phytophthora ramorum*	*Pythium acanthicum*	*Globisporangium debaryanum* *Globisporangium proliferum*	*Phytopythium helicoides*	
Rosemary	*Phytophthora cryptogea*				
Runner beans					*Aphanomyces euteiches*
Saintpaulia	*Phytophthora cactorum* *Phytophthora cryptogea*	*Pythium aquatile* *Pythium diclinum*	*Globisporangium intermedium*		
Sage	*Phytophthora cryptogea*	*Pythium aphanidermatum*			
Schizanthus	*Phytophthora cactorum* *Phytophthora cinnamomi*				
Spathiphyllum	*Phytophthora niederhauserii* *Phytophthora nicotianae*				
Spinach	*Phytophthora cryptogea* *Phytophthora erythroseptica* *Phytophthora megasperma* *Phytophthora nicotianae*	*Pythium anandrum* *Pythium aphanidermatum* *Pythium dissotocum* *Pythium monospermum* *Pythium salpingophorum*	*Globisporangium debaryanum* *Globisporangium irregulare* *Globisporangium spinosum* *Globisporangium sylvaticum* *Globisporangium ultimum*	*Phytopythium helicoides* *Phytopythium polytylum* *Phytopythium vexans*	*Aphanomyces cochlioides* *Aphanomyces cladogamus*
Strawberry	*Phytophthora bisheria*	*Pythium anandrum*	*Globisporangium debaryanum*	*Phytopythium helicoides*	

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	Phytophthora cactorum *Phytophthora citrophthora* *Phytophthora cryptogea* *Phytophthora fragariae*	*Pythium aphanidermatum* *Pythium dissotocum* *Pythium hydnosporum* *Pythium myriotylum* *Pythium perniciosum*	*Globisporangium echinulatum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium sylvaticum* *Globisporangium ultimum*		
Fragaria vesca	*Phytophthora cactorum* *Phytophthora fragariae*	*Pythium middletonii*	*Globisporangium intermedium* *Globisporangium paroecandrum*		
Sweet Pea	*Phytophthora cactorum* *Phytophthora pisi*		*Globisporangium ultimum*		*Aphanomyces euteiches*
Sweet William	*Phytophthora cryptogea*				
Tomato	*Phytophthora cactorum* *Phytophthora capsici* *Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora cryptogea* *Phytophthora hydropathica* *Phytophthora infestans* *Phytophthora irrigata* *Phytophthora mexicana* *Phytophthora nicotianae* *Phytophthora richardiae* *Phytophthora verrucosa*	*Pythium acanthicum* *Pythium afertile* *Pythium anandrum* *Pythium aphanidermatum* *Pythium aquatile* *Pythium diclinum* *Pythium hydnosporum* *Pythium myriotylum* *Pythium perniciosum* *Pythium salpingophorum* *Pythium tracheiphilum*	*Globisporangium carolinianum* *Globisporangium debaryanum* *Globisporangium intermedium;* *Globisporangium irregulare;* *Globisporangium megalacanthum* *Globisporangium proliferum* *Globisporangium paroecandrum* *Globisporangium spinosum* *Globisporangium splendens* *Globisporangium ultimum*	*Phytopythium vexans*	*Aphanomyces cladogamus*
Tulip	*Phytophthora cactorum* *Phytophthora cryptogea* *Phytophthora erythroseptica*		*Globisporangium debaryanum* *Globisporangium ultimum*		
Veronica	*Phytophthora ramorum*		*Globisporangium*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
			megalacanthum		
Viburnum	*Phytophthora cinnamomi* *Phytophthora hedraiandra* *Phytophthora ramorum*		*Globisporangium irregulare*		
Viola/pansy/ violet	*Phytophthora cryptogea*	*Pythium aphanidermatum* *Pythium hydnosporum* *Pythium perniciosum*	*Globisporangium debaryanum* *Globisporangium intermedium* *Globisporangium irregulare* *Globisporangium mamillatum* *Globisporangium spinosum* *Globisporangium violae*		*Aphanomyces euteiches*
Wallflower	*Phytophthora cryptogea* *Phytophthora megasperma*		*Globisporangium ultimum*		
Walnut	*Phytophthora cactorum* *Phytophthora cinnamomi* *Phytophthora cambivora* *Phytophthora citrophthora* *Phytophthora cryptogea* *Phytophthora parsiana*				
Watercress	*Phytophthora cryptogea* *Phytophthora nicotianae*	*Pythium diclinum*	*Globisporangium paroecandrum*		
Water lily		*Pythium hydnosporum*			
Winter cherry (Solanum capsicastrum)	*Phytophthora nicotianae*				
Yew	*Phytophthora cinnamomi* *Phytophthora citricola* *Phytophthora cryptogea* *Phytophthora citrophthora* *Phytophthora cryptogea*		*Globisporangium attrantheridium* *Globisporangium heterothallicum* *Globisporangium intermedium* *Globisporangium irregulare*		

Horticultural crop	Oomycete pathogen genera				
	Phytophthora	**What was/is *Pythium***			**Other genera**
		Pythium	***Globisporangium***	***Phytopythium***	
	Phytophthora gonapodyides *Phytophthora megasperma* *Phytophthora ramorum* *Phytophthora syringae*		*Globisporangium mamillatum* *Globisporangium sylvaticum* *Globisporangium ultimum*		
Zinnia	*Phytophthora cryptogea*	*Pythium aphanidermatum*	*Globisporangium debaryanum* *Globisporangium mamillatum* *Globisporangium spinosum*		

Species known in the UK **Species known in Europe = potential risk to UK**

References: Abad *et al*. (2008); Ainsworth (1937); Álvarez *et al.*(2007); Bala *et al*. (2010); Blair *et al.* (2008); Cline *et al*. (2008); Denton (2008); Donahoo *et al*. (2006);Érsek *et al*. (2008); Erwin & Ribeiro (1996); Farr *et al*. (1996); Ginetti *et al.* (2014); Grünwald (2003); Henley *et al*. (2009); Henricot & Waghorn (2014); Henricot *et al*. (2004); Hong *et al.* (2010); Larsson & Olofsson (1994); Li *et al*. (2014); Man in 't Veld *et al*. (2002); Moore (1959); Moorman *et al.* (2002); Moralejo *et al.* (2008); Mostowfizadeh-Ghalamfarsa *et al*. (2008); Mostowfizadeh-Ghalamfarsa *et al*. (2010); Mrázková *et al*. (2011); Muthukumar & Venkatesh (2012);Orlikowski *et al.* (2007); Rangaswami (1962); Robertson (1976) & (1980); Schuerger & Hammer (2009); Smith (1975); Strouts (1981); Tsukiboshi *et al*. (2007); Uzuhashi *et al*. (2010); Van der Plaats-Niterink (1981); Van Os (2003); Van Os & Van Ginkel (2001); Yoshimura *et al*. (1985); Zentmyer (1980).

Stem rots (also ‘collar rots’ and ‘crown rots’), are probably the most variable symptoms category and can result from the internal spread of aggressive root infections under appropriate conditions as mentioned above, from the splash of inoculum from contaminated growing substrates, structures or other infected plants, or from contaminated irrigation water. The aetiology of stem rots often reflects the growth and developmental stage of the host plant, for example strawberry crown rot caused by *Phytophthora cactorum* can progress quite slowly and pass through long periods of virtually symptomless quiescence (‘silent infections’) or rapidly degrade the vascular tissues in infected crowns, depending on the growth stage of the host plant and external weather conditions, whereas *Phytophthora cryptogea* crown rot of fast-growing asters is relatively fast-acting. The first obvious symptoms of stem rots are usually the rapid discoloration and irreversible wilting of shoots –often giving the (sometimes false) impression of rapid infection. Pathogen species that regularly are associated with stem rots are often specialised in their ability to initiate infections in these areas, for example *Phytophthora cinnamomi* zoospores are chemotactically attracted to emerging axillary roots and the stem regions of thin or discontinuous periderm surrounding axillary shoots where they readily initiate infections in woody hosts (O’Gara *et al.*, 2015).

Commonly considered the territory of fast-growing *Pythium* species pre- and post-emergence damping off or seedling rots (or rotting-off of cuttings) are caused by members of all three pathogen genera under consideration in this review. The factors that can encourage damping off are well defined and include; contaminated seed/propagation material, contaminated growing media, water or containers, and over-watering. The symptoms consist either of non-emergence of affected seed/seedlings, or early wholesale collapse and death of newly-emerged seedlings or freshly-stuck cuttings, which often show water-soaked lower stem lesions or are entirely rotted-off within days of emergence.

There are two groups of oomycete pathogens that commonly cause damping off symptoms: a) aggressive/opportunistic species (often fast-growing species of *Pythium* e.g. *P. ultimum*) or b) species more frequently associated with root or crown rots that have been under conducive conditions (e.g. *Pythium sulcatum* on carrot seedlings Davison *et al*., 2003, and *Aphanomyces cochlioides* in beet and spinach, Islam *et al*. 2005). If they survive the early stages of damping off, plants can survive and ‘grow-through’ the disease, but are still likely to sustain low-level root infections as a consequence, especially if infected by pathogens in the latter group.

There are analogies to be drawn with the ‘growing through’ concept and the pathogens’ preference for attacking juvenile tissues, between survival of damping-off, which seems linked to the establishment non-juvenile roots and collar tissues, and the planting out of propagated plants where the development of vigorous established roots greatly increases chances of survival. For example in root rot of chrysanthemum caused by *Pythium sylvaticum*, where, if a sufficiently vigorous root system can be formed in propagation blocks before planting out, plants have a

greatly increased chance of maintaining height in the first weeks after planting in contaminated soil (Pettitt & Langton, 2002 – AHDB Horticulture PC157) and the survival and fruiting of papaya plants established in 'virgin soil' propagules prior planting *Phytophthora*-contaminated soil (Ko, 1982).

Some pathogen species are highly host-specific, for example *Phytophthora primulae* and its close relatives in *Phytophthora* Clade 8b that are all host-specific, slow-growing and infect specific herbaceous crop species at relatively low temperatures (Bertier *et al.*, 2013). These more host-specialised species appear to be strongly co-evolved with their hosts and likely follow similar, highly evolved 'zig-zag-zig' pathways of molecular interactions and counter-interactions that determine ultimate disease outcomes in more heavily studied pathogens like *Phytophthora infestans, P. sojae* and the downy mildews (Hein *et al*. 2009, Tör, 2008, Fry, 2008).

These interactions involve the secretion of effector molecules which are secreted into host cells and help overcome plant immune systems in susceptible hosts. An important group of effector molecules are those with the RXLR motif that enables their entry into the cell (Whisson *et al*., 2007, Jiang *et al*., 2008, Tör, 2008, Haas *et al*., 2009, Kamoun *et al*., 2014) and these have been found in all *Phytophthora* and downy mildew species assessed but interestingly not in the non-host-specific species *Pythium ultimum* which appears to possibly produce a large range of its own, different effectors, a factor that may be linked to the less specific more opportunistic pathogenicity of this and most other *Pythium* species (Levesque *et al*. 2010). It will be interesting to see whether any of these groups when fully characterised, are also found in some of the species of *Phytophthora* (e.g. *P. cactorum, P. cinnamomi and P. ramorum*) with broader host ranges.

Zoospores are undoubtedly the key dispersal propagule of many oomycete plant pathogens and are certainly of central importance in the key species, *P. infestans; P. ramorum* and *P. cinnamomi*, on which a large proportion of research effort is spent, although in the former two sporangia are also infectious units of some importance. Consequently, a large amount of research effort has focused on the zoospore infection model whereby zoospores swim towards host tissues, find potential infection-sites, encyst and adhere to the host tissue surface, germinate and form appressoria which by a combination of enzyme secretion and the application of pressure (MacDonald *et al*., 2002) penetrate the host tissues (Hardham, 2001), after which a 'molecular battle' ensues between pathogen and potential host involving the 'zig zag zig' process (Hein *et al*. 2009; Tyler, 2009) of effector production by the pathogen and counter-measures by the host until one or the other runs out of cards to play, depending on which, either resistance or disease result. A very neat (and also highly photogenic! *viz.* images of germinated cysts of *Phytophthora cinnamomi* on host surfaces in Hardham, 2001) model system that is being widely studied in molecular labs and throwing up much potentially useful information, it is definitely *not* the whole story with regards oomycete infection of plants. For example, several of the most pathogenic and fast-spreading *Pythium* species; *P. ultimum*, *P. sylvaticum*

and *P. violae* are not thought to produce zoospores, indeed *P. violae* has even been found to have a 'stop codon' in its genome for the flagellum gene rendering any zoospores (if formed) immobile (Robideau *et al*., 2014).

Inoculum:

Reliable and accurate (not *necessarily* ultra-sensitive or precise) inoculum quantification over time and space, in relation to infection and disease development, is crucial to gaining a full insight of disease progress, the proper definition of disease risks and developing an understanding of the impact of cultural practices and the efficacy of management and control treatments on disease. This is well established with airborne oomycete diseases, especially the two important 'airborne' *Phytophthora* species; *P. infestans* late blight for which the classic Beaumont- and Smith- blight warning periods (Smith, 1956) were successfully developed, and *P. ramorum* where airborne inoculum has been quantified and related to infection likelihoods (Webber *et al.*, 2010). However, for purely soil- and water-borne oomycete pathogens of horticultural crops the situation is not quite so well covered (e.g. Hong 2014). In soil, studies on the density of resident inoculum, as opposed to introduced experimental preparations, in relation to disease development are difficult to carry out and relatively infrequent in the literature. Nevertheless, such studies if carried out over time and space can reveal useful information on the dynamics of disease and the impacts of cultural operations (Vawdrey, 2001; Cacciola & Magnano di San Lio, 2008; Pettitt *et al.,* 2011). A major drawback to such studies, and probably the reason why so many studies rely on artificially-introduced inoculum, is the difficulty in separating and identifying the disease-causing propagules from 'background' oomycete populations.

Immunodiagnostic techniques have been effectively used to determine the distribution of *Phytophthora* propagules in field soils (Miller *et al*., 1997). This work has shown that symptom incidence and severity in field-grown peppers and soybeans are related to the concentration of *Phytophthora* inoculum in the soil. Fields affected by *Phytophthora* were intensively sampled and this showed a high degree of heterogeneity of colonisation, and *Phytophthora* propagules were found to be highly aggregated, a situation found with many other diseases (e.g. *Phytophthora parasitica* in citrus, Timmer et al., 1989), and 20 or more samples were needed to give reliable estimates of the mean density of pathogen (Miller *et al*., 1997). A limitation of this work was the specificity of the antibody used, which in this case was only genus-specific. In a study of *Pythium* root rot of chrysanthemum using conventional dilution plating (Pettitt et al., 2011), species specificity was bypassed by the use of a simple detached leaf pathogenicity assay which enabled quantification of pathogenic colony forming units (cfu). This study revealed that reduction in stem height (and therefore yield) was inversely proportional to the number of pathogenic *Pythium* cfu g^{-1} dry weight of soil. Slopes for this relationship varied with sampling

time and location but interestingly a fairly consistent threshold of 2000 cfu g^{-1} was the level of soil colonisation at which 10% symptom severity was observed.

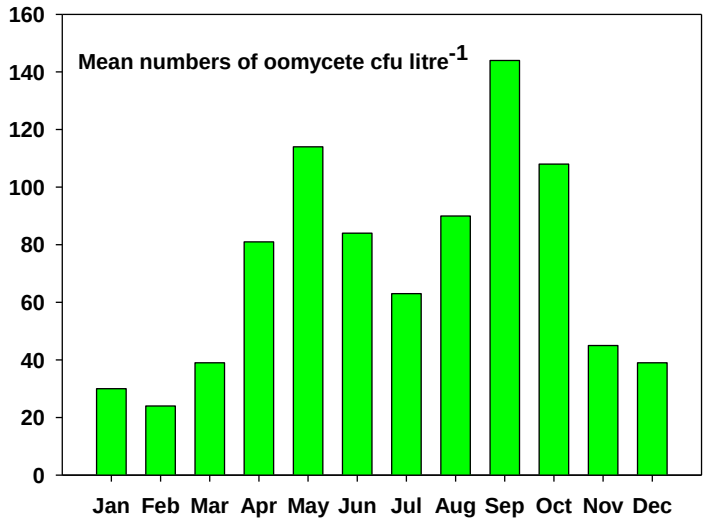
This study is unusual in that a simple and rapid pathogenicity test was possible, directly linking soil colonisation with potential disease outcomes. Unfortunately, in the majority of cases this is not feasible, especially in situations where mixed plantings are being studied, for example on HNS production nurseries. This is where techniques like multiplex quantitative PCR can be very powerful tools, and this approach has more recently been used to assess groups of oomycete pathogens in a range of host species (e.g. *Pythium* populations Kernaghan *et al*., 2008; Li *et al*., 2014; Le Floch *et al*., 2007, *Phytophthora* populations Ippolito *et al*., 2004; Schena *et al*., 2006, and mixtures Lievens *et al*., 2006). There is some value in combining conventional dilution plating with real-time PCR as done by Kernaghan *et al*. (2008), as plating provides a useful source of living reference cultures (Cooke et al., 2007) that can be used for population assessments of pathogenicity (Pettitt et al. 2011), and, by careful microscope assessment of colonies at an early stage of development, can even provide an indication of propagule types present in samples (Pettitt & Pegg, 1990). In addition, discrepancies between results of the two approaches can highlight subtleties that would be missed by one technique used in isolation (Kernaghan *et al*., 2008). Despite the power of these techniques, so far there have been disappointingly few studies relating field inoculum density to disease occurrence and severity.

In citrus production where plating, immunodiagnostics and plating techniques have been intensively used to study *Phytophthora* populations (Timmer et al., 1989 & 1993; Ippolito, 2004), attempts have been made to define inoculum thresholds, with 1-20 cfu g^{-1} being identified as a 'normal' orchard population, >100 cfu g^{-1} associated with disease outbreaks and a threshold for the application of intervention treatments of 10-20 cfu g^{-1} (Cacciola & Magnano di San Lio, 2008). Nevertheless, the determination of 'disease thresholds' has mostly been attempted by inoculation studies. The fairly low cfu counts for the citrus thresholds imply oospore inoculum and comparable levels of oospore inoculum were used by Berger *et al*. (1996) to achieve close to 100% infection by inoculations of growing media with *Pythium* ultimum (10 oospores g^{-1}), *Phytophthora cactorum* and *P. megasperma* (100 oospores g^{-1}). Similarly, Mitchell (1978) found that between 15-43 *Pythium* oospores g^{-1} in flooded soil gave 50% in a range of plant host species. Pure oospore inoculum is difficult to prepare and properly quantify and there is always the potential for either adding much more potentially infective pathogen biomass, in the form of mycelial fragments, than determined by straight spore counts, or of drastically altering the spores behaviour (and possibly inoculum potential?) by vigorous extraction procedures. Many more studies have deployed zoospore inoculum as this is more straightforward to prepare and quantify – although its relationship to 'natural' soil inoculum is debatable. Studies with both *Pythium* and *Phytophthora* indicate that applications of 200-300 zoospores per plant are capable of causing 50% infection in

a range of plant species including tomato, watercress, cotton, amaranthus and strawberries (Mitchell, 1978; Pettitt, 1989; Davis et al., 1997). More recently, inoculations with suspensions of sporangia were used by Tooley *et al.* (2013 & 2014) to establish threshold inoculum levels of 36-750 *Phytophthora ramorum* sporangia ml^{-1} for 50% disease in unwounded plants of a range of species and 100-250 sporangia ml^{-1} with wounding.

As stated previously, in a recent review on the role of irrigation water in plant disease epidemiology Hong (2014) could only find three published studies that reported suitably quantified water-borne oomycete pathogen inoculum (MacDonald *et al*., 1994; von Broembsen & Wilson, 1998; Reeser *et al*., 2011) and even these did not relate inoculum to disease. In preparation for hydroponics pathology trials at HRI Wellesbourne (Calvo-Bado *et al*., 2006), infection–inoculum density relationships were measured and inoculum concentrations of 400 zoospores ml^{-1} for *Phytophthora cryptogea* and 600 zoospores ml^{-1} for *Pythium* group F were found to initiate 50% infection of tomato plants in a small-scale hydroponics system. Concentrations of oomycete inoculum in water can vary enormously with season, and observations from practical irrigation water testing for plant pathogens indicate that there are two peaks in detectable oomycete cfu coinciding with late spring and late summer/autumn usually with a drop in numbers over the summer (see **Figure 4**). This is in contrast to total filamentous fungal counts and the numbers of *Fusarium* cfu which generally reach a single peak in August/September. A similar distribution with distinct peaks of detected cfu in spring and autumn, is seen in citrus orchard soils (Cacciola & Magnano di San Lio, 2008), although this represents the annual progress curves of two different species of Phytophthora (*P. citrophthora* and *P. nicotianae*) separated by what must be assumed would be an arid 'Mediterranean summer'. This data indicates that whilst it is advisable to regularly monitor irrigation water, if doing so infrequently probably the best times to sample will be late spring or early autumn.

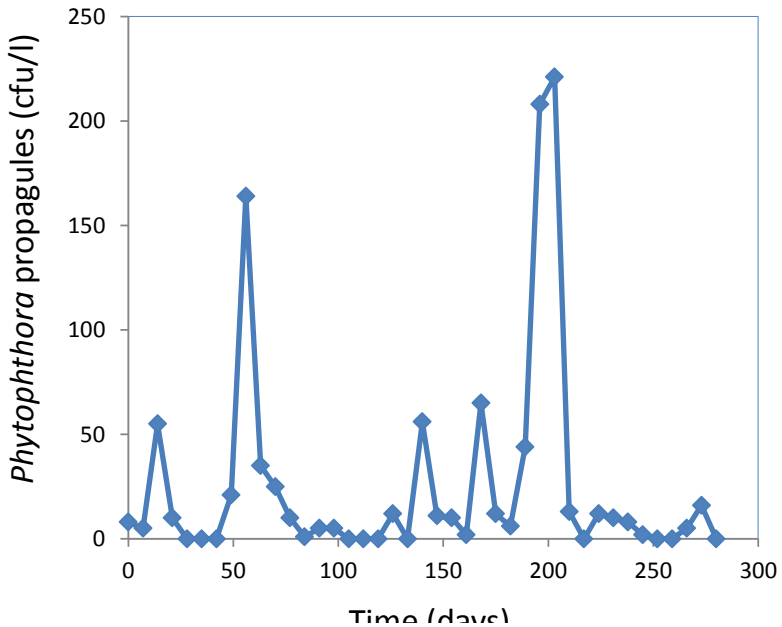
Figure 4: *Variation in total number of oomycete colony forming units (cfu) detected per litre of irrigation reservoir water with season (15 years' of pooled clinic sample data from Stockbridge Technology Centre, HRI Efford and Tim Pettitt).*



In addition to seasonal variation in inoculum, short-term surges can occur (Pettitt *et al*., 1998). These are often initiated by changes in environment like increased irrigation frequency or by rainfall (Ristaino, 1991; Café-Filho *et al*., 1995), but can also result from subtle changes in cultural practice, for example sudden reductions in the root zone temperature in hydroponic crops (Kennedy & Pegg, 1990). **Figure 5** shows the numbers of *Phytophthora cryptogea* cfu detected in runoff water from HNS production beds containing infected *Chamaecyparis* plants in an experiment started at the end of February (Pettitt *et al*., 1998, unpublished).

Whilst inoculum was released from infected plants, numbers of cfu were not consistent but appeared to peak in almost cyclical 'surges'. This work shows that in HNS production systems it is advisable to frequently monitor irrigation water if it is being collected for re-use as these short-lived 'spikes' of inoculum could represent discrete periods of high disease risk. Hong (2014) has considered such scenarios and compared single doses of high inoculum concentration with repeated ('recycling') lower concentrations and found that the latter caused more disease in *Catharanthus* inoculated with *P. nicotianae*. However, this area of work is still in its infancy and more detail is needed on the precise environmental conditions that pertain to inoculum surges to determine whether these a) are also conducive to increased infection and b) whether they might be predicted or avoided.

Figure 5*: Number of Phytophthora cryptogea cfu released over time from early March, from infected Chamaecyparis plants growing in 2 litre pots maintained on a free-draining gravel HNS production bed.*



In inoculations of strawberry crowns Pettitt (1989) obtained widely varying results, with concentrations up to 1000 *Phytophthora cactorum* zoospores plant^{-1} needed to initiate 50% infection in the susceptible variety Tamella and even on one occasion applications of 10000 zoospores plant^{-1} resulted in no infections whatsoever! This variation was largely due to the physiological state of the host plants which were more susceptible when in flower and showed greatly enhanced susceptibility to both infection and crown rot symptom development post-cold-storage treatments when 100% infection and symptom development was achieved with 25 spores plant^{-1}. Yarwood (1976) defined predisposition as 'the tendency of treatments and conditions acting prior to inoculation to affect disease susceptibility'.

By this definition predisposing factors may increase or decrease plants' susceptibility to disease and these can be the result of seasonal changes in the environment and their effect on host plants. For example in the *Pythium* root rot disease system described above, there are periods during the year from late spring through to late autumn when little or no symptoms of disease will be observed even when the inoculum 'threshold' is exceeded. This is likely linked to the plants' increased photosynthesis enabling faster root regeneration combined with the more 'stable' irrigation regimes possible during this period leading to less over-watering. Plant stress is an important predisposing factor (Schoeneweiss, 1975), probably the most important stress factors relating to oomycete root and stem rots are the effects of water-logging and transplanting stress. Much of the later stress can be avoided by making sure that root tissues are at the right stage of active growth at transplanting.

Ivors & Moorman (2014) state that ‘the tremendous physiological variation in *Pythium* and *Phytophthora* spp., as well as their pathogenicity, will preclude the formation of broad generalisations that can be exploited by growers to manage crop losses’ meaning that in their view no generalised thresholds of oomycete pathogen propagule concentrations can be determined. As can be seen here, the truth is that much more of the variation seems to be due to environmental and host factors than on differences between pathogen species *per se.* Nevertheless, current understanding of the dynamics of inoculum production and disease risks is still very limited and more detailed study of the inoculum of several carefully-selected horticultural disease problems will be of wide benefit

OOMYCETE DETECTION AND DIAGNOSIS

Background:

Economic losses resulting from disease development on crops can be reduced by accurate and early detection of plant pathogens. Early diagnosis can provide growers with useful information on optimal crop rotation patterns, varietal selections, appropriate control measures, harvest date and post-harvest handling. Unfortunately, the methods commonly adopted for the isolation of pathogens are slow and normally deployed only after disease symptoms have become apparent.

In an industry where profit margins are narrow and a policy of 'zero-tolerance' of disease expression is generally applied by retailers, a culture of blanket fungicide applications has developed and until recently been tolerated in Europe. However the recent introduction of the Sustainable Use Directive (SUD) is set to change this (http://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides/index_en.htm). Producers will now be required to demonstrate that they have taken alternative integrated pest management (IPM) measures to prevent pest & disease development before the use of spray applications of insecticides and/or fungicides. This will increase the pressure on producers and their staff to monitor and identify potential disease and pest problems quickly.

Pathogen detection prior to infection can reduce or even prevent disease epidemics by identifying when and where treatments and avoidance measures need to be applied. The timely detection and identification of economically important diseases in a commercial cropping environment will provide the initial key to drive a successful and informed control strategy. It is however only part of the solution, the success of which will depend on how the information is evaluated and incorporated within an integrated disease management system (IDMS).

'Conventional' detection and diagnostics: culturing live pathogen and identifying by morphological characters:

Conventional plating of plant tissue, water filtrate or soil suspensions onto semi-selective agars containing antibiotics is a simple and useful procedure for isolating and identifying *Pythium*, *Phytophthora and Aphanomyces* species (Papavizas & Ayers, 1974; Ribeiro, 1978; Tsao, 1983; Hong *et al*., 2002; Pettitt *et al.*, 2002). Early diagnosis of their presence provides growers with vital information regarding the effectiveness of nursery sanitization processes, source contaminants, control-measures to prevent spread, disease containment or eradication.

However, these methods tend to be used more only after disease symptoms are observed and then take valuable time to implement. The detection methods commonly used are those of baiting, culture plating, or a combination of both (Pittis & Colhoun, 1984). Whilst these procedures are useful and relatively simple to carry out, their interpretation requires time and skill and they can give variable results,

especially with plant tissues. Quantification of pathogen propagules or inoculum can be achieved from soil by dilution plating (Tsao, 1983), from water by membrane filtration-resuspension plating (Pettitt *et al*., 2002) and from plant tissues by comminution followed by plating dilutions onto selective agar plates and counting the resulting colonies (Pettitt & Pegg, 1991). Baiting techniques have been used since the 1960s for both *Phytophthora* and *Pythium* detection in water and in soils (Werres, Ghimire & Pettitt, 2014), and can be very effective, although of variable sensitivity, as they are dependent on the quality and physiological state of the plant tissues being used as baits.

Baiting procedures are also likely to give a skewed picture of the potential pathogens present (Arcate *et al*., 2006) and are really best deployed for the detection of specific pathogen species using specific plant tissues. Nevertheless, they can provide confirmation of disease presence with a limited capacity for quantification *e.g*. by the MPN method (Tsao, 1960 & 1983).

The main drawback of these 'conventional' techniques is the time required to generate information; measured in days rather than hours which is often too slow to assist growers in making disease management decisions. This has led to a situation of routine, often prophylactic deployment of fungicides (oomyceticides?!) generally leading to ineffective targeting and overuse, and consequently resulting in the build-up of widespread fungicide resistance (White & Wakeham, 1987).

In the UK, the current best practice 'conventional' diagnostic tests for root and stem rot oomycetes take upwards of 24 hrs with float tests (Ribeiro, 1978; Dhingra & Sinclair, 1995 – specific examples: 24h *Phytophthora* in strawberry crowns, Pettitt & Pegg, 1994; overnight in HNS roots Pettitt *et al*., 1998) and between 3 and 10 days by conventional agar plating methods (Fox, 1993).

Molecular Approaches to Disease Diagnosis

Immunoassays:

Immunoassays have been investigated for their use to provide simple, inexpensive and robust diagnostic tools to monitor disease epidemics. Following the work of Yalow & Berson (1959), using anti-insulin antibodies to measure hormone levels in blood plasma, immunological assay systems have provided an important contribution to analytical diagnostic test development. With an array of different labels and detection systems available, measurement of the antibody (diagnostic probe) and antigen (target analyte / disease propagule) can be made quantitative or qualitative.

This system has been found to be highly transferrable from a commercial centralised laboratory offering a test with high throughput, specificity and sensitivity (for example the enzyme-linked immunosorbent assay (ELISA)) to a simple point of care test system (POC) operated by a non-specialist. The latter of which is designed to be used at or near the site where the problem is located, does not require a

permanent dedicated space and can provide results quickly (within minutes). Originally these types of tests were devised for a clinical setting but are used now used as frequently in patients' homes. They can provide quick feedback in many sorts of investigations *i.e.* enzyme analysis, drugs of abuse, infectious agents, toxic compounds, metabolic disorders, allergens, ovulation and pregnancy testing.

Immunoassays using polyclonal antisera (antibodies isolated from blood serum of immunised animals) were first reported for the detection of viruses and bacterial plant pathogens in infected plant tissues (Voller et al., 1976; Clark & Adams, 1977; Nome *et al*., 1980). The potential of this approach for fungi was demonstrated by Casper & Mendgen in 1979. Later, Johnson (1982) reported the diagnosis of *Epichloe typhina* colonization in tall fescue (causing toxicity syndrome in cattle) using a polyclonal antiserum.

However discrimination of the pathogen was limited to genus level. Where the use of this technology was being successfully applied worldwide for screening plant material for viruses (Raju & Olson, 1985; Burger & von Wechmar, 1988) the poor specificity achieved to the structurally more complex fungal plant pathogens (Drouhet, 1986) hampered the development of immunologically accurate diagnostic probes for commercial applications (Mendgen, 1986, Barker & Pitt, 1988).

As with fungi, the oomycetes share a complex array of antigenic sites that can induce a highly immunogenic and immunodominant response in the immunised animal. These include carbohydrate and protein complexes, of which the *Phytophthora* cellulose binding elicitor lectin (CBEL-1) has been established as playing an important role as a cell surface biomarker (pathogen associated molecular patter (PAMP)) (Larroque *et al*., 2013).

Mannose-containing heteroglycans such as galactomannans and rhamnomannans have also been identified as important derivatives of cell wall substances with importance towards immunogenic dominance. For example, enzymatic digestion and competitive inhibition tests showed that galactosyl residues with beta linkages are immunodominant for *Aspergilus*, *Geotrichum* and *Cladosporium* antigens. Mannosyl residues with alpha linkages provide immunodominance for Mucor antigens (Tsai & Cousin, 1993). The structure and complexity of these pathogens can thus lead to the production of antibodies able to bind selectively to both related and non-related species (Mohan, 1989a & b; Notermans & Soentoro, 1986; Da Silva Bahian et al., 2003; Viudes, et al., 2001; Priestley & Dewey, 1993). In test application towards a specific disease this would be an undesirable attribute and likely prevent successful uptake of the test.

The advent of hybridoma technology (Köhler & Milstein 1975) and, more recently antibody engineering using phage display technologies, has however allowed the generation of highly specific monoclonal antibodies (MAbs) or single-chain antibody variable fragments (scFvs) (Arap, 2005). Targeted to single epitope sites these

immunological probes provide the opportunity to discriminate not only between groups of organisms, but also between different genera, species and isolates of pathogenic fungi (Dewey *et al*., 1990; Priestley & Dewey, 1993; Keen & Legrand, 1980; Hardham *et al*., 1986).

For oomycetes, the ability to identify molecules at a specific stage in a pathogen's life cycle (e.g. zoospores or cysts) has been reported (Estrada-Garcia *et al*., 1990). Whilst this is desirable for detailed epidemiological research, such probe specificity has the potential to be problematic in commercial test development in pathogens where multiple life cycle stages exist. For this reason the organism and the application of the test should be well understood. To overcome these issues the combination of antibody types (monoclonal and polyclonal) has been found beneficial to achieve an appropriate test specificity and/or sensitivity. Equally where non-specific binding to host tissue is observed the use of antibody combinations for capture and labelling of the target antigen (target disease component) has also been found useful (Priestley *et al*., 1993).

These early successes have resulted in a rapid expansion of MAb-based immunoassay diagnostic procedures for the qualitative and quantitative measurement of fungal and oomycete pathogens (Dewey *et al*., 1993, Karpovich-Tate *et al*., 1998; Wakeham & Kennedy, 2010; Wakeham *et al*., 2012; Dewey *et al*., 2013 & Thornton & Wills, 2015). Availability of these probes from maintained cell lines may in the future prove a useful resource for fundamental host-pathogen interaction studies.

In test development, Clark and Adams (1977) introduced the use of the enzyme-linked immunosorbent assay for the quantification of plant viruses in host tissues. This system is now used routinely in laboratories worldwide to provide high throughput, quantitative measurement of contamination of viral, bacterial and fungal plant pathogens in a range of environmental samples (Singh & Singh, 1995). For viral and bacterial samples many of the commercial ELISA systems use a double antibody sandwich format (DAS ELISA). This can prove useful in capture and isolation of a target pathogen from a complex material and provide improvement of specificity with attachment of a second antigen specific labelled antibody.

A second type of ELISA is the plate trapped antigen (PTA ELISA). This assay is often reported for use in the diagnosis of fungal and oomycete plant pathogens. Antibodies raised to these targets are often directed to glycoprotein structures which bind readily to the solid phase surface of an ELISA process and so do not require a capture antibody. Secondly, many soluble glycoprotein structures do not lend functionally to the binding of two antibody types at one time. Where these structures prove heat stable this characteristic can be used in sample treatment to mitigate issues of antibody cross-reactivity (Dewey *et al*., 1997). The third type of ELISA system used routinely is the competitive ELISA (c ELISA).

This format is used extensively in the detection of mycotoxins in food, pesticides in ground water and has been reported for the measurement of some soil-borne fungi and oomycetes in plants and soil for example with *Pythium violae* and *P. sulcatum* cavity spot in carrots (Lyons & White, 1992). However the usefulness of these assay systems for the measurement of plant pathogens in environmental samples, in particular soil, has its challenges.

Soil case study:

In the UK, cavity spot on carrot roots is caused by the soil borne Pythiaceous plant pathogens *Pythium violae* and *Pythium sulcatum* (Hiltunen & White, 2002). Once infected, carrots can quickly develop cavities which are sunken, with circular to elliptical lesions, usually less than 10 mm across and sometimes surrounded by a pale halo. The cavities develop rapidly on roots that are close to harvest; severely affected carrots are unmarketable. Infection and progress of the disease is affected by environmental conditions during the season. Both of these *Pythium* species are able to survive in soil as thick walled oospores, and in the absence of a carrot crop can survive for some years. Both are pathogenic on a wide plant host range so crop rotation as a disease measure can be problematic. Disease severity can increase rapidly in wet conditions (Suffert & Montfort, 2007).

A laboratory diagnostic competitive ELISA has been developed to monitor oospore concentrations in soils (White *et al.* 1995, 1996, 1997). However it has been found to be of limited use for cavity spot disease prediction as *Pythium* oospore concentrations were found not to correlate well with cavity spot incidence (Wynn *et al.* 2000). However this disparity could result from the environmental conditions that prevail during the cropping periods, the soil composition and the use of polyclonal antisera within the assay format. Many oomycete species are found naturally occurring in soil. This may have led to reactivity of the antibodies with these or antigenically related fungal species. Issues of soil inhibitors, assay sensitivity and non-specific binding have been reported for other soil immunoassays (Kageyama *et al.* 2002, Otten *et al.* 1997). Also, the range of soil compositions with differently sized aggregates and irregular distribution of microbial populations, presents challenges for the use of immunoassays directly in soil.

To overcome these potential challenges to immunoassay efficacy, workers have attempted to develop simple and efficient extraction processes for isolation of the target pathogen from the soil. However this has proven one of the biggest hurdles in the development of quick and sensitive plant pathogen diagnostic immunoassays (Dewey & Thornton, 1995). Of the soil-based tests developed many have had to resort to the use of a biological amplification stage (soil-baiting) to provide target analytes at concentrations suitable for readability (Yuen *et al.* 1993; Thornton *et al.* 2004).

A benefit of this is that these tests can provide valuable information on viability of the target organism. However, they are generally reduced to being qualitative or semi-quantitative and can prove as time consuming as conventional media based isolation tests. Other approaches have been to develop tests which require a pre-treatment, for example drying, grinding, centrifugation and floatation processes, to recover pathogen resting structures. These processes often prove both laborious, lack economy of scale and, require considerable laboratory space prior to analysis (Wallis *et al*., 1995; Wakeham & White, 1996; Miller *et al*., 1997). The ability to develop highly sensitive and inexpensive assay is somewhat irrelevant if the extraction process is lengthy, laborious and costly in time and labour.

For this reason, simple and rapid processes are required to isolate and concentrate disease propagules from soil. Separation of bacteria has been achieved by immunomagnetic capture with isolation, concentration and detection reported from contaminated feedstuffs (Johne et al. 1989; Mansfield et al. 1993), faeces (Luk and Lindberg, 1991) aquatics (Bifulco and Schaefer, 1993) and soil (Mullins et al. 1995). Recently, this approach has been adopted to isolate and concentrate resting spores of the clubroot plant pathogen from infested UK horticultural and agricultural soils (Kennedy & Wakeham, 2015). Monoclonal antibodies specific to *Plasmodiophora brassicae* (causal agent of clubroot) and conjugated to super paramagnetic spheres have been used to 'fish' soil for *P. brassicae* resting spores. The labelled spores are isolated from the soil matrix and concentrated by exposure to a magnetic field. Quantification of the isolated spores is determined either by quantitative polymerase chain reaction (qPCR) (Lewis, 2011) or by using an on-site ten minute lateral flow test (Wakeham et al. 2012).

New technologies:

A refinement of the magnetic capture concept offers opportunities to develop immuno-array tests (multiplex testing for more than one pathogen). These tests can be used to measure multiple plant pathogen incidence in complex environmental samples such as soil. An example of this being the magnetic microsphere capture immunoassay system (Luminex MAGPIX technology). The technology employs a set of 50 different fluorescence colour coded magnetic microspheres of which each coloured microsphere set can be coated either with target analyte or a target pathogen-specific probe. Using a 96 well ELISA format, samples for testing are aliquoted (20-100µl per well). Within each well there is the potential to deploy 50 bead types at once each seeking and binding to a specific homologous target pathogen analyte.

By applying a magnetic field, the beads with bound target material can be withdrawn from the sample and retained and separated from potential assay inhibitors. After this an ELISA process is carried out and the magnetic sphere bound target analyte is identified by linking with a fluorophore (R-phytcoerythrin) conjugated detector antibody. The MAGPIX system is able to identify the colour-coded magnetic bead

and measure the fluorescence of the detector antibody to provide quantification of multiple target pathogens in a sample. This approach provides a versatile multiplexing platform capable of performing qualitative and quantitative analysis of up to 50 target analytes in a single reaction volume and, in a variety of sample matrices.

The assay time of the microsphere immunoassay (1hr) is much shorter than for a standard ELISA system (approx. 4hr).There have been several reports using this new technology to detect foodborne pathogens and toxins (Kim *et al*., 2010), three potato viruses in infected host tissues (Bergervoet *et al*., 2008) and a multiplex plant pathogen assay designed for use in seed screening to simultaneously detect four important plant pathogens: a fruit blotch bacterium (*Acidovorax avenae* subsp. *Citrulli*), and three viruses (chilli vein-banding mottle virus, watermelon silver mottle virus and melon yellow spot virus) (Charlermroj *et al*., 2013). The platform should prove highly versatile for epidemiological studies and crop clinic work assaying for, isolating, concentrating and quantifying multiple plant pathogens in potentially complex samples, such as soil, plants or water, at moderate cost.

Nevertheless, there is a requirement for inexpensive tests that can be used on-site for routine sampling. For example, to determine the efficacy of sanitation processes and in early disease detection/warnings on pre-symptomatic crops.

Existing assays such as the ELISA often can translate to the simplified POC format whilst retaining the tests' original performance characteristics. This process eliminates the requirement for laboratory equipment and highly trained personnel whilst providing a quick test turn-around time of approximately 10 minutes. The results can be qualitative (yes/no) or made quantitative by using a digital reader. The Clearblue Advanced Pregnancy POC system combines two tests within one system and an inbuilt digital reader to report to the end user a written display of "Pregnant" or "Not Pregnant". A quantitative reading of 1-2, 2-3 or 3+ is displayed to indicate by how many weeks.

This integrated technology is currently limited to the pharmaceutical industry where a strong global market can support the financial investment required for test development and delivery. In plant production, where the financial return is not so great, test development is restricted to a stand-alone reader for quantitative measurement of pathogen incidence. Initially these readers were developed for use solely with a specific product line. Charm Sciences offers the ROSA-M reader system which is a hand-held instrument designed to electronically read and quantitate results from ROSA POC strips for feed, grain and wine mycotoxin tests (www.charm.com/instruments/instruments-rosa-reader).

However with the surge in POC development to ever expanding markets, more companies are coming on line to deliver generic POC readers which can be tailored to specific product lines. These smart readers are able from the POC test barcode to

identify the correct analysis to be applied for quantitative measurement and display, print, email or download the results to a computer. The Vertu reader has been tailored to deliver POC mycotoxin testing to food and agriculture producers worldwide to protect humans and animals from potentially lethal effects of contamination (www.vicam.com/vertu-lateral-flow-reader).

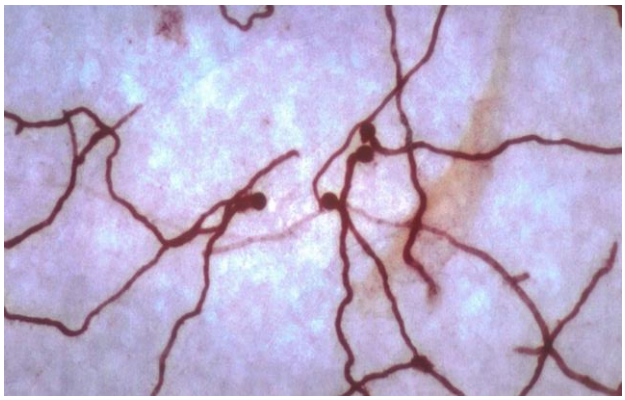
The reader in its generic form is an ESE-Quant Lateral Flow System and can be purchased from QIAGEN (www.quiagen.com). Skannex (http://www.skannex.com) offers the SkanSmart hand held system which can be developed to analyse POC-specific test formats. It has been developed to provide ultimate flexibility by providing a capability to analyse tests in multiple design formats: single and multiplex analysis of a strip, single and multiple strip cassette strip formats.

The development and use of these POC assays has reduced the time taken to achieve reasonably accurate diagnosis of plants infected by some diseases. Originally developed by Agri-Diagnostics Associates as flow through tests (Ellis & Miller 1993) , these or variants, for example immuno-chromatographic test strips (Wong & Tse, 2009), are available worldwide for on-site testing of a range of viral, bacterial and fungal plant pathogen infections (www.neogen.com; http://www.envirologix.com; www.pocketdiagnostic.com). Although on site testing has been found useful to quickly determine oomycete infections, currently-available tests have limitations in their ability to discriminate at the species and, at times even to the genus level. Although, this is perhaps not so problematic for *Phytophthora*, where the majority of species might be considered a potential risk to cropping systems, the same is not the case for *Pythium* species, a large proportion of which are saprophytic and not pathogenic to horticultural crops. In addition, as outlined in the 'Oomycetes as pathogens' earlier in this review, at least four species, *Pythium oligandrum*, *P.nunn*, *P. perioplocum* and *P. acanthicum*, are mycophagous and therefore potentially beneficial in disease control (Martin & Hancock, 1987; Ali-Shtayeh & Saleh, 1999; Paulitz, *et al*., 1990: Vallance *et al*., 2009). The value of these immunoassay tests has also not been assessed for some environmental samples (e.g. growing substrates) or for the pre-symptomatic infection of plant material, although their use in conjunction with plant tissue baits has been assessed with some promise in AHDB Horticulture project HNS/PO188 (Wedgwood, 2014).

Importantly, these tests as they stand fail to distinguish between live and dead pathogen propagules. Although, Cahill & Hardham (1994b) overcame this to some extent by exploiting zoospore chemo taxis and developed a test which could be carried out in water and on-site by unskilled operators. However, often only a limited proportion of the total number of zoospores present in a water sample are detected using this method (Pettit *et al*., 2002), and it may be wise to include a step inducing cyst germination to prove viability as opposed to relying solely on chemotaxis (or apparent chemotaxis), since apple bait pieces were found to pick up non-viable pathogen material under comparable circumstances in AHDB Horticulture project HNS/PO188 (Wedgwood, 2014).

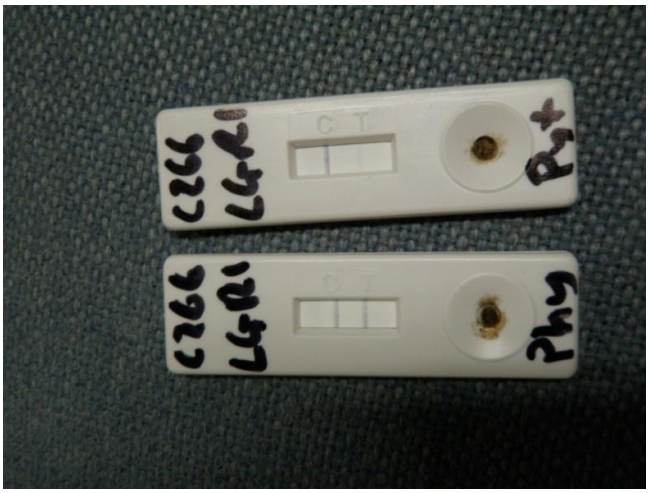
This limitation could be very important in irrigation water supply where the number of zoospores per unit volume may be very low. Other workers have tried to overcome this by the development of a zoospore trapping immunoassay (ZTI – Wakeham *et al*., 1997). This process concentrates material from irrigation water by filtration onto a membrane. Following a short incubation with a selective medium the viable zoospore-germlings, if present, can be visualised using a specific antibody probe conjugated to a coloured marker (see **Figure 6**). To date this is one of the most sensitive test procedures to have been successfully deployed in routine water assessments for the measurement of viable oomycete propagules (Pettitt *et al*., 2002). However, as a commercial system, the supply of the polyclonal antiserum has over time proven the test to be self-limiting. Nevertheless, new monoclonal antibody probes are now under development for this purpose in AHDB Horticulture project CP136.

Figure 6: *Developed ZTI membrane showing trapped and germinated Phytophthora zoospore cysts.*



On site immunoassays are increasing in popularity and look like they are here to stay, and for the moment, the immuno-chromatographic test strip (lateral flow) is proving a successful format. Lateral flows consist of a carrier material containing dry reagents that are activated by applying a liquid sample. Movement of this liquid allows passage across various zones where molecules have been attached that exert specific interactions with target analytes. Results are usually generated within 5-10 minutes with the formation of a control and test line as appropriate to the sample and the test (**Figure 7**). They are designed for single use, can be quantitative in measurement and can provide a limited multiplex test platform. In plant protection they are increasingly used to provide a first line rapid defence screen.

Figure 7: *Lateral flow device*



This is amply demonstrated in forestry disease management where a genus *Phytophthora* test device has been used in the UK by Fera Plant Health and Seed Inspectorate to monitor the spread of the oomycete pathogens *Phytophthora ramorum,* the causal agent of sudden oak death and dieback/leaf blight in a range of tree, shrub, and herbaceous species, and the recently described pathogen *Phytophthora kernoviae.* Initial positive diagnosis of the pathogen has enabled the effective management of the disease on horticultural nurseries by immediate quarantine and containment measures (Kox *et al*., 2007; Lane *et al.*, 2007). Once a sample is identified as a potential risk from infestation confirmatory tests are undertaken to fully characterize the strains involved using DNA-based molecular techniques (www.fera.defra.gov.uk/plants/publications/documents/factsheets/pramparks.pdf).

Lateral flow tests have also been used as a quality control diagnostic tool to provide immediate on-site results of product suitability. The importance of *Fusarium* mycotoxins to human and animal health is well documented. To identify levels of contamination in crops, monoclonal antibody immunoassays have been developed specific to fusarins, T-2 toxin, zearalenone (F-2 toxin) and DON (Barna-Vetro´ et al., 1994; Casale et al., 1988; De Saeger& Van Peteghem, 1996; Maragos et al., 2008).

These assays have improved the sensitivity, specificity and speed at which the mycotoxins can be detected. Through the development of rapid on-site immunoassays for use in farms, storehouses and factories Envirologix, under their

QuickTox label (http://www.envirologix.com) supply a range of immuno-chromatographic tests to provide quantitative and traceable test results for mycotoxins in commodity grains. Within this product range a lateral flow device is also available to rapidly determine levels of stable *Botrytis* antigens in table and dessert wines (Dewey *et al.,* 2013). Lateral flow assay systems have also been developed and used to track horticultural biocontrol agents. Using a monoclonal antibody probe active propagules of *Trichoderma* species can be detected in soil samples within 15 min of antigen extraction. The device can also be used to detect human infections (Thornton & Wills, 2015).

Although the lateral flow POC assay has its strengths there are potential weaknesses of the test format (Poshuma-Triumphe *et al.,* 2009). As with DNA-based detection assays, the total volume of the sample that can be applied to a test kit is quite small and this could lead to a limit on sensitivity. This may be addressed by a pre-extraction treatment such as immuno-magnetic capture. However, additional sample processing adds a level of complexity and detracts from the simplicity of the one step test approach. Application of the sample to the lateral flow is often drop-wise and this has the potential to lead to a level of imprecision, especially if tests are being measured with a reading device.

In complex environmental samples, for example soils, food, or estuarine water, there is the capacity for the test strip to become blocked and inhibit the assay process. Suppliers of these test components have, to a large extent, overcome these problems by producing pre filtration materials that can be incorporated within the lateral flow format. Equally they have been quick to react to sample volume issues by supplying a range of sample pads that allow increased volumes to be held prior to the immunoassay stage (www.millipore.com/diagnostics; www.whatman.com/DiagnosticComponents). Measures should also be taken to determine the shelf life stability of the product over a range of environmental conditions. Often a requirement of these tests is global shipment and this may involve periods of time in transit where extreme temperatures can exist prior to reaching the final country of destination. However, the specificity, sensitivity and robustness of tests over extended time periods and with global distribution have proven to be strengths of this type of test (Unilever Pregnancy Test, malaria and HIV testing). Ultimately, it is the antibody probes used within each POC test format that will prove key to determining whether the required sensitivity and specificity can be attained and, at a level suitable for the application and commercialisation of the test.

Where this is seen as problematic, this type of test can combine nucleic acid molecular techniques (nucleic acid lateral flow (NALF)) to provide an on-site solution. This approach has recently been applied for the detection of oomycete pathogens *Phytophthora ramorum* and *P. kernoviae* from infected plant tissue (Tomlinson *et al*., 2010). After application to a chromatographic test strip, DNA is isolated and extracted from the membrane in <5 min with manual shaking in a small vial containing an extraction fluid. After extraction and applying loop-mediated

isothermal amplification (LAMP), the target DNA is amplified using labelled specific primers.

Detection of these labelled amplicon products is then performed in a lateral flow test strip. Each of these steps (manual shaking to disrupt the sample before application onto the membrane, placing a section of the membrane into pre-prepared LAMP reaction mix and incubation in a heated block or water bath, and dilution of the LAMP reaction and application onto the chromatographic test strip) is deemed as sufficiently simple to potentially allow this method to be performed outside a conventional laboratory facility without extensive prior training.

A result can be obtained in just over an hour. A LAMP assay for the detection of plant DNA (cytochrome oxidase gene) can be used in conjunction with pathogen-specific assays to confirm that the assay is working when it gives negative test results (Tomlinson *et al*., 2010). This technology is currently being used by trained operators, for example the UK plant health inspectorate, it will be interesting to see whether it is taken up by industry for use at grower holdings to evaluate the risk of disease epidemics and the efficacy and timing of control measures.

Nucleoide (mostly DNA-based) assays:

There has since the 1980's been a rapid development of molecular DNA-based technologies which can be applied to the on-site testing of plant samples. Molecular methods, essentially based upon Polymerase chain reaction (PCR), have evolved from a complex test procedure to become an indispensable, routine tool used widely in the diagnosis of infectious diseases. Over the past two decades PCR and quantitative PCR techniques (q PCR) have expanded to become one of the most widely used laboratory assays for the direct detection of low levels of pathogenic microbes in environmental samples (Theron *et al.,* 2010). The increasing ability to sequence pathogen genomic content provides a capability to design specific and sensitive primer sets to amplify target pathogen DNA by PCR to detectable levels.

The internal transcribed spacer (ITS) regions of ribosomal DNA are reported to be the most widely sequenced DNA regions of fungi (Peay K.G *et al*., 2008). It has been recommended as the universal fungal barcode sequence (Schoch, 2012), and as a consequence, has also been adopted for studies of oomycetes (Lévesque, 2011). Consisting of alternating areas of high conservation and variability it has proved popular for the development of highly specific and sensitive primer sets for use in PCR based diagnostic tests to discriminate target fungal plant pathogenic species in complex environmental samples (Klemsedal *et al*., 2008; Lees *et al*., 2012).

These processes have been successfully applied to develop molecular probes which are able to discriminate and measure plant pathogenic oomycete species (Cooke *et al*., 2000; Lévesque & De Cock, 2004; Beakes *et al*., 2012). Of use also, other regions of the genome have been sequenced to reveal nucleotide base pair

differences for the phylogenetic characterisation of *Phytophthora* and *Pythium* species. These include the mitochondrial cytochrome oxidase (cox 1 and cox 2) spacer regions and the nuclear translation elongation factor 1alpha and β-tubilin gene (Kroon et al., 2004; Villa *et al*., 2006; Blair *et al*., 2008). There are several sequence databases where information for these species is held and available for use (www.phytophthoradb.org; www.phythophthora-id.org; www.q-bank.eu; www.boldsystems.org) .

In some cases additional information is provided, for example key morphological features and biology. Using BLAST analysis (Basic Local Alignment Search Tool) the use of these sites provide a good resource for identification of Pythiaceous isolates and towards primer design for test application.

Quantitative PCR, a process by which DNA copy generation is monitored by conformational change of a fluorescently labelled probe, provides a platform to measure target disease concentration in a sample with reference to a standard curve. This system is often referred to as real-time PCR as the fluorescently labelled PCR products produced during each amplification cycle can be monitored as the reaction progresses. Although widely used, PCR diagnostic testing is still somewhat confined to larger central laboratories where special room requirements are required to eliminate aerosol contamination (Regis *et al*., 2006).

The 'closed' qPCR process can to some extent overcome this requirement and has been shown to have advantages of speed, accuracy, and sensitivity over conventional PCR-based techniques (Schaad & Frederick, 2002). However, the purchase costs of a 'real time' laboratory operating system are expensive (circa £45 k), making this an unaffordable option for many. Nevertheless, where speed, specificity and sensitivity are priorities regardless of cost, analysis by qPCR can be performed outside of the conventional laboratory using a system originally developed for the US military to provide on-site capability to monitor bioterrorism related outbreaks of anthrax. The real time platform was at that time supported by a portable battery and packaged in a large brief case to allow movement to a field situation.

The portable sampler has since been made commercially available (Cepheid Smartcycler Inc., Sunnyvale, California) and assessed for quantitative capability of infectious agents (Bélanger *et al*., 2003; Tomlinson *et al*., 2005). The fully automated sample preparation system is designed to work with a disposable cartridge that accepts up to several millilitres of an unknown aqueous sample. The sample preparation procedure is performed in less than five minutes and within the single platform provides real time detection for limited multiplex diagnostic capability. However, the molecular detection of fungal pathogens in plant material requires the pre-extraction of DNA (Schaad, 2009).

For this reason the on-site molecular testing of environmental samples has demanded not only a portable real time PCR platform but also a simple and robust DNA extraction method. For infected plant material this perhaps is not the issue that it was once perceived. A DNA extraction and the use of a portable real-time PCR platform has been used for the detection of *P. ramorum* from symptomatic plant material with a proposed use time of less than two hours.

Further, using NALF this process has been demonstrated to measure disease on site and, using the loop-mediated isothermal amplification (LAMP) method (Notomi *et al*., 2000), it does not require a costly PCR platform (Tomlinson *et al*., 2010). With the strong possibility of prices going down with economies of sales, this has the potential to make this type of molecular technology affordable for wider on site use. For procedures requiring a PCR platform for DNA amplification, the Smartcycler II Laptop platform which retails in the UK at circa £32K with an optional £1.8k maintenance contract might be appropriate. Alternatively, a number of other portable real-time PCR platforms are commercially available for on-site molecular testing: the R.A.P.I.D. system and RAZOR instrument (IdahoTechnologies, Salt Lake City, UT), and the hand held BioSeeq instrument (Smiths Detection, Edgewood, MD).

Simpler, less expensive technologies have been sought to allow molecular based assays to be translated from the laboratory to the field. LAMP provides a novel nucleic acid amplification process under isothermal conditions (60 to 65°C). For this reason simple incubators, such as a water bath or a block heater, are sufficient for DNA amplification. As a by-product of the reaction a white precipitate of magnesium pyrophosphate is produced, which enables the visual judgment of amplification by 'naked eye'. LAMP has been reported to be less affected by inhibitors (Francois *et al*., 2011) and, because of its speed, robustness and simplicity is increasingly used for diagnostics in human medicine (Parida *et al*., 2008) and, more recently, in plant health (Kubota *et al*., 2008; Tomlinson *et al*., 2010; Bühlmann *et al*., 2013).

In the United States the development of a 'grower performed LAMP PCR' has been assessed for the detection-based management of spray programmes for grapevine powdery mildew in vineyards (Mahaffee *et al*., 2011). Based on two years of results, a commercial company ran a feasibility trial to offer a grower based test service. Estimates were that it would require $2100 in capital equipment, $60 dollars in reagents and 25 minutes labour with a 1.5 hr time to process 10 samples. This did not however include the cost of a sampler for collection of field aerosols.

The LAMP process consisted of several steps including extraction, heating, and centrifugation, and, although it could be operated in a grower's office with desktop equipment, it was found that participants were not consistently successful when interpreting the results. The company considered performing the LAMP service 'in-house' however opted to partner with a commercial laboratory to offer a laboratory quantitative PCR service (Reiger, 2013). As a result of the high sensitivity of the

test, it was observed that one of the biggest concerns in the collection of samples for a commercial DNA based testing service was the cross-contamination of samples. Spores could be easily picked up and moved on peoples clothing and hands. For this reason they instituted clean practices whereby samplers wear gloves and protective clothing, which is changed between traps. Mahaffee and his team at the United States Department of Agriculture continue to work with growers to develop field tests that are more economical and easier to use. They are currently investigating the use of a hand-held, portable device called the Smart-DART (www.diagenetix.com/product-and-technology/smart-dart-platform) which allows the LAMP process to be performed on site and provides an application to an Android phone device for quantitative measurement of the assay process (**Figure 8**).

If successful the grower will still have to perform the DNA extraction process. Mahaffee estimates a grower could set the complete system up for less than $2000 in initial capital equipment with an annual operating cost of $400 for test reagents. Labour costs to operate the system were not included within this analysis. If successful this system could prove useful in a field situation where speed, sensitivity and specificity are key to a successful outcome and, with an economy of scale for use within Integrated Disease Management Systems.

Figure 8: *The Smart-DART™ platform and application for Android devices*

Nevertheless the ability to perform molecular tests in the field remains a challenging goal for complex environmental samples (plant tissue, soil), largely due to the need for (often complex) pre-processing of samples (nucleic acid extraction), which for environmental samples, such as soil, is still a rate and skill limited step due to the relatively complex nature of current nucleic acid extraction methods (King *et al.,* 2008).

If portable real-time PCR platforms are to be used successfully they should ideally consist of completely closed systems capable of performing all steps of the assay. These steps include (1) nucleic acid extraction, (2) PCR set-up, (3) amplification and (4) unambiguous calling of results (Mikidache *et al.,* 2012). A significant driver for use of these systems in the field will be ease of use and test reliability. For on-site

testing, it is likely that only those molecular technologies that are cost-effective will be used in plant pathogen diagnostics. This is a particular consideration for many plant cropping systems where the profit margins and emotional attachment to crops are low. The cost of equipment, expensive reagents and a requirement of skilled staff would not be easy to justify. Where legislative issues are a factor and potential of quarantine outbreaks a concern the demand for specificity, sensitivity and speed may, however, to prove an overriding factor to cost.

Where a laboratory/clinic environment is feasible, advances in molecular diagnostic test technology have provided the opportunity to couple PCR with high throughput pathogen detection multiplex arrays. These array systems were originally designed for gene expression profiling, gene discovery and single nucleotide polymorphism (SNP) analysis (Lockhart & Winzeler 2000; Mei *et al*., 2000). PCR-based multiplex arrays generally consist of a high density of selected and synthesised immobilized nucleic acid sequences spotted onto a solid platform such as glass microslides, beads or nylon membranes (Eptstein & Butow, 2000, Ishii *et al*., 2008).

Following sample DNA extraction of the environmental sample, amplicons of a target DNA region are generated by PCR and bound with a fluorescent, biotinylated or enzyme label. Following a process of DNA hybridisation, amplicons which are able to bind selectively to immobilised target sequences of the array are visualised, either by direct fluorescence scanning or enzyme-mediated detection, to yield a semi-quantitative result (de Boer & Beurmer, 1999). In general, target amplification is based on the use of universal primers that recognize conserved sequences flanking variable domains in housekeeping genes, such as the ribosomal RNA gene. In this way, numerous targets can be amplified with a single primer pair, while target discrimination is performed afterwards on the array (Lievens *et al*., 2003 & 2011).

DNA arrays have been developed for the detection of plant pathogens in a range of environmental samples (Boonham *et al*., 2007; Mumford *et al*., 2006; Lievens *et al*., 2012). For *Pythium*, a DNA array containing 172 oligonucleotides complementary to specific diagnostic regions of the internal transcribed spacers (ITS) has been developed for the identification and detection of more than 100 species (Tambong *et al*., 2006). More recently a membrane-based oligonucleotide array has been developed to detect *Phytophthora* spp by using three DNA regions (ITS, cox1 and cox2-1 spacer).

The array was validated with 143 pure cultures and 35 field samples, and proved sensitive, being able to detect as few as 50 pg of PCR amplicon from pure laboratory cultures. Using a multiplex real-time PCR approach, other workers have reported a detection sensitivity ranging from 1 fg (gene with multiple copies) to 100 fg (single-copy genes) of target *Phytophthora* DNA (Schena *et al.,* 2006; Tooley *et al*., 2006). However each of these plant tissue assays was limited to the measurement of a few target *species*; *Phytophthora ramorum*, *P. kernoviae*, *P. citricola* and *P. quercina* in

symptomatic leaf samples and the latter test only Phytophthora *ramorum* and *Phytophthora pseudosyringae*.

As a laboratory tool, the nucleotide-based array system can provide a highly specific and sensitive assay for the simultaneous detection of multiple diseases present in a cropping system (Robideau *et al*., 2008) and has also been used to identify species with fungicide resistance (Ishii *et al*., 2008). In general, macro arrays (immobilized nucleic acid sequences spotted onto reusable membranes) have been used for plant disease diagnosis as a result of cost, sensitivity and more modest equipment requirements (Lievens *et al*., 2012). For commercial applications, Bio-art bvba (Belgium) have demonstrated the usefulness of this multiplex approach and report detection of a range of fungal, oomycete and bacterial plant pathogens (DNA MultiScan®, http://www.bio-art.org). Meanwhile, continuing, considerable advances in the areas of genomics and bioinformatics mean that ever more powerful molecular diagnostic methodologies continue to be developed. For the oomycetes, sequence data continue to generate and provide additional information for phylogenetic analysis and update species classifications (Kamoun *et al*., 2014).

The development of second generation sequencing provides the capability to analyse and compare whole genomes of plant pathogens. The *Pythium ultimum* genome (42.8 Mb) is reported to encode for 15,290 genes of which extensive sequence similarity and synteny with the potato blight pathogen *Phytophthora infestans* is reported (Lévesque *et al*., 2010). More recently, analyses on the sequencing, assembly, and annotation of six *Pythium* genomes (*P. aphanidermatum*, *P. arrhenomanes*, *P. irregulare*, *P. ultimum var. sporangiiferum P. vexans* and *P. iwayamai)* provides comparison with other plant pathogenic oomycetes including *Phytophthora* species*, Hyaloperonospora arabidopsidis*, and *Pythium ultimum* var. *ultimum* as well as related animal pathogens such as the important fish pathogen *Saprolegnia parasitica* (Bishwo *et al*., 2013).

Next generation sequencing (NGS) also offers a diagnostic tool that requires no previous knowledge of either a specific host or pathogen (Schuster, 2008). It is a high-throughput approach that generates thousands to millions of DNA sequences. However as a diagnostic technique, obtaining and making sense of these sequences involves several complex stages, both at the lab bench and at the computer desk. With more and more organisms being sequenced, a flood of genetic data is being continually made available (Liu *et al*., 2012). Distilling meaningful information (bioinformatics) from the millions of new sequences and interpreting this from voluminous, noisy, and often partial sequence data presents a serious challenge.

Analysis requires considerable skill and understanding to avoid potential pitfalls and challenges in the process (Dewoody *et al*., 2013). NGS has however the capability to analyse complex environmental samples and from this identify uncultured known, unknown and novel pathogen variants (Adams *et al*., 2009, Harju *et al*., 2012, Be *et al*., 2013 , Breitbart *et al*., 2008). For plant virus identification Adams *et al*. (2009)

report a cost of £1000 per sample analysis but that this sum could reduce considerably in the future. For the moment however, NGS is likely to remain a sophisticated laboratory tool which will underpin fundamental genetic based studies to provide a new perspective to host-pathogen interactions and ecological studies. It will provide considerable support to the development of new diagnostic, molecular-based technologies.

The specificity that can be achieved by nucleotide based molecular methods remains persuasive in diagnostics for plant pathogenic fungi and oomycetes. Target organism genomic sequences can readily be compared using DNA-similarity searches like BLAST (Altschul *et al*., 1997) and DNA and RNA sequence databases, such as the International Nucleotide Sequence Database (INSD). However, caution is still needed in interpreting results of comparisons since for fungal species, it has been reported that less than 1% of the estimated 1.5 million viable species have been sequenced for the ITS region, and that as much as 20% of all fungal sequences deposited in the INSD may be incorrectly annotated to species level (Bridge *et al*., 2003, Nilsson *et al.*, 2006). There are also concerns over the classification of species solely based on results of DNA region/gene analysis.

Classical identification of plant pathogens has relied heavily on morphological and biological features (van der Plaats-Niterink, 1981). These relationships are not always conveyed when compared by genomic analysis. Will (2004) reports on the myth of the DNA barcode for species classification and reasserts the requirement for morphological analysis in the identification and classification process. Interestingly, in the field of medical mycology the uptake of PCR as a diagnostic tool has been constrained by the lack of standardization, such that PCR is not an accepted diagnostic criterion for the detection of human fungal diseases according to 2008 EORTC/MSG guidelines (De Pauw *et al.,* 2008).

Aside from this, careful consideration should be given to sample coverage and size along with a suitable extraction and/or concentration process to enable efficient and reliable amplification of low numbers of target genomic sequences. Careful optimisation and evaluation of the PCR should be made. This should include melting and annealing temperatures to prevent the formation of undesirable secondary structures such as primer dimers (Saiki *et al*., 1988; Atlas, 1991). Potential sample inhibitors need to be determined and accounted for in extraction and assay procedures. These include humic substances, pesticide residues and organic material, all of which are reported to inhibit the DNA polymerase enzyme (Kong *et al*., 2003), as well as colloidal matter, which has a high affinity for DNA (Way *et al*., 1993; Wilson, 1997).

The presence of these in field samples has the potential to affect the amplification process and test sensitivity (Lombard *et al*., 2011; Stewart-Wade, 2011). Also the test parameters should consider whether the ability to discriminate dead/inactivated species from viable disease is relevant and, at what concentration. The testing of

recycled irrigation water still provides a challenge as, following treatments to kill plant pathogens, many dead pathogen cells and particles of debris can still be present and differentiating between the infectious (viable) and non-infectious (non-viable) state remains a limitation of PCR (Stewart-Wade, 2011) as DNA persists for significant periods of time after the death of cells (Master *et al*., 1994).

Bettraino *et al*. (2010) and Chimento *et al*. (2012) approached this problem for the detection of *Phytophthora cambivora* and *P. ramorum* by targeting the mRNA of the cox genes for reverse transcription followed by PCR amplification. As an indicator of viability, mRNA is considered an appropriate target since most mRNA species have a short half-life. In bacteria this amounts to just a few minutes (Kushner 1996), whilst in fungi, the determination of mRNA half-lives for *Candida albicans*, suggest an enhanced period of between 4-168 min (Kebaara et al. 2006)

Prospects for uptake of molecular diagnostics in Plant Disease Management Systems (PDMS):

Plant diseases impact significantly on crop yield and quality on an annual basis. A major problem for producers is that diseases are moving targets that evolve in response to agricultural practices and environmental change. This is a fluidic process which can change not only on a seasonal, but a daily basis. Although early disease diagnosis and pathogen detection remain central to the ability to protect crops, the success of this will depend on how the information is derived, evaluated and then incorporated within an integrated disease management system. For example, once a disease is identified, information about the presence of sufficient pathogen concentration coupled to the associated environmental parameters is required to determine accurate disease thresholds at which damage may occur (Scherm & van Bruggen, 1995).

Consideration of the cultivar grown may also be appropriate for some diseases as might diagnosis of the pathogen to an isolate or race level. This information needs then to be translated in a meaningful, timely and accessible way to growers for targeted and cost effective control measures to be taken for disease containment or eradication. For this purpose when considering test development, extensive ecological studies should be conducted, studying the responses of a pathogen in relation to both biotic (microbial, plant) and abiotic factors (light, temperature, humidity etc.) of its environment (Lievens & Thomma, 2005). Early detection allied to key environmental parameters to control disease at the onset can lead to an increase in production, an improvement of resource efficiency and make a substantial contribution to food security (Wakeham & Kennedy, 2010).

Test sampling procedures and sample size must also be considered for spatial variation of pathogen incidence within a cropping system. With the development of new diagnostic technologies, often the material required for analysis reduces as test sensitivity increases. The use of nanotechnology will drive even smaller sample

volumes. This has the potential to be problematic in cases where detection of disease potential ahead of infection or during pre-symptomatic infection is a requirement. For example, the assessment of plants or soil from large cropping acreages like those used in the production of outdoor vegetables. Equally, in plant health quarantine, seed and certification of transplant stock, the sample size and sampling strategy is critical to identifying and determining an accurate disease potential.

However, this issue may be overcome by the isolation and concentration of the target pathogen(s) from the sample medium ahead of testing. Nevertheless, sampling should be performed in a manner that ensures a statistically representative sample (Ranjard *et al*. 2003). Whatever the process, the suitability of use and cost returns of the test for the end user should be fully evaluated ahead of prototype development. Growers are unlikely to invest in equipment that; proves tedious in operation, expensive, possibly requiring an annual maintenance contract, staff to operate or the equivalent of a small laboratory to operate.

As described earlier, the detection of pathogens in soil or water samples is also difficult and has its challenges in test development and commercialisation of the process once developed. Soil provides a diverse matrix which can alter considerably within a sampling area and influence pathogen distribution. Issues of sensitivity, specificity, non-specific binding of the diagnostic probe and soil inhibitors are well documented in assay development. Difference in soil types across regions and the effect of this on assays should be evaluated. Often biological amplification is required by soil baiting, and although this can provide information on viability of the target organism it makes quantitative readings more difficult. While some of this information is available, the format for new tests will depend on the pathogen(s) and may require additional experimentation and validation studies.

A further consideration for the successful delivery of a test is whether a capability to differentiate between viable and non-viable organisms is important. As described earlier, this can prove critical in nursery irrigation systems or soil/composting materials where treatment processes can lead to detectable pathogen presence in the absence of disease risk. Equally test specificity should be at an appropriate level and not jeopardise indigenous biocontrol agents. A consideration is the existence of fungal species that contain pathogenic and non-pathogenic or even beneficial strains. This is a known phenomenon for complex species such as *Fusarium oxysporum*, *F. solani*, and *Rhizoctonia solani* (Recorbet *et al*., 2003). Similarly, for *Pythium,* where a number of species are mycophagous parasites of fungi and other oomycetes and provide real potential as useful horticulture and agricultural biocontrol agents (Paulitz *et al*., 1990; Martin & Hancock, 1987; White *et al*., 1992).

Other commonly-present *Pythium* species are primarily saprobes and not pathogenic. In horticulture, where many different *Pythium* species are present and occur across a range of cropping systems, the challenge in developing a suitably

specific and sensitive probe will rely on the capability to identify and detect pathogens responsible for specific crop diseases. This holistic approach to probe selection, and assay development is critical if a useful test is to be delivered to the end user.

To address issues in *Sclerotinia,* Abd-Elmagid *et al*. (2013) developed a multiplex PCR test able to discriminate between four key plant pathogenic *Sclerotinia* species (*Sclerotinia homeocarpa*, *S. minor*, *S. sclerotiorum*, and *S. trifoliorum*) in a single PCR reaction. Lievens *et al.* (2006) described the development of molecular qPCR to measure the concentration of a number of economically important fungal pathogens of tomato in soils and plant material (*Fusarium solani*, *Rhizoctonia solani*, *Verticillium* species responsible for tomato wilt and *Pythium ultimum*).

Once a suitable diagnostic prototype is available it is essential that it is extensively validated and compared with existing adopted systems (for example the isolation of pathogens by use of selective media, culture based morphological analyses and baiting using plant tissues), and that this process is carried out across the range of environments in which the test will be used. Equally, if the test is to be carried out by non-scientists, the robustness of the system should be assessed in supported trials with multiple 'non-skilled test' end users. Early collaboration with design engineers to make ergonomic improvements may provide optimal test delivery and speed up commercialisation of the product. The development and successful uptake of any test will therefore require careful planning and optimisation of the process for each target, with a robust validation period.

TREATING WATER TO CONTROL OOMYCETE DISEASE SPREAD

The importance of irrigation water in the spread of plant pathogens, especially the oomycetes, and the prospects of and available methodologies for their management and control, have been the subject of several recent reviews in the scientific literature (Ehret *et al.*, 2001; Hong & Moorman, 2005; Pettitt, 2003; Raudales *et al.*, 2014a; Stewart-Wade, 2011; Zappia *et al.*, 2014), as well as an excellent book published by the American Phytopathological Society (Hong *et al.*, 2014). Hong & Moorman (2005) and Stewart-Wade (2011) gave good general overviews of likely pathogens and their management in irrigation water, whilst the less recent reviews of Ehret *et al.* (2001) and Pettitt (2003) were focused on control of pathogen spread. Zappia *et al*. (2014) reassessed current understanding of fungal and oomycete plant pathogens known or suspected to be spread and possibly even exacerbated by irrigation water, whilst Raudales *et al*. (2014a) aimed to summarise the current state of knowledge on control treatments and effective doses for controlling plant pathogens, biofilms and algae as well as reported toxicity thresholds.

Together with Hong *et al.* (2014), these studies have helped identify where our understanding is reasonably good (for example certain aspects of water disinfestation) and key areas where current knowledge and understanding are weak and published information in the public domain is thin or non-existent. By and large the reviews mentioned above have taken a generic stance to plant pathogens and necessarily draw the majority of their information from the public domain. Here we focus on oomycete control and draw on considerable unpublished information and experience gained within large experiments carried out at Efford and Stockbridge House Experimental Horticulture Stations and from 20 years of water sampling and clinic work for the UK horticultural industry.

Deciding whether water treatment is necessary – disease risks associated with water source:

Table 3 summarises the risks of spreading oomycete diseases associated with the main categories of water available for irrigation. The information collected for this table is based mostly on studies where pathogen species known to attack specific crops have been detected in significant quantities in particular water sources or where water samples have been assessed in the clinics at Efford, Stockbridge or Eden Project. As discussed elsewhere, very few studies make direct links between specific inoculum levels and outbreaks of disease.

Nevertheless, consistent absence of inoculum makes a convincing case for the safety of a water source as do persistently high levels of specific inocula for the converse. Generally, mains and borehole-derived water are safe to use so long as they are stored properly (see Table 3 ‘Uncovered tanks’) and the irrigation system is kept clean, whilst surface-derived waters (ponds, ditches, reservoirs, rivers and runoff) carry moderate to very high disease risks. Treatment of surface-derived

water to control oomycete plant pathogens before use for irrigation is therefore highly desirable/recommended.

The available choice of water treatment options is large and selection of a treatment or combination of treatments for individual nurseries is very much a case of ‘horses for

Table 3 *Water sources potentially available for irrigation and their associated level of risk of carrying and spreading oomycete stem and root rots*

Water source	Oomycete pathogen risk	Crops/sectors where risks have been identified	References
Mains	Very, very low/none	All	Bewley & Buddin (1921); Pettitt (2003); Moorman *et al.* (2014); Pettitt ({0/>50} unpublished*)
Bore hole/ Well	Low/none	All	Bewley & Buddin (1921); Pottorff & Panter (1997); Themann *et al.* (2002), Pettitt ({0/>45} unpublished)
Uncovered tanks	*Pythium* Moderate-High	All	Based on clinic data of tests carried out on tanks known to be totally or partially uncovered & predominantly outdoors: *Pythium* spp. {111} *Phytophthora* spp. {12}
	Phytophthora Unknown		
Reservoirs/ Ponds/Lakes	*Pythium* High	HNS; Tomatoes; Protected Ornamentals; field vegetables; cotton	Bewley & Buddin (1921); Bush *et al*. (2003): Pittis & Colhoun (1984); Shokes & McCarter (1979); STC & Pettitt ({>300} unpublished)
	Phytophthora High	HNS; Tomatoes; Protected Ornamentals	Bewley & Buddin (1921); Ali-Shtayeh & MacDonald (1991); Bush *et al*. (2003); Ghimire *et al.* (2009 & 2011); Hong *et al.* (2008); Orlikowski *et al.* (2009); Pittis & Colhoun (1984); Werres *et al.* (2007); STC & Pettitt ({>300} unpublished)
	Aphanomyces Unknown	Field vegetables	Pettitt ({2} unpublished)*
Rivers/ Streams/ Canals/ ditches	*Pythium* High	HNS; strawberries; field veg; tomatoes	Bewley & Buddin (1921); Bush *et al*. (2003); Pittis & Colhoun (1984); Ali-Shtayeh & MacDonald (1991); MacDonald *et al.* (1994); Pettitt ({24/52}unpublished)
	Phytophthora High	HNS; Fruit and nut trees; strawberries; tomatoes	Ali-Shtayeh & MacDonald (1991); Bewley & Buddin (1921); Klotz *et al.* (1959a & b); McIntosh (1966); Mircetich et al. (1985); Orlikowski *et al.* (2009); Reeser et al. (2011); Hansen & Delatour (1999); Pettitt ({33/52} unpublished)
	Aphanomyces Unknown	Field vegetables	Pettitt ({4/52} unpublished)*
Roofs/Paved areas	*Pythium* Moderate-High	Protected ornamentals; Cucumbers; Tomatoes; Research station pack-house roof	Bewley & Buddin (1921); Pettitt (2003); Pettitt ({38/55} unpublished*)
	Phytophthora Low-Moderate	Tomatoes; Sweet Peppers; Strawberries; Protected ornamentals; HNS	Bewley & Buddin (1921); Pettitt ({8/55} unpublished*)
Run-off from fields or production beds	*Pythium* High	HNS	Bush *et al*. (2003); Pittis & Colhoun (1984); Ali-Shtayeh & MacDonald (1991); MacDonald *et al.* (1994); Pettitt ({63/100} unpublished*)
	Phytophthora High	HNS, Vegetables	Bush, Hong & Stromberg (2003); Ghimire *et al.* (2009 & 2011); Klotz *et al.* (1959b); MacDonald *et al.* (1994); Middleton (1985); Pettitt *et al.* (1998); Werres et al. (2007); Roberts *et al*. (2005); Pettitt ({25/100} unpublished*)
	Aphanomyces [High]?	Field vegetables	Cook & Papendick, (1972); Hughes & Grau (2007)
Recirculated nutrient solution	*Pythium* High	Tomatoes; Cucumber; Lettuce; Chrysanthemums; protected ornamentals	Calvo-Bado *et al.* (2006); Postma *et al.* (2001); McPherson *et al*. (1995); Jenkins & Averre (1983); Pettitt (2001); Thinggaard & Middelboe (1989)
	Phytophthora High	Tomatoes; protected ornamentals	Calvo-Bado *et al.* (2006); McPherson, Harriman & Pattison (1995); Strong *et al.* (1997); Thinggaard & Middelboe (1989)
* Results from commercial clinic samples - values in brackets {x} = number of isolations **OR** number positive isolations/total number of tests			

courses' (Pettitt & Hutchinson, 2005, Büttner *et al.,* 2014) and has to take a wide range of factors other than immediate concerns with plant pathogens, weeds and/or bio-fouling into consideration, including general horticulture, water chemistry and microbiology as well as engineering, economics and even local politics (Fisher, 2014), not to mention perceptions of the complexity, suitability and availability of the various techniques and systems possible (Raudales *et al*., 2014b).

Over the last five or so years the recycling and therefore the disease risks and the potential treatment of irrigation water have become 'hot' topics in the USA and Canada, with strong extension and research groups establishing at Virginia Polytechnic, Pennsylvania State University, Florida State University and the University of Guelph. The Extension departments of these institutes, especially at University of Florida are starting to provide some useful practical information to help with decision-making (see 'Education Resources' at end of references section).

Pasteurisation:

Based on the procedure used to pasteurise milk, Pasteurisation was developed for horticultural use at IMAG-DLO in the Netherlands (Runia *et al*., 1988; Van Os *et al*., 1988). The process uses heat to disinfest water and was developed with a broad range of potential horticultural pathogens in mind, including tobacco mosaic virus (TMV) as well as oomycetes and fungi like *Fusarium* and *Verticillium* spp. This resulted in the still currently used recommended settings for treating water of heating it to 95^{o}C for 30 seconds – conditions that are theoretically more than adequate to treat water for the eradication of oomycete propagules (see **Table 4** for lethal temperatures and exposure times measured for various oomycete species), and this has been demonstrated convincingly in several large scale trials and in commercial production over the last 15 or so years (McPherson et al., 1995; McPherson, 1996 {AHDB Horticulture PC60}; Rey et al., 2001; Newman, 2004). More recently, the possibility of reducing the temperature and keeping treated water hot for longer periods has been considered as this can reduce the energy consumption considerably, and an alternative setting of 85^{o}C for 3 minutes has been found to be as good as 95^{o}C for 30 seconds (Runia & Amsing, 2001a; Atwood, 2014).

A relatively compact, flexible, very effective treatment, that has little impact on the chemical qualities of the treated water and no noxious chemical inputs or residues, Pasteurisation has two major drawbacks: the cost of installation and running, and the environmental impact of conspicuous energy consumption. It has been estimated that 1.25-1.5 m^3 gas is needed to treat 1 m^3 (220 gallons) of water (Runia *et al*., 1988; Atwood, 2014), although this can increase to as high as >20 m^3 in some circumstances (Newman, 2004 {270-530 ft^3/US gallon water = 20.21-39.65 m^3 gas/m^3 water}; Hao *et al*, 2014).

This has so far limited uptake of Pasteurisation so far mainly to the Netherlands where many nurseries were able to afford the capital costs in the 1990s and energy

concessions enabled the economic deployment of the technique. There are two possible routes to improving the economic and environmental viability of Pasteurisation in the future.

The first concerns further investigation into the parameters of operation; using modified equipment Runia and Amsing (2001b) were able to reduce the effective temperature to 60ºC by extending the exposure period to 2 minutes and thereby reducing energy input by 42%.

The second is the source(s) of energy used and the means of supplying and applying heat. Renewable energy sources may be deployed as and if these become more economic for example geothermal energy (Cosgrove, 2013; Lund et al., 2005), whilst in areas (probably not the UK!) where there is adequate solar radiation, solar power may be used as the energy source for the Pasteurisation process (e.g. Duff & Hodgson, 2005).

- In summary, pasteurisation is a very effective method for eliminating oomycete pathogens from irrigation water. Its main drawback is its consumption of energy. At present no further research is needed in this area

Table 4: *Lethal temperatures and exposure times measured for various oomycete horticultural stem and root rot pathogen species*.

Pathogen species	**Inoculum/propagule type**					**Heat treatment parameters**		**Reference**
	Mycelium/ hyphae	Oospores	Chlamydospores	Zoospores/ cysts	Infected plant tissues/ debris/soil	**Temp**	**Duration**	
Aphanomyces cochlioides		+				45ºC	72 h	Dyer *et al*., 2007
						45ºC	4h/day (4 days)	
						50ºC	6 h	
Phytophthora cactorum	+	+			+	45ºC	30 min	Juarez-Palacios *et al.,* 1991
Phytophthora capsici	+					42.5-45ºC	30 min	Bollen, 1985
		+				>50ºC	30 min	
					+	53ºC	1h	Etxeberria *et al.,* 2011
		+				53ºC	12 min	
		+			+	40ºC	4h/day (28	

Pathogen species	Inoculum/propagule type					Heat treatment parameters		Reference
	Mycelium/ hyphae	Oospores	Chlamydospores	Zoospores/ cysts	Infected plant tissues/ debris/soil	Temp	Duration	
							days)	
Phytophthora cinnamomi	+					38ºC	1-2 hours	Gallo *et al*., 2007
			+			40ºC	1-2 hours	
	+					45ºC	15 min	
	+		+		+	45ºC	20 min	Juarez-Palacios *et al.,* 1991
	+					39ºC	90 min	Benson, 1978
						44ºC	4.5 min	
Phytophthora cryptogea		+				40-42.5ºC	30 min	Bollen, 1985
	+			+		95ºC	30 sec	McPherson *et al*., 1995
	+			+		44ºC	15 sec	Runia & Amsing (2001b)
Phytophthora infestans		+				40ºC	12 h	Fay & Fry, 1997
						46ºC	2 h	
Phytophthora kernoviae	+				+	32.8ºC	5 days	Noble *et al*., 2011
***Phytophthora megasperma* (low temp. isolates)**	+	+			+	45ºC	20 min	Juarez-Palacios *et al.,* 1991
Phytophthora nicotianae			+		+	47ºC	2 h	Coelho *et al*., 2000
						50-53ºC	5 min	
			+	+		48ºC	6 h	Hao *et al*., 2012
Phytophthora pini		+				48ºC	6 h	Hao *et al*., 2012
Phytophthora pseudosyringae	+				+	25.9ºC	10 days	Noble *et al*., 2011
Phytophthora ramorum	+				+	56ºC	45 min	Tubajika *et al*., 2008
						60ºC	30 min	
	+		+		+	50ºC	30 min	Linderman & Davis, 2008
	+		+		+	40ºC (*in*	1 day	Noble *et al*.,

Pathogen species	Inoculum/propagule type					Heat treatment parameters		Reference
	Mycelium/ hyphae	Oospores	Chlamydospores	Zoospores/ cysts	Infected plant tissues/ debris/soil	Temp	Duration	
						vitro)		2011
	+		+		+	41.9°C (*in vivo*)	5 days	
Pythium aphanidermatum	+			+		95°C	30 sec	McPherson *et al*., 1995
	+			+		51°C	15 sec	Runia & Amsing (2001b)
		+			+	>52.5°C	30 min	Bollen, 1985
Pythium irregulare		+			+	50°C	30 min	Linderman & Davis, 2008
Pythium sylvaticum		+				47.5-50°C	30 min	Bollen, 1985
	+					45-50°C	30 min	
Pythium ultimum	+	+				37°C	18 days	Pullman *et al.* 1981
						50°C	33 min	

Chemical disinfestation of irrigation water:

A number of different sterilisation chemicals with broad-spectrum anti-microbial activity can be used to effectively eliminate oomycete pathogens from irrigation water supplies. Fungicides formulated for the control of oomycetes are not considered here, although some can give a measure of disease control in irrigation water (Smith, 1980; Vanachter *et al.*, 1983a & b; Price & Fox, 1986). Fungicides are generally not effective or appropriate for cleaning water supplies because those currently available are largely fungistatic in action, rarely achieving total control, and are not formulated or registered for treating water (Pettitt, 2003). The majority of chemicals that can be used for effective and safe water decontamination are oxidising agents of one form or another. The other main groups that can be deployed in oomycete control are metal ions and surfactants, although these last two groups at present represent only a tiny proportion of chemical use for irrigation water treatment (Ehret *et al.,* 2001; Pettitt, 2003; Stewart-Wade, 2011).

Oxidising agents are strongly reactive with organic matter including micro-organisms and therefore plant pathogens. Oxidation reactions result in changes in the chemical structure of organic matter and when such materials are parts of living organisms these changes are often lethal. Oxidising agents change form and are 'consumed' in

oxidation reactions, the concentrations of agent needed therefore vary depending on the concentrations of microbes and other reactive material present. Thus, as with most other water disinfestation treatments, the efficacy of treatments with oxidising agents can be improved by pre-filtration. Filtration has the added benefit of removing much infected plant debris (often some of the toughest infective material to eliminate) and generally lowering the numbers of infective units in contaminated water; in observations of commercial and experimental samples at HRI Efford, filtration to 100 microns can remove at least 30% of pathogen propagules (Pettitt *et al*., unpublished).

When using oxidising agents for control of micro-organisms it is important to maintain the treatment dosing at effective levels without causing damage to the crops being protected. ORP or Oxidation-Reduction Potential is effectively a measure of the amount of oxidising and/or reducing agents present in water. ORP sensors work by using an inert metal electrode, usually platinum, that has low resistance and will readily give up electrons to oxidising agents or accept them from reducing agents. This generates a voltage which is compared with a reference electrode and this voltage gives a measure of the ORP. ORP measurement can be used to monitor the concentrations of oxidising agents added to water, but this needs to be carried out with caution and a good understanding of the chemistry of the system involved. This is because the voltage measured by an ORP sensor is logarithmically dependent on the concentration of the oxidising agent by the Nernst equation and is also strongly dependent on other solution components such as pH and other oxidising/reducing agents likely to be present. For example to measure the chlorine concentration from adding hypochlorite to water (effectively the hypochlorous acid concentration), the total chloride ion [Cl^-] concentration and the pH [H^+] must also be measured or carefully controlled as they will affect the ORP sensor readings. Nevertheless, in a well-understood system, the ORP value can give a good indication of anti-microbial oxidative activity, so long as it is used with caution. Simple colorimetric tests for chlorine and for peroxide concentration are readily available, more straightforward and reliable, and can be used alone or in support of ORP measurement.

Ozone: Ozone is a powerful oxidising agent with the highest redox or oxidation potential (2.07 V at 25^{o}C for ozone {O_3} and 2.72V for hydroxyl radicals {$^{\cdot}HO$}, Stanbury, 1989) of all oxidising water treatments (Hoigné & Bader, 1976; United States Environmental Protection Agency, 1999), and it has been used to treat water for over 100 years (Elmer *et al*., 2014). Ozonation is widely used in drinking water treatment facilities worldwide for the safe and effective disinfection of drinking water contaminated with enteric bacteria and viral pathogens (Wolfe *et al*., 1989). The process is also widely used in the food and beverage industry (Kim *et al*., 1999).

As it is unstable, ozone gas (O_3) is produced *in situ* by either of two main types of ozone generator; corona discharge or UV, the details of these processes are beyond the scope of this review and they are considered in detail elsewhere (Degrémont, 2007; Elmer *et al*., 2014; United States Environmental Protection Agency, 1999; Raudales *et al*., 2014a; Summerfelt, 2003). The gas is bubbled through water being treated and

reacts with target micro-organisms and organic matter either by direct oxidation or by the production of short-lived, highly reactive hydroxyl free-radicals and superoxide ions (Hoigné & Bader, 1983a & b; United States Environmental Protection Agency, 1999). Although there is some variation in effective doses and exposure times, ozonation is a highly effective treatment for the control of bacteria (Kobayashi et al., 2011), viruses (Runia, 1994a), fungi, algae (Yun *et al*, 1997), protozoa (Owens et al., 2000) and oomycetes in water systems.

In irrigation water Runia (1995) considered an O_3 concentration of 10 mg l^{-1} and a contact time of 1 h sufficient to kill all phytopathogens present. This high dose/contact time recommendation takes highly resistant pathogens such as tobacco mosaic virus into consideration and for oomycete control, effective doses have been found to be considerably lower. For example Ogawa *et al.* (1990) found that 3.8 mg l^{-1} for 2 minutes and 1.5 mg l^{-1} for 20 minutes inactivated both *Phytophthora parasitica* and *P. nicotianae,* whilst Beardsell & Bankier (1996) eliminated *Phytophthora cinnamomi* chlamydospores by exposure to a starting concentration of 2.4 mg l^{-1} (this declined to approx. 0.6 mg l^{-1}) for 16 minutes and *Pythium ultimum* oospores 1.2-1.5 mg l^{-1} for 4 minutes in tap water and 0.4-0.7 mg l^{-1} for 8 minutes in 'dam water'. These small-scale, mostly *in vitro*, studies are also supported by the results of large-scale trials on a range of plant pathogens (Runia, 1994a & 1995), including important oomycete species; *Phytophthora cryptogea* and *Pythium aphanidermatum* in protected vegetable crops in recirculating hydroponic systems (McPherson *et al*., 1995), and in Hardy Nursery Stock growing in recycled irrigation water (Pettitt, 1996 unpublished).

As ozone is highly reactive, there is not likely to be much residual left in treated water (Singer 1994); Raudales *et al.* (2014a) cite Hayes *et al*. (2009) stating that 'a residual dose of under 1 mgl^{-1} is typically suggested for greenhouse irrigation', although this concentration is unlikely to be exceeded as the solubility of O_3 under normal circumstances falls between <0.1-1.0 mgl^{-1} (United States Environmental Protection Agency, 1999). An optimum pH range for ozonation is often quoted as pH 4-4.5 (Atwood, 2014), although this properly refers to the stability of ozone rather than its efficacy as a oxidant/biocide, since its decomposition is much slower over the range pH 4 – 6 than at pH 7 or above (Ku *et al*., 1996) and at higher pHs the production of strongly oxidative hydroxyl radicals is markedly increased – a phenomenon that provides the basis of one form of advanced oxidation process (AOP) whereby greatly enhanced oxidation is achieved by increased hydroxyl radical formation caused by raising the pH, a similar reaction can be induced by the addition of hydrogen peroxide with ozone (Andreozzi *et al*., 1999; Wolfe *et al*., 1989). Other AOP are possible in combination with UV and this is considered further at the end of the section assessing UV treatments.

Despite its promise as a treatment for irrigation water, ozonation has not been widely adopted for this purpose. This is most likely the result of a number of factors, the most important of which are the running and installation costs which remain high despite the optimistic projections of the 90s (Beardsell & Bankier, 1996). The technique is also

widely perceived as being too ‘high tech.’ a situation not helped by the limited provision of potentially suitable equipment and the comparatively small amount of scientific information available on which to base good guidelines for optimal effective application for the control of plant disease spread. The final problem is shared with other techniques, especially UV, and that is the lack of any significant residual disinfectant effect post treatment, as O_3 is so rapidly broken down by its high reactivity.

- Ozonation is an effective treatment for eliminating all oomycete propagules from irrigation water. Further data on dose rates and longevity of residual in irrigation systems would be desirable but given the high costs and low availability of ozonation equipment, such studies would have a low priority. AOP is worth further consideration and this is mentioned also in the section considering UV treatments.

Hydrogen peroxide and activated peroxygens: With a redox potential of 1.76 V at 25^{o}C, hydrogen peroxide (H_2O_2) has been widely used as an effective oxidising sterilant and antiseptic for many years (Elmer *et al*., 2014). Pure hydrogen peroxide can be used as a water treatment but is quite unstable and has a short shelf-life. It is therefore marketed in a number of ‘stabilised’ forms that often enhance its antimicrobial activity (Toté *et al*., 2009). These can consist of mixtures with organic acids such as acetic and formic acids which react and form stable equilibrium with peroxyacetic acid (e.g. Jet 5®) or peroxyformic acid (e.g. Reciclean®) respectively, or with compounds like chelated silver (e.g. Intra Hydrocare) {other stabilisers traditionally used are various metal chelating agents and colloids including stagnates, pyrophosphates and organophosphonates – the levels of these vary with grade and intended use}.

Since their primary break-down products are water and oxygen, hydrogen peroxide formulations are widely used as additives to recycled water, particularly in situations where other techniques available may not be practicable. For example in the forcing of chicory where there is a rapid turnover of very large volumes of solution within a restricted space (Pettitt, 2003). Whilst it has a broad efficacy against pests and pathogens (Runia, 1995; Runia & Amsing, 1996; Vänninen & Koskula, 1998; Newman, 2004; Van Os, 2010), hydrogen peroxide is considerably weaker than ozone, requiring longer exposure times to achieve similar levels of biocidal effect (Domingue *et al*., 1988) raising the potential problem of phytotoxicity (Menzies & Bélanger, 1996; Ehret *et al*., 2001).

The information available on hydrogen peroxide phytotoxicity is limited and is likely to be influenced not only by the concentration of H_2O_2 but by the types and concentrations of stabilisers and activity enhancers used in each formulation. Symptoms include stunting (Nedderhoff, 2000; Vines *et al*., 2003), spots and blotches on foliage and petals, drying out and necrosis (Copes et al., 2003), and in extreme cases, wilting and (young) plant mortality (Van Wyk et al., 2012). Phytotoxicity is dependent on both the concentration and the type of exposure. When applied directly to hydroponic solutions, reported phytotoxicity thresholds range from as low as 8 mgl^{-1} reported for lettuce

seedlings (Nedderhoff, 2000) to 125 mgl^{-1} in cucumbers in rockwool (Vänninen & Koskula, 1998), to 50ppm in closed or recirculating hydroponic systems for cucumbers and tomatoes (McPherson, 2000). There was also an indirect problem of blockage of irrigation lines or 'drippers' due to fungal growth in solution where specific formulated products based on per-acetic acid/hydrogen peroxide was used in these studies. This was considered to be due to the presence of a carbon source in the hydroponic solution preferentially supporting mycelial growth. Less damage appears to result from foliar applications; Copes *et al*. (2003) report using rates of a formulation containing 27% H_2O_2 and 2% peroxyacetic acid (PAA) at rates of up to 2700 mgl^{-1} peroxide on a wide range of plants with very little damage, whilst Pettitt (2003) applied overhead irrigation containing 500 mgl^{-1} peroxide to young plants of *Chamaecyparis, Berberis, Pyracantha* and *Calluna* for 7 months without observing any significant increases in damage compared to untreated controls.

Direct measures of the doses and contact times required for control of oomycete pathogen are relatively scant with most studies involving *in vitro* testing and not stating the pathogen propagules/inoculum assessed. In reports cited by Raudales *et al*. (2014a), Choppakatla (2009) found that H_2O_2 plus PAA at 12.3 and 8 mgl^{-1} gave 100% inactivation of *Pythium* and *Phytophthora* spp., whilst Steddom & Pruett (2012) found that a similar preparation gave 100% mortality of *Phytophthora* sp. When added to nursery runoff and pond water at a rate of 185 mgl^{-1} H_2O_2.

In comparative field trials on hardy nursery stock species at HRI Efford, Pettitt (2003) found that a H_2O_2/PAA product (Jet5®: a mixture in equilibrium of PAA (5%), H_2O_2 (20%), acetic acid (10%) and water) at a concentration of 40 mgl^{-1} H_2O_2 was highly efficient at eliminating *Phytophthora cryptogea* disease spread in recycled water whilst in vitro applications of 20 mgl^{-1} H_2O_2 resulted in 100% mortality of mixtures of zoospores, zoospore cysts and mycelial fragments (Pettitt & Wainwright, 1997). Also, despite phytotoxicity effects in cucumber using the same product, McPherson (2000) obtained control of Phytophthora in hydroponic tomato crops growing on rockwool at a rate of 50ppm/mgl-1 per-acetic acid/H2O2. McPherson (2000) obtained control of Phytophthora in hydroponic tomato crops growing on rockwool at a rate of 50ppm/mgl-1 per-acetic acid/H2O2. Currently, the peroxyacetic acid product registered in the UK for use as a sterilant is Jet 5 and this is a different formulation to that tested in the studies mentioned above. It is also important to note that unfortunately Jet 5 can only be used to treat irrigation systems and cannot be intentionally applied directly to plants for the purpose of disease control or added routinely to treat irrigation water. Other stabilised hydrogen peroxide products are now available in the UK (e.g. Quill Intra Hydrocare: H_2O_2 stabilised with chelated silver – www.quillproductions.co.uk/Water-Sanitisers-c-531/) that are cleared for water treatment, but still not for treating diseased plants. Peroxide concentrations can be maintained using an ORP sensor but this requires regular calibration using colour test strips and can easily become dirty and malfunction (Howarth, 2007).

- Hydrogen peroxide treatments show enormous promise for eliminating oomycetes from irrigation water – either as a primary treatment or as a 'polishing' step (e.g. for biofiltered water that is to be used in a 'high biosecurity' area such as in a woody cuttings propagation house). Further work is needed on the effective doses, survival and efficacy of residual in systems and the impact of water quality on these parameters.

Chlorination: Probably more widely assessed for its activity against plant pathogens than other chemical water treatments (Fisher *et al*., 2014), chlorination has had a comparatively long history of use for treating potentially contaminated surface-derived water sources for horticultural use (Bewley & Buddin, 1921; Smith, 1983) and, more recently, recycled water in hardy nursery stock and the production of other ornamentals in the UK (Pettitt, 2003). Worldwide the three most commonly used sources of chlorine are chlorine gas, sodium hypochlorite (NaOCl) solution and calcium hypochlorite ($Ca(OCl)_2$ solid, and the most commonly used of these, especially in the UK, is sodium hypochlorite.

All three materials hydrolyse when added to water to form hypochlorous acid (Morris, 1946), which in turn dissociates into hypochlorite. The balance of hypochlorite to hypochlorous acid is determined by the pH and below approximately pH 7.5 hypochlorous acid predominates whilst above 7.5 hypochlorite predominates (Suslow, 1997). This is important as hypochlorous acid is a much stronger oxidising agent and biocide, and it is for this reason that it is advisable to acid dose alkaline water sources before applying chlorination treatments, but not below pH 6 as chlorine gas starts to form and escapes from solution. Hypochlorous acid reacts readily by oxidation and by electrophilic transfer of chloride ions (chlorination), with organic substances, especially amino acids and simple proteins, certain minerals (iron and manganese) and nitrogen salts especially ammonium and nitrites in water (Suslow, 2001). The latter reaction occurs rapidly forming chloramines which although biocidal in their own right, are estimated to be only about 4% as effective as hypochlorous acid (Black & Veatch Corporation, 2010), whilst being more phytotoxic (Date et al., 1999), although concentrations up to 2.9 mgl^{-1} are still considered safe for most plants (Skimina, 1992).

For this reason the efficacy of chlorination on solutions containing soluble fertilisers can be limited, although potassium nitrate and urea solutions alone do not appear to react in the same way (Fisher *et al*., 2014). This, and the potential build-up of sodium in the case of sodium hypochlorite use, has meant that chlorination has not generally been used for treating recycled hydroponic nutrient solutions (Ehret *et al*., 2001). Chlorination of organic matter results in the formation of organo-chlorinated bi-products some of which (e.g. the trihalomethanes THMs) are harmful to human health (Palmstrom *et al*., 1988).

The amount of chlorine consumed in reactions with salts and organic matter (including pathogen spores!) is generally referred to as the chlorine demand, whilst the chlorine

remaining after these reactions is known as free or available chlorine. The amount of chlorine bound by reactions with organic matter etc. varies greatly with both site and season but can be readily determined by testing the water colorimetrically (US EPA, 1978 - method 330.5, Spectrophotometric DPD) or with ORP meter/sensors (Suslow, 2004). From this the chlorine demand can be determined and the dose adjusted to give appropriate levels of free chlorine for pathogen control and maintaining a residual to eliminate new contamination.

Following efficacy assessments against zoospores and mycelium of several important *Phytophthora* spp. (*P. cinnamomi, P. citricola, P. citrophthora, P. cryptogea, P. megasperma* & *P. nicotianae*), Hong *et al.* (2003) set the recommended target for dosing to be 2 mgl^{-1} free chlorine detectable at discharge points (e.g. sprinklers and risers). This level is well within the risk threshold of 10 mgl^{-1} free chlorine established for phytotoxicity in a wide range of hardy nursery stock at HRI Efford (Scott *et al*., 1984), although Cayanan *et al.* (2009a) observed some limited foliar symptoms in *Salix, Hydrangea, Prunus, Weigela* and *Physocarpus* after 11 weeks of overhead application of irrigation water containing 2.4 mgl^{-1} free chlorine.

Smith (1979) also reported 100% zoospore mortality in *Phytophthora cinnamomi* after exposure for 1 minute to NaOCl at 2 mgl^{-1} free chlorine, but observed that killing mycelium and preventing new sporulation required 100-200 mgl^{-1}, a concentration that caused phytotoxicity symptoms in *Rosmarinus, Caryopteris, Abelia* and *Fuchsia* when applied in routine watering treatments. Bewley & Buddin (1921) killed mycelium of *P. cryptogea* with 20-50 mgl^{-1} chlorine (200-500 mgl^{-1} NaOCl), while Berenguer *et al*. (2001) found significantly greater survival and yield in tomato plants infected with a mixture of *Pythium* and *Phytophthora* spp. that were irrigated with 5 mgl^{-1} chlorine.

Surprisingly few other studies have been carried out on oomycetes, mostly focusing on zoospores of *Pythium* and *Phytophthora* and finding 100% mortality resulting from concentrations of 0.3-2.42 mgl^{-1} chlorine for contact times from 0.25-10 minutes (Bush *et al*., 2003; Hong *et al*., 2003; Hong & Richardson, 2004; Lang *et al*., 2008; Cyanan *et al*., 2009b; Roberts & Muchovej, 2009; Granke & Hausbeck, 2010; Raudales et al., 2011; Steddom & Pruett, 2012). Other structures, where they have been assessed, do appear to be more resistant, for example chlamydospores and zoospores of *Phytophthora* sp. Required 50 mgl^{-1} (Grech & Rijkenberg, 1991).

This raises the question of how resistant oospores might be and how likely would their appearance in contaminated water be? Nevertheless, chlorination is a well-established method and is reasonably efficient for the treatment of water of low electroconductivity (e.g. reservoir water or recycled irrigation water in HNS production), so long as the chlorine dosing is carefully monitored, either automatically or by hand. In conclusion, trials at HRI Efford in the early 1990's showed that when using sodium hypochlorite for chlorination it is important to obtain a high or 'horticultural' grade material and not use

cheaper materials, e.g. ‘dairy grade’ as these are likely to contain contaminants, especially chlorates (e.g. $KClO_3$) which are herbicidal in action and highly toxic to some plant species (Stanford et al., 2011; Pettitt, 2003; Powell Inc., 2014)

- Effective chlorination is probably the most well-known and widely applied of all the treatments available for the control of oomycete pathogens in water and yet clear guidelines on doses and contact times (concentration x time (*Ct*) relationships) needed for these pathogens and how these translate into practice in different horticultural sectors plus the impacts of water quality parameters have not been developed. Neither are comprehensive data on the potential for phytotoxicity and the generation of unwanted bi-products readily available.

Chlorine dioxide: Chlorine dioxide is unlike other chlorine treatments as it reacts predominantly by oxidation and not by transfer of chloride ions (chlorination) and thus does not form potentially carcinogenic and undesirable trihalomethanes (THM) or haloacetic acids (HAA) on reacting with organic molecules, or produce chloramine by reaction with ammonium (Aieta & Berg, 1986; Copes *et al*., 2014). In tests on surface waters from diverse locations in Italy, Sorlini & Collivignarelli (2005) showed that both chlorine dioxide and ozone produced 98% less THMs than free chlorine.

Chlorine dioxide dissolves readily in water but does not react with it to form hypochlorous acid, and maintains optimal biocide activity over a wide range of pH (United States Environmental Protection Agency, 1999), with its highest reduction potential in acid conditions. Chlorine dioxide is a more powerful oxidant than other chlorine compounds and under acidic conditions is comparable to hydrogen peroxide and second only to ozone in activity (Deininger, Ancheta & Ziegler, 2010).

It does, however, react to form chlorite and chlorate (Aieta & Berg, 1986; Singer, 1994; Lee *et al*., 2004) as end products which are toxic at high concentrations (Chauret *et al.,* 2001), and in comparisons with free chlorine treatments chlorine dioxide produced higher percentages of ‘unknown organic halogens’ (Hua & Reckhow, 2007). The potential toxicity of these and other oxidation bi-products has been much less studied than those from chlorination and more work is needed in this field (Gómez-López *et al.*, 2009). Disproportionation of chlorine dioxide to chlorite and chlorate is also catalysed by UV light (Stevens, 1982; Cosson & Ernst, 1994), and exposure of treated water to direct sunlight results in ready decomposition to chlorate (Zika *et al*., 1986).

Water treated with chlorine dioxide must, therefore, be kept in darkness as much as possible to minimise degradation. The anti-microbial effect of chlorine dioxide on bacteria appears to be the result of non-specific oxidative damage to cell membranes resulting in lost permeability control (Gómez-López *et al.*, 2009). In *Bacillus subtilis* spores treated with chlorine dioxide the initial stages of germination proceed but then development stops, possibly as a result of membrane damage (Young & Setlow, 2003), and in *B. cereus*, treated cells appear elongated and show surface roughness and

indentations (Peta *el al*., 2003), the elongation may be due to the inhibition of cell division. As chlorine dioxide dissolves in water with little dissociation, it is able to readily permeate through cell membranes as well as deep into biofilm layers often resistant to conventional 'free chlorine' treatments (LeChevallier *et al*., 1988), greatly enhancing its biocidal effects (Junli *et al*., 1997 a & b).

Chlorine dioxide is generally considered more expensive than other forms of chlorine (Deininger, Ancheta & Ziegler, 2010) but its greater stability over a broad range of conditions usually results in a lower total active ingredient demand and excellent residual disinfection within distribution pipework. A good example of the efficacy of chlorine dioxide in comparison with free chlorine in treating drinking water comes from studies of *Cryptosporidium parvum*, a protozoan waterborne human parasite. Whilst the resting oocysts of *C. parvum* are resistant to the concentrations of free chlorine normally used in drinking water treatment, chlorine dioxide is generally more efficient against this parasite (Carpenter *et al.,* 1999; Gyurek & Finch, 1998; Peeters *et al.*, 1989).

However, there does appear to be wide variation in the resistance of oocysts of different strains of *C. parvum* to chlorine dioxide treatment (Chauret *et al.*, 2001), the possibility that this phenomenon might be exhibited by the oospores and other resistant resting structures of oomycete pathogens does not seem to have been investigated in any depth and could have some significance; probably the most effective way to study this would be to establish *Ct* (concentration of disinfectant in mgl^{-1} x time in minutes) relationships (e.g. Clark *et al.,* 2003 – established for *Cryptosporidium* oocysts). The potential value of this is indicated by the results of Beardsell & Bankier (1996) indicating that the chlorine dioxide dose might be reduced by increasing the exposure time.

These authors also present some of the only data on oospore mortality (0.9 mgl^{-1} for 12 min for *Phytophthora cinnamomi* and 0.5 mgl^{-1} for 2 min for *Pythium ultimum* gave mortalities of 92 and 99% respectively). In studies on *Phytophthora capsici* Lewis Ivey & Miller (2013) found *in vivo* bait tests of Chlorine dioxide-treated 'ditch water' still detected viable infections after treatments of 1 mgl^{-1}, and in *in vitro* tests, zoospore mortality was 0-42.3% and 6.8-24.3% at doses of 1 and 3 mgl^{-1} (measured = 0.8-1.0 & 2.7-2.9 mgl^{-1}) respectively, with even less impact on sporangial and mycelial inoculum viability. These results are in marked contrast to the other few reported assessments of chlorine dioxide efficacy against oomycetes, predominantly carried out *in vitro* against suspensions of zoospores.

In these studies, the lethal range for zoospores falls between 0.9 and 4 mgl^{-1} chlorine dioxide (Mebalds *et al*., 1995; James *et al*., 1996; Pettitt, 2014; Fisher *et al*., 2009), whilst possibly not at odds with observations of 100% mortality of spores of *Fusarium oxysporum, Septoria tritici* and *Phytophthora* sp. at 10 mgl^{-1} chlorine dioxide (Lovatt, 2014, unpublished), presented in promotional literature by the company Ximax (see website list at end of references section).

Slightly higher doses of chlorine dioxide are required to kill cells off distantly-related algae, with 5 mgl^{-1} for 30 min resulting in 100% mortality of filamentous green alga *Ulothrix* sp. and of unicellular *Ankistrodesmus* sp. (Junli *et al*., 1997a), whilst 100% of unicells of *Chlorella vulgaris* were killed by 2 mgl^{-1} for 15 min (Rav-Acha *et al*., 1995), although it is of interest that 5 mgl^{-1} for 30 minutes was only sufficient to kill 75% of the flagellate unicells of *Chlamydomonas* sp. (Junli *et al*, 1997a).

The symptoms of phytotoxicity induced by chlorine dioxide consist of yellowing leaf margins, sometimes leading to scorch, drying and necrosis. Also spots and blotches on leaves and flowers and reduced plant size. At dose rates of 1-2 ppm chlorine dioxide Fisher *et al*. (2009) found phytotoxicity symptoms when water was applied repeatedly to impatiens and geranium foliage in mist propagation, whilst periodic applications to roots or foliage seemed to cause much less damage than continuous mist applications. Rens (2011) investigated the possibility of phytotoxicity to hydroponic greenhouse-grown bell peppers and observed reduced leaf areas, plant heights and dry weights from 10 down to 2.5 mgl^{-1} chlorine dioxide, although the effects were dramatically reduced in plants grown in pine bark media as opposed to perlite, probably as a result of the pine-bark medium reducing the available chlorine dioxide.

At Eden Project an early dosing fault with misting lines resulted in 5 mgl^{-1} being indirectly applied to foliage in parts of the Rainforest Biome instead of the normal level of <0.5 mgl^{-1}, which resulted in scorched leaf margins and necrosis of some young emerging shoots and leaf flushes (Pettitt et al., 2009). Other workers have encountered less phytotoxicity, for example Copes et al. (2003) found that rates of 5 and 50 mgl^{-1} sprayed 5 times at 3 day intervals did not damage most bedding and shrub plants tested, and Carrillo et al. (1996) found that whilst high concentrations (1000 mgl^{-1}) of chlorine dioxide (Halox E-100) caused damage to radish and lettuce seedlings, rates of 10-100 and 40-200 mgl^{-1} respectively did not cause any phytotoxicity.

To avoid phytotoxicity, Fisher *et al*. (2009) suggest that plants should not be exposed to more than 0.25 ppm residual chlorine dioxide (that is the concentration leaving the irrigation pipework and incident of the plants which will be somewhat lower than the concentration dosed into the system depending on how much oxidisable material there is present in the untreated water), unless specified otherwise on the product label. The focus on the residual concentration is of key importance both for phytotoxicity and control of disease spread, and it is not just the water quality that impacts on this as demonstrated by Krauthausen et al. (2011) who found that overhead irrigation can cause significant losses to ‘outgassing’ and that nozzle types had a major impact on this. The highest chlorine dioxide losses were with deflector nozzles causing 93% loss compared to approximately 72 and 66% with standard flat spray and floodjet nozzles respectively, but despite this, Krauthausen et al. (2011) were still able to deliver final concentrations greater than the minimum of >0.21 mgl^{-1} required for control of *Xanthomonas* brassica blackrot, using an infeed concentration of 3 mgl^{-1}.

- Water treatment with chlorine dioxide shows great promise as an effective method for controlling water-borne oomycete inoculum in irrigation systems. Again, clear, reliable data on doses and treatment times (concentration x time (*Ct*) relationships) are lacking. This situation is compounded by the fact that there are a number of different processes for generating the active ingredient available that appear to carry varying risks of generating phytotoxic bi-products. The impacts of different horticultural environments on stability, efficacy and the production and potential build-up of bi-products have not been comprehensively explored. Finally, chlorine dioxide does not form hypochlorous acid in water and as a consequence is quite distinct from chlorination and this important fact needs to be more clearly explained and presented to the industry.

Ultra-violet irradiation:

Ultra-violet (UV) light is electromagnetic radiation of wavelength between 100 and 400 nm. The magnitude and types of biological effects of UV radiation vary greatly with wavelength and for this reason the UV spectrum is broadly categorised according to wavelength as UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm) following the convention established by the Second International Congress on Light in 1932 (Diffey, 2002; Newman, 2014). Short wave UV-C rays are unlikely to be seen in terrestrial sunlight except at high altitudes and are the most effective wavelengths for killing microbes, their anti-microbial properties and potential for disinfecting water having long been appreciated (Hijnen *et al*., 1984).

UV systems use either high or low pressure lamps. High pressure lamps emit UV-C between 200-280 nm, whereas low pressure lamps emit UV-C predominantly at the most effective anti-microbial wavelength of 253.7 nm (normally rounded 254 nm) (Gelzhäuser *et al*., 1989; Burgener, 2006). In addition, high pressure lamps are less energy efficient, with only 10% of their power consumption converted to UV-C compared to 40% for low pressure lamps (Runia, 1995).

Nevertheless, the efficiency of high and medium pressure lamps is improving and for organisms like the relatively robust protozoan parasite *Cryptosporidium parvum*, UV wavelengths over the range 250-275 nm have been demonstrated to be equally germicidal (Linden *et al*., 2000). UV treatment of recirculating irrigation water and nutrient feeds has been widely tested since the early 1980s, and shown much promise in experimental systems (Adams & Robinson, 1979, Buyanovsky *et al*., 1981, Ewart & Chrimes, 1980, Daughtrey & Schippers, 1980, Menzies & Bélanger, 1996, Runia, 1994b, Stanghellini *et al*., 1984, Wohanka, 1992). Runia (1994b) reported that a UV dose or fluence (see Table 7) of 100 mJ cm^{-2} reduced *Fusarium* spp. in tomato nutrient solution by 99.9% and tomato mosaic virus by 90%, although for a complete kill a much higher fluence of 250 mJ cm^{-2} was recommended (Runia 1995, Van Os & Stanghellini, 2000).

This recommendation is supported by the work of Chang *et al*. (1985) who looked at a range of micro-organisms and found those with resistant spores required a high fluence (*Bacillus subtilis* = 135 mJ cm^{-2} and *Acanthamoeba castellani* = 225 mJ cm^{-2}), although only 15 mJ cm^{-2} proved lethal for *Escherichia coli* and 14.2 mJ cm^{-2} for *Cryptosporidium parvum* in fresh apple cider (Hanes *et al*., 2002) and for *Phytophthora capsici* zoospores in surface irrigation water (Jones *et al*., 2014). Mebalds *et al.* (1996) found that slightly higher fluences of 40 and 43 mJ cm^{-2} were required to inactivate *Pythium ultimum* and *Phytophthora cinnamomi* respectively whilst in several large-scale trials, UV systems of various sizes and fluences ranging from 100-250 mJ cm^{-2} (in the McPherson et al work quoted we used 100mJ cm-2 as a standard UV dose using a Vialux UV system from Priva for work in both tomato (Phytophthora cryptogea) & cucumbers (Pythium aphanidermatum). It is important to recognise that it isn't just the UV dose that is important with UV systems either) have proven effective against oomycete pathogens (McPherson *et al*., 1995; Wohanka, 1992; Van Os *et al*., 2004; Pettitt *et al*., 2002).

High intensity UV treatment can be detrimental to the health of plants downstream of treatment (Schwartzkopf *et al*., 1987). This is thought to be due to the formation of destructive concentrations of ozone and/or free radicals in the nutrient solution being treated (Blazka & Prochazkova, 1983). UV treatment systems pass UV radiation through the water being treated from a lamp located inside a transparent cell. It is important that no particulate matter remains in suspension as this is likely to shield potential pathogens as well as cast shadows; absorbing and scattering the UV light (Caron *et al*., 2007; Christensen & Linden, 2003; Linden & Darby, 1998), and this potential for suspended matter to interfere with light penetration in water with high turbidity has led some to discount the use of UV treatment for recycled nursery water (Skimina, 1992).

Perhaps more reasonably, some form of effective pre-filtration should be installed and indeed some commercially-available UV treatment rigs incorporate this as standard. Transmittance (UV transmittance {UVT} or T_{10}, see **Table 5**) is a measure of the fraction of UV light remaining after passage through 10 mm of the water being treated and is of more importance than turbidity alone in determining the efficacy of UV treatment. The two are linked, with transmittance and bactericidal efficacy generally decreasing as turbidity increases although not directly so as (a), transmittance is also strongly affected by substances in solution such as iron salts and a wide range of organic compounds as well as colloids (Jones et al., 2014) and (b), some suspended particles do not absorb UV light but scatter it, thus contributing to increased turbidity but not necessarily reduced biocidal activity (Qualls *et al*., 2013).

The key parameter of UV water treatment is the fluence , often referred to as the UV dose, which is the amount of energy reaching the target (fluence rate, often referred to incorrectly as light intensity, see **Table 5**) multiplied by the exposure time (Bolton, 2000, see **Table 5**). Transmittance directly influences UV treatment efficacy as the fluence decreases with decreasing transmittance, furthermore this relationship is not linear and

the fluence drops off dramatically at transmittance values below 70%. In order to maintain a required dose when the transmittance is low, either the UV radiation intensity or the exposure time (or both) need to be increased, greatly affecting economic efficiency. Thus, since the transmittance of used and recycled hydroponic solution (drain water) can be very low (normally 20-40% Runia (1994b)) and even surface-derived supplies can be less than an acceptable threshold transmittance of 50% due to the presence of organic compounds and chelated iron (Mebalds *et al*., 1995), it is highly recommended that water from such sources is diluted at least 1:1 with rainwater of high transmittance to maintain UV treatment efficacy (Runia, 1995).

The UV cell and the lamp are not readily visible from the outside of the treatment unit and it is very important to be able to make sure that the lamp is functioning properly and that the walls of the cell are clean and clear and transmitting the UV radiation. Many modern UV treatment systems deploy self-cleaning cells, nevertheless, since the UV-C output of some lamps declines with age, it is important to be able to regularly monitor their performance and adjust settings or replace lamps accordingly, and good quality UV units incorporate monitoring devices and inspection ports to permit this. Another problem often associated with UV treatment is the potential effects of the high radiation fluence on oxidisable components of plant nutrient solutions, especially iron chelates which react in UV radiation to form insoluble precipitates causing fouling of UV cells and iron depletion from the nutrient solution (Albano & Miller, 2001).

The key factors in this process are the radiation fluence, the pH and the chemical stability of the chelates used. Acher *et al.* (1997) demonstrated that the best pH range was 4.5-6.0 and that out of three chelates tested; (FeEDDHA {Fe-ethylene-diamine-dihdroxyphenyl acetic acid}, FeNaEDTA {Fe-ethylene-diamine-tetraacetic acid} and FeDTPA {Fe-diethylene-triamine-pentaacetic acid}), FeEDDHA was by far the most stable in UV treatments (stable after 42 sec at UV radiation fluence of 80 mJ cm^{-2} at pH 6). Greater disease spread than expected has sometimes been encountered in UV-treated systems, from infected plants introduced downstream of treatment. Zhang & Tu (2000) suggested that in the case of *Pythium* root rot of hydroponic tomatoes this may be linked to an overall reduction in the total bacterial population in the rhizosphere, possibly reducing the natural suppression of pathogen activity.

In addition this might be exacerbated by the lack of a 'residual' anti-microbial effect in UV-treated water, which is both a benefit (i.e. no chemical residues) and a limitation to the commercial application of the technology (Menzies & Bélanger, 1996), necessitating strict measures for the protection of treated water from recontamination (Newman, 2014). Nevertheless, there are some excellent UV irrigation water treatment rigs available with monitoring and regulation to maintain a consistent fluence or UV dose (e.g. Priva Vialux rigs http://www.priva-international.com/media/61328/vialux.pdf and the smaller-scale Hannovia units http://www.hanovia.com/uv-products/uv-systems/), and the technique is widely employed in the UK and northern mainland Europe, particularly in protected cropping systems and for treating water with high % transmittance (e.g. recycled roof water).

The next development in UV treatment appears to be the UV-oxidation process or Advanced Oxidation Process (AOP – Van der Velde *et al*., 2008), which combines the addition of hydrogen peroxide with UV. The UV radiation converts hydrogen peroxide into highly oxidative hydroxyl radicals, these react strongly with organic materials including pathogens and contaminants like pesticide residues, and meanwhile the UV radiation is still operating as described above. This is a relatively new technology and its full application, efficacy, economic efficiency and potential for bi-products has yet to be fully investigated.

- UV treatment of irrigation water is a well-established and effective method for cleaning irrigation water. Parameters for treatment are reasonably well established. UV-oxidation or Advanced Oxidation Processes are a relatively new and potentially more effective and economic alternative to conventional UV and these systems warrant further investigation both in terms of availability and economics, and direct research on their efficacy against oomycete pathogens in realistic horticultural systems.

Table 5: *Explanation of some important terms used in UV-treatment of water*.

Term	Symbol	Units	Explanation
Wavelength	λ	nm	UV is electromagnetic radiation; UV light particles travel in a wave and the wavelength, the distance over which the wave's shape repeats, determines its position in the electromagnetic spectrum and its physical properties e.g. the germicidal effects of UVC wavelengths 280-100 nm.
Fluence rate (light intensity)	*E'*	mW cm^{-2}	Total radiant power passing through a point
Irradiance	*E*	mW cm^{-2}	Total radiant power incident on a surface point – this term is often used interchangeably with fluence although the latter is derived differently and is more appropriate for UV treatment of a liquid
Fluence or UV-dose (radiant exposure)	*H'*	mJ cm^{-2} or mW s cm^{-2}	Fluence is a measure of the total radiant power incident on the target micro-organisms. It is calculated by multiplying the fluence rate by the exposure time. Fluence is dependent on transmittance with which it increases more or less exponentially.
Transmittance (Ultra-Violet Transmittance UVT)	*T*	%	*T* is determined by the ratio of the transmitted fluence to the incident fluence as a beam of UV light passes through a medium (in this case the water being treated) over a path length *l*.
	T_{10}	% cm^{-1}	T_{10} is the transmittance determined for a path length of 10 mm. T_{10} values of 60% or more are needed for efficient biocidal UV treatment of water.

Turbidity	-	NTU (Nephelometric Turbidity Units)	Turbidity is a measure of the cloudiness of a liquid, which is caused by tiny suspended particles scattering the light that passes through. Turbidity is measured with a nephelometer which detects the amount of light scattered by the liquid as a proportion of that passing straight through..
References: Bolton (2000); Bolton & Cotton (2008); Diffey (2002); Hijnen et al. (2006)			

Biofiltration

The term biofiltration has been used here to cover slow sand filtration (SSF) and essentially similar processes that use filter media other than sand. Whilst sand is the filter medium most commonly used for such processes, a wide range of materials including crushed coral, volcanic ash, rockwool granules and even burnt rice husks have been successfully used in SSF-type biofilters for cleaning water (Huisman & Wood, 1974, Ellis, 1986), and in systems for treating horticultural irrigation water, pumice (Runia, 1996a & b), Seramis®, anthracite and especially rockwool (Grodan® type 012519 fine) have been successfully used (Wohanka & Helle, 1996; Wohanka *et al*. 1999).

Slow sand filtration/biofiltration: SSF has a long history of use for cleaning drinking water, and was successfully deployed long before its full benefits (i.e. the removal of harmful pathogens such as cholera bacteria) were understood. The adoption of the technique in horticultural practice, however, has been a very recent development from the pioneering work of Wohanka (1988 & 1992), who used it for disinfecting nutrient solutions in closed cultivation systems at the Forschungsanstalt, Geisenheim. Following on from this work, research programmes in the Netherlands, England and Australia (Van Kuik, 1994, Runia *et al.*, 1996, Pettitt, 1996, Barth *et al.*, 1997) have also demonstrated the value of SSF for treating recycled irrigation water in a wide range of cropping systems.

Figure 9: *Diagram showing basic elements of SSF design*

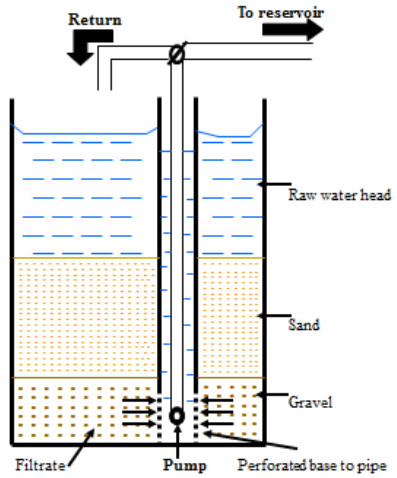
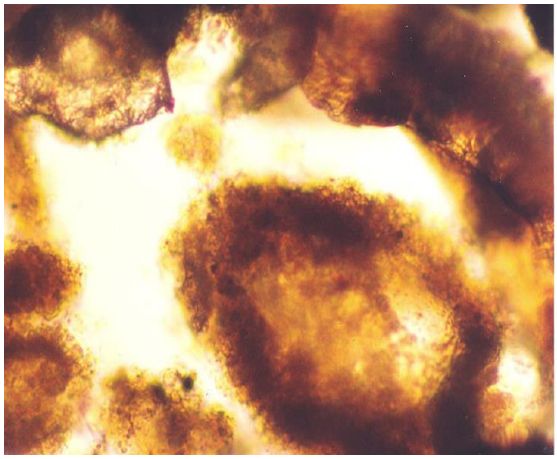


Figure 10: *Quartz sand grains from a mature SSF showing biofilm layer on their surfaces.*



Basic operation and efficacy: SSF operate by a combination of physical, physico-chemical, and biological processes (Huisman, 1978; Weber-Shirk & Dick, 1997 a & b; Brand & Wohanka, 2001; Calvo-Bado *et al*., 2003; Haig *et al*., 2011). Raw water percolates through a sand layer between 0.4 and 1.5 m deep under the force of gravity and the head pressure of an approximately 1 m deep layer untreated water on top of the filter (**Figure 9**). The slow passage through the sand breaks up the flow of the water being treated. The filter sand grains intercept suspended particles, including pathogen spores (Ives & Gregory, 1967; Ison & Ives, 1968). In addition, many particles are drawn towards and, more importantly, held by sand grains by physico-chemical forces (Ives & Gregory, 1966). The sand grains are coated by a biofilm layer that builds up as the filter matures and organic particles, including spores, are trapped and consumed by the complex ecosystem that composes this layer. The distribution of biofilm on the sand grains is uneven in appearance (**Figure 10**), which reflects the surface topography, charge characteristics and the localised water flow characteristics (Shani *et al*., 2008; Ives & Gregory, 1966). SSF only show full efficacy when they are 'fully primed' (Furtner *et al*., 2007; Calvo-Bado *et al*., 2003; Mine *et al.*, 2003), that is when the biofilm layer has fully developed on the sand grains.

Some studies have implied that while true fungi are removed by redominantly biological processes, control of *Pythium* spp. and *Phytophthora cinnamomi* may occur solely by physical means (Déniel *et al*., 2004; Van Os *et al*., 1999). However, using treatments that kill the biofilm has demonstrated that the efficacy SSF against oomycete plant pathogens is strongly dependent on biological activity (Brand & Wohanka, 2000; Pettitt, 1999), and filters are designed and operated to optimise this. Detailed aspects of the construction, maintenance and the efficacy of slow sand filters in protected horticultural use are very well covered by Ehret *et al.* (2001) and in an AHDB Horticulture Grower Guide (Pettitt & Hutchinson, 2005).

The precise acvtivity of SSF against specific plant pathogens is still not fully understood, although with improving molecular techniques, knowledge about details of biological activity is increasing (Calvo-Bado *et al*., 2003; Hunter *et al*., 2012; Haig *et al*., 2011). Often research groups have tried to improve efficacy by 'boosting' the microflora with inoculations with known biological control agents or with isolates of micro-organisms from effective filters.

This approach has met with varying success, Déniel *et al*. (2004) found additions of strains of *Pseudomonas putida* and *Bacillus cereus* improved control of *Fusarium oxysporum,* whilst others have found that inoculants have little impact (Furtner *et al*., 2007; Hunter *et al*., 2012), such differences may be linked to the level of maturity or 'ripeness' of non-inoculated control SSF used in such studies. It can be problematic ripening SSF when they are running within closed, protected cropping systems – a situation that practical experience with commercial scale SSF treating water for protected vegetable crops shows can be remedied by topping up the raw water supply from exterior ponds or reservoirs (Pettitt, unpublished).

SSF efficacy has been demonstrated to be linked to active microbial biomass in the sand (Campos *et al.*, 2002), the wide variation in microbial diversity between different effective SSF (Hunter *et al*., 2012; Renault *et al*., 2012; Haig *et al.*, 2014a) indicates that functional groups of organisms and mass trophic interactions (Haig *et al*., 2014b) are of more importance to anti-pathogen activity than the presence/absence of individual species. This concept is supported by the detection of a range of cell wall-degrading enzymes (CWDE), including protease and hemicellulase, in the most biologically active layers of SSF by Brand & Alsanius (2004a).

These authors found that CWDE and consequently anti-pathogen activity could be stimulated by adding lyophilised mycelial extracts to filters. However, care needs to be taken with the addition of 'supplements' to SSF, as significant breakdowns in efficacy have been observed in UK commercial SSF following applications of propriety biocontrol agent mixes, humic extracts, seaweed preparations and 'home brew' plant extracts (Pettitt, 1996-2006, unpublished). In many of these cases increased levels of soluble carbohydrates might have had some influence, which links well with the concept of CWDE activity as high levels of such materials may induce catabolite repression of enzyme synthesis with a concomittent decline in anti–pathogen activity.

In SSF (and possibly all biofilter systems) we have a system with the potential capacity to build up populations of naturally disease-suppressent micro-organisms and act as a reservoir that slowly distributes suppressive inocula downstream (Déniel *et al*., 2004; Postma *et al.*, 2000). This possibility is supported by observations of differences between 'active' and 'passive' water treatments by McPherson *et al*. (1995) where 'passive' treatments like SSF showed evidence of natural disease suppression down stream of treatment Pettitt (2006).

This has led some workers to propose that in a closed recirculating system there may not be a need for complete elimination of all pathogen propagules with every pass through a filter (VanOs & Postma, 2000). This is a difficult area and work is still needed to determine whether suppression means infection is suppressed or eliminated OR if symptom expression is suppressed. The latter scenario is potentially very problematic and is akin to the over use of fungistatic fungicides – running the risk of widespread infections below detection thresholds and providing a route for new invasive species such as *Phytophthora ramorum* to become quickly established. Nevertheless, in crops like tomatoes where the rootsystems remain and are not for sale experimentation with altered disease thresholds is worthwhile.

Sand quality: Two parameters of sand grain size are used to judge whether a sand is suitable for SSF use. The effective size (ES), and the uniformity coefficient (UC). The effective size of a sand is the sieve mesh diameter through which 10% by weight of the sand will pass. Reasonably uniform sand is required for SSF (although this requirement is not as critical as in fast pressurised sand filtration) and a measure of this is obtained using the UC which is the sieve diameter through which 60% by weight of the sand passes divided by the ES.

The majority of successful horticultural SSF using sand have been constructed following the guidelines set out by Visscher *et al.* (1987); an ES in the range 0.15-0.30 mm and a UC less than 5 and preferably less than 3. In horticultural applications there has so far been little work testing the boundaries of these prescribed sand characteristics on SSF efficacy, although a range of different quality locally-sources 'builder's sharp sands' were assessed in AHDB Horticulture funded research (Pettitt, 2000, HNS88a). These fell within the ES range (approximately 0.2 mm) but had comparatively low levels of uniformity, nevertheless all performed very well in eliminating all oomycetes (not just the target pathogen species) from treated water.

SSF design and configuration: One of the most attractive features of SSF is its flexibility in terms of design, allowing filters from as small as approx. 50 cm^2 (a filter this small would only treat approximately 1 litre per hour!) of surface area upwards to be easily constructed. At Eden Project a filter capable of treating 30 m^3day^{-1} was constructed re-using an old oil storage tank, the other main components being a 3m length of PVC sewer pipe, a new borehole pump, switching gear, and china-clay sand, for an estimated capital cost of £350 (Pettitt & Cutler, 2006 unpublished). As long as the basic principles of the process are adhered to, effective water treatment will be achieved. These are described in an AHDB Horticulture grower guide (Pettitt & Hutchinson, 2005) and also outlined by Atwood (2014). Essentially the water to be treated is passed through a column of sand of minimum depth 40 cm, at a flow rate of 0.1 – 0.3 m^3/m^2 of filter surface area per hour. (Flow rates are often referred to as the depth of water passing through the filter per hour, e.g. 0.1 – 0.3 m/h. The volume per hour in m^3 is then easily calculated by multiplying this figure by the surface area of the filter in m^2).

The fundamental filter arrangement is illustrated in **Figure 9**. In this illustration, the treated water is lifted from the filter using a bore-hole type pump controlled by float switches and housed in a drain pipe that has perforated walls in the bottom 25 cm or so of its length – the part that goes into the gravel under-drain layer. The majority of horticultural SSF installed in the UK have used this pump-lift approach to remove filtered water.

However, simple under-drain systems work equally well and are very easy to construct, especially on a small scale. For example, a good small-scale filter made using a proprietary rain water butt and capable of treating 1 m^3 per day would be best operated by under-drainage and controlling the flow rate using the exit tap positioned at the base of the butt. The main potential drawback to a simple under-drain system is an increased chance of leaks resulting from the placement of a valved outlet low on the side of the filter unit. The rate of flow of water through the filter is controlled either by an exit valve on an under-drain system, or by the flow rate of the pump in a lifting system.

The main consideration for design is the container for the filter. Anything will do for this, as long as it is watertight and can hold the sand column, allowing easy removal of treated water from the bottom and access to the sand for cleaning operations. The two most widely used approaches to filter design have been circular butyl-lined corrugated steel water tanks or lined holes dug in the ground. Either technique works well, although the latter can only be operated using pump-lift. Ultimately design decisions depend on costs, operational demands, site suitability and local planning authority rulings.

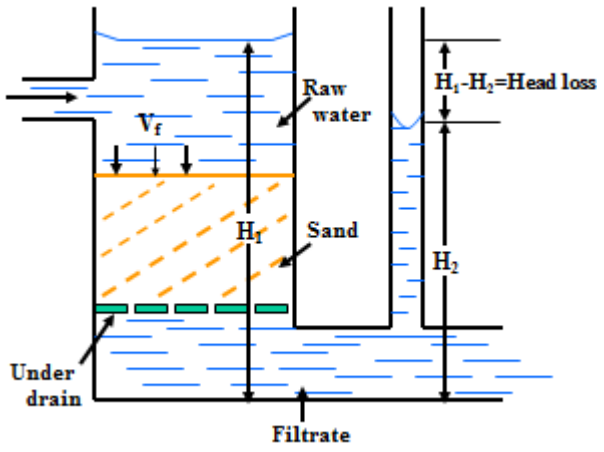
Head loss and filter blockage: SSF operate effectively at flow rates between 0.1 and 0.25 m/h. The flow rate of water through sand depends on three factors: the raw water head in m (H_1), the filtrate (or effluent) water head in m (H_2) and the rate of filtration in m h^{-1} (or velocity of flow, V_f) that is the total volume passing per hour divided by the surface area of the bed. These factors are related according to Darcy's law which states that the velocity of flow is proportional to the head-loss:

$$V_f = \alpha (H_1 - H_2) \qquad (1)$$

where α = is a coefficient dependent upon the hydraulic characteristics of the complete filter. The head-loss is the resistance to flow offered by the filter and can be determined manometrically (**Figure 11**) to give a measure of the increasing resistance due to accumulated particles on and in the filter sand during a filter run. As the filter pores gradually become blocked by particles settling out of suspension, the flow rate (V_f) through the filter decreases. However, V_f can be regulated by the rate of pumping in a lifting system or by gradually opening the exit valve aperture in an under-drain system. The size of filter needed is governed by the operational flow rate. However, when deciding on what size of filter to install it is better to make a conservative estimate of the expected flow rate egg 0.1-0.15 m/h. **Figure 12** gives an indication of the daily volumes of treated water that can be expected with increasing filter size. Usual practice is to aim

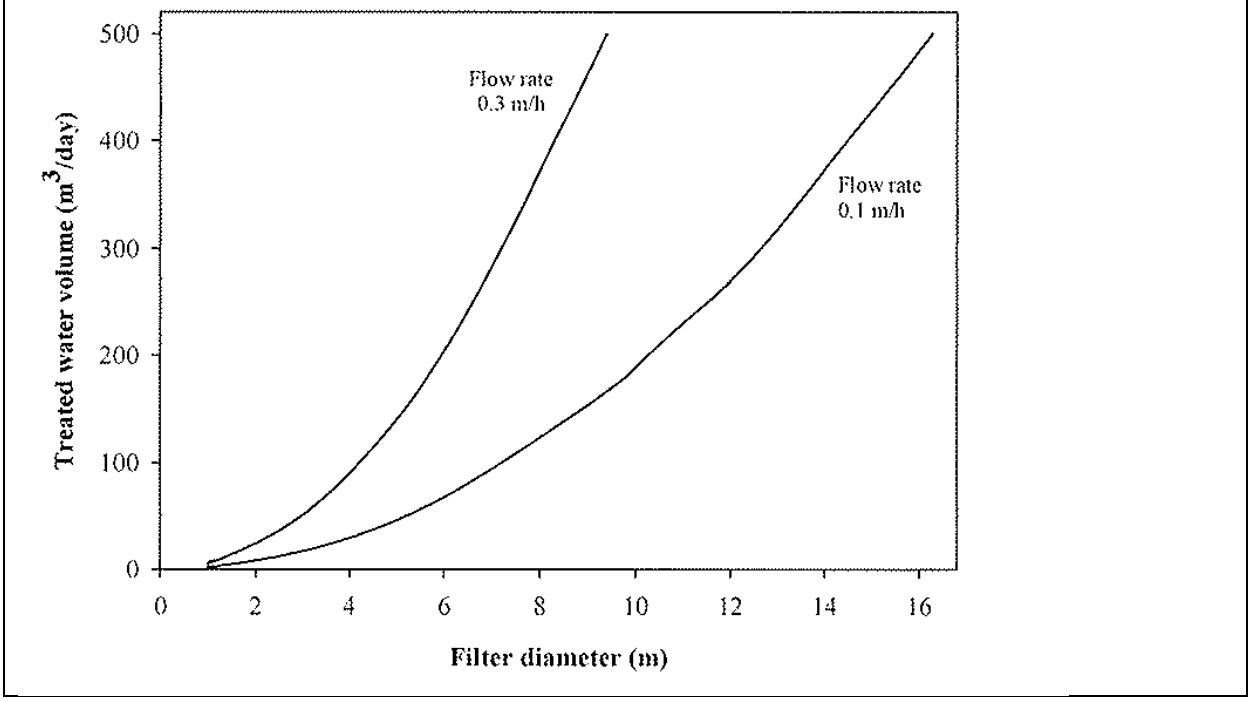
for a SSF to be capable of producing enough water in 24 h for one day's watering at maximum demand plus a safety margin of approximately 10%.

Figure 11: *Schematic diagram of a SSF illustrating the derivation of head-loss.*



During a filter run the head-loss continues to gradually increase until a point when the rate of flow cannot be further regulated by the methods indicated above, and will rapidly decline. The frequency of this happening depends on the quality of the raw water and the pre-filtration treatment. This rapid decline in filter flow rate can be predicted by measuring the filter head-loss. Head-loss measurement can be used to determine when the SSF needs cleaning (if at all!) and cleaning is generally advisable once the head-loss exceeds 60% of the height of head above the filter sand (Raw water, **Figure 11**).

Figure 12: *Theoretical range of treated water outputs from a SSF depending on the filter size and the flow rate.*



Cleaning blocked SSF: A properly maintained SSF with good pre-filtration should not require cleaning more than once per season. Filter clean-ups are straightforward, but are ideally kept to an absolute minimum as they can be disruptive and add significantly to filter running costs by:

(a) causing the filter to be out of production for 1-2 days;

(b) labour inputs required to scrape the clogged sand out (approximately 30 m^2 of filter surface can be scraped in 1 man-hour);

(c) causing sand loss (the more frequent the clean-ups, the more often sand will need to be replaced).

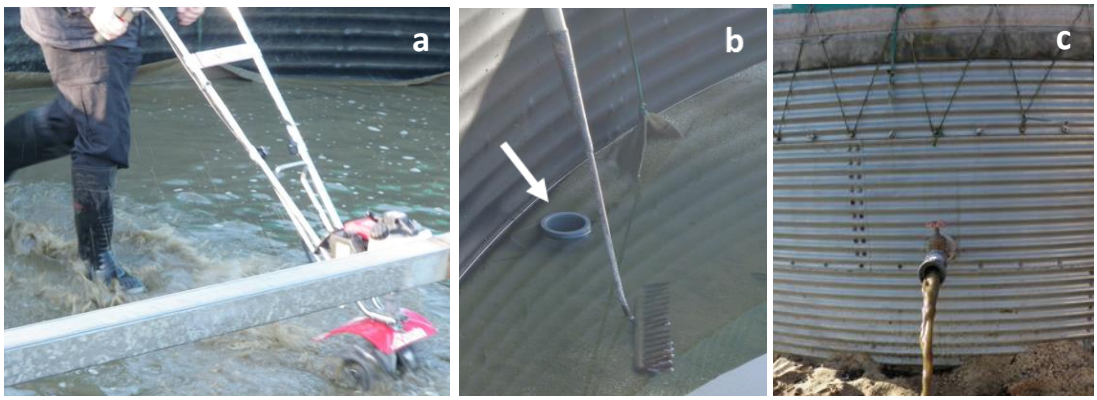
A clean-up consists of the removal of the clogged surface layer of sand (approximately 1-3cm) with a shovel after allowing the water to drain below the sand surface. After levelling the scraped surface with a rake, the sand is recharged with clean water from below until the water level is about 5-10 cm above the sand surface. This allows the sand surface to settle, prevents the raw water inlet from scouring the sand surface and reduces the formation of air pockets in the filter profile. Once the water depth above the sand is between 5-10 cm, the raw water inlet can be switched back on and the SSF is run back to the raw water collection reservoir for 24 h to reprime, after which it can be switched back into production.

Without adequate pre-filtration removing suspended particles down to at least 50 µm, SSF frequently become blocked and require cleaning. This has harmed the uptake of this technique for water treatment in the UK, although those nurseries that have invested in suitable prefiltration in combination with SSF have found the process has worked extremely well

Speeding up SSF and reducing blockages: An experimental sand filter was constructed at Eden Project using coarse sand (actually marketed as 'horticultural grit'). The sand used is a china-clay bi-product, has an ES of 0.5 mm and a UC of 2.8 (Diaz & Pettitt 2007, unpublished) and was placed in the filter to a depth of 1 m with approximately 80 cm of head water above it. The filter has been run effectively at a flow rate of 0.3-0.5 mh^{-1} and regular testing by plating and baiting following the procedures of Pettitt *et al*. (2002) has shown that it consistently removes all oomycete propagules, which were sometimes at levels exceeding 300 cfu l^{-1} in the untreated water (Pettitt, 2006-2014, unpublished).

With this sand the biologically highly active zone appeared to be much deeper, at >100 mm and <200 mm, than with 'regular' sand and despite the dirty raw water and use of limited pre-filtration, head loss was slow to develop with the sand requiring cleaning after 3 years continuous use. Significant penetration of fine particles was observed to a depth of 120 mm, whilst single-celled algae only reached 60-80 mm and oomycetes 60 mm (Pettitt, 2009, unpublished).

Figure 13: *(a) Using rotivator to agitate the surface 30 cm of grit on a commercial SSF; (b) view of SSF surface drained to allow agitation process – white arrow marks the drainage for dirty water on completion of agitation process; (c) dirt water outlet.*



Cleaning was not by the usual scraping method as the usual clogging crust had not developed, instead the top 300 mm of sand were removed and washed by agitation and rinsing in a cement mixer before being returned to the filter, which then ran well for a further 3 years without problems. This result inspired the installation of the same sand (grit), in a supplementary layer approximately 30 cm deep, on the top of a fully operational commercial SSF, following a procedure based on the results of AHDB Horticulture funded research (Pettitt, 2002, HNS 88b). This filter runs at a constant rate of 6 m^3h^{-1} producing between 100-140 m^3day^{-1} depending on how much is drawn off for use, and was frequently becoming blocked by water rich in single-celled algae (e.g. *Staurastrum* sp.).

The installation of the grit layer increased the time between filter clean-ups from once per month to once per year, and the cleaning procedure has been changed from scraping off the top 1-2cm of blocked sand to agitating the top 30 cm layer with a 'Rotivator'and draining off the small volume of re-suspended blocking particles into a settlement channel/ditch returning to the reservoir (**Figure 13** a-c).

Other biofiltration approaches: The combination of coarser medium and faster throughput of water alluded to above is more akin to other biofiltration methods, indicating the mechanisms involved are probably related. Most prominent amongst these are 'lava filters' which use pumice or 'lava granules' as a medium, and have been shown to be 100% effective against *Pythium* and *Phytophthora* propagules at double the flow rates normal for SSF (Ufer *et al*., 2008a; Ehret *et al*., 1999). As a consequence of this, they take up much less space than conventional SSF. Commercially-produced lava filters, for example the design produced by SHIEER Group (the SCHIEER BioFilter®) contain complex systems for injecting air bubbles into the filter column to improve aeration and anti-pathogen microbial activity.

Whilst these systems have been recorded as effective against both *Pythium* and *Phytophthora* spp. (Runia, 1996a & b; Van Vliet, 2005), it is vital that the bubbling

process is carried out in such a way as to not disrupt the biofiltration process itself – one unit (not by Shieer) tested by the author on a recirculating system growing Gerbera on a nursery near den Haag, had an extremely vigorous bubbling system but was an ineffective disinfestation system as it only reduced the numbers of *Phytophthora cryptogea* propagules by approximately 15% (Pettitt, 2000, unpublished). Possibly as a result of their increased engineering or the extra expense of the pumice, these filters cost on average about three times as much as SSF (Stewart-Wade, 2011), which might explain their scarcity (Werres & Wohanka, 2014).

A lower cost system has been developed in the UK that uses a slow rate of aeration that is claimed to break down the organic matter that normally accumulates in biofilters and to increase the potential flow rate compared to conventional SSF (thus reducing the demand for space), as well as encouraging the development of populations of beneficial fluorescent Pseudomonad bacteria (http://www.fpl.irrigation.com/mf01.htm - The Manchester Filter System, Flowering Plants Ltd).

This approach appears to improve the clarity of irrigation water and increase populations of Pseudomonad bacteria but requires further research to fully understand its biology and the limitations of its efficacy. A wide range of materials other than pumice have been tested in biofilters such as Seramis® porous clay granules, sintered glass, vermiculite, perlite, rockwool (e.g. Grodan®) and even polyurethane foam (Park *et al*, 1998; Wohanka, 1995 & 1996; VanOs *et al*., 2001), but none have really excelled over pumice or sand and most carry higher costs and/or potential waste-disposal problems.

Constructed wetlands-type systems including Iris Beds: Whilst 'lava filter' systems are rare, pumice is more often used as a medium in various types of constructed wetland filter (Werres & Wohanka, 2014). There are two main types of constructed wetland that can be used in horticultural water systems; the surface flow (or free water surface) and the subsurface flow (horizontal or vertical flow) (White *et al*., 2011), and within these categories there are a great many different designs (Berghage *et al.,* 1999) exploiting a range of different media including pumice, and a range of different aquatic plants including the common reed, *Phragmites australis* (Cav.) Trin. *ex* Streud., and yellow iris, *Iris pseudocorus* L. (Vacca *et al*., 2005).

Much work has been done to assess the effectiveness of these wetlands at removing human pathogens such as *E. coli* from contaminated water, but relatively few studies have focused on plant pathogens (Werres & Wohanka, 2014). Of these, several have demonstrated activity against *Pythium* and *Phytophthora* (Gruyer *et al*., 2013: Headley *et al*, 2005; Ufer *et al*., 2008b). A key parameter governing efficacy of constructed wetlands is the retention time, often referred to as the Hydraulic Residence Time or HRT (Persson *et al*., 1999). This is essentially a measure of flow rate through the system and combined with the structure of the wetland (depth and type of medium and possibly the plant spp. used, Vacca *et al*., 2005) it will determine the efficacy of the system against plant pathogens.

The required HRT can be used in a similar way to the recommended flow rate for a SSF to determine the size of wetland required by a nursery to treat its water – generally these systems need a comparatively large amount of space. Care is also needed in their operation, and if not properly operated, efficacy against *Phytophthora* and *Pythium* may be lost (Ufer *et al*., 2008b). Constructed wetlands appear to be a promising low-maintenance prefiltration method that might be deployed prior to other methods of purification such as SSF, UV or even chemical dosing. In operating such a combined treatment, the required HRT and therefore the demand for space would be likely to be greatly reduced.

Iris beds are a specialised type of constructed wetland specifically designed for elimination of plant pathogens (especially oomycetes), and they consist of a system very similar to surface flow constructed wetlands but not using any medium to support aquatic plants which are supported on the water by rafts. This approach was developed in Zundert in the Netherlands (Jochems, 2006) and was originally inspired by a flourishing fish pond outside the De Douglas Nurseries office in Wernhout that was receiving no 'maintenance' whatsoever! In some ways similar to the Root Zone Method (RZM) of Rivera *et al*. (1995) where anti-pathogen biological activity is thought to be supported in the rhizosphere, the 'Ditch System' (or Iris Beds) of Jochems (2006) utilises a series of butyl-lined channels, populated by aquatic plants, that feed one to

Figure 14: *General view of iris bed as installed at John Richards Nurseries (a); young root systems of iris plants a couple of months post planting (b) and mature iris plants one season later (c).*

the next shallow weirs that allow oxygenation and exposure to the UV in sunlight. An adaptation of these systems using dense plantings of *Iris pseudacorus* on expanded polystyrene rafts that cover the water channels has been developed on two UK HNS nurseries (Carr, 2010; Atwood, 2014) (a-c). Microbiological monitoring has shown these systems to be complex but to show great promise (Pettitt, 2008-2014, unpublished). Essentially *Phytophthora* and, much more commonly, *Pythium* propagules have been detected entering the systems but their numbers rapidly decline with progress along the channel, and none have been detected exiting. However, unlike in SSF, other oomycete species (members of the Saprolegniales) appear to thrive throughout the iris beds! This selective pathogen removal appears to be consistent on both nurseries deploying the system, although the precise nature of this

efficacy, whether or not it is due to chance and its limitations of use and application require and warrant further research.

- Slow sand filtration (SSF) has received a good deal of research effort and has been demonstrated to be a highly effective method for the elimination of oomycete propagules from irrigation water. A relatively simple process to set up and run, SSF is still often misunderstood or misinterpreted (often by people who should know better!). Highly adaptable, SSF is best operated within a broader policy encompassing integrated predominantly biological pest and disease management, although this is by no means essential for it to be effective. The basic parameters for successful SSF have been established and the main areas where improvements in efficacy might be obtained would transform slow sand filters into faster biofilters. They are: (1) the careful consideration and design of filter media to increase effective surface area and biological activity per unit filter volume; (2) examining procedures (physic-chemical and structural) that can increase the depth of the active layer (e.g. as already achieved by using a coarser grit and faster flow rates; (3) closer investigation of potential inoculants; (4) reducing times between clean-ups and designing to increase the efficiency of clean-ups.

- Of the other forms of biofiltration the most exciting category are those that seem to be operating on the RZM (root zone method) principle for pathogen removal e.g. Iris Beds. These require more intensive microbiological assessment and testing to determine their mode(s) of operation, their impacts on the microbiology of the irrigation system downstream and the limits of their efficacy, to identify possible improvements and provide guidelines for their reliable installation and operation.

- Combinations of biofilters are another possibility, for example the deployment of a slow sand channel at the end of the final channel in an Iris Bed system might greatly improve the quality of the finished water, or the use of biofilters as providers of ‘biological residual’ post ozonation or UV.

Potential problems with biofilms in irrigation systems?

Biofilm formation in irrigation lines can be a serious problem on nurseries across all horticultural sectors, typically resulting in reduced water flows, increased pump resistance with associated wear on irrigation rig components and blockages to nozzles and drip lines. Recently there have also been claims that biofilms harbour oomycete plant pathogens, especially *Pythium* spp. (e.g. Dramm corp., 2011). Whilst there is some evidence that bacterial human pathogens such as *E coli* can accumulate shelter and multiply in biofilms in irrigation rigs distributing untreated reservoir water (LeChevallier *et al*., 1988; Pachepsky *et al.*, 2011), there is no evidence yet to support

the existence of a similar situation with *Pythium* or other oomycete pathogen species. In fact, available evidence would suggest quite the opposite in closed hydroponics systems (McPherson *et al*., 1995; Brand & Alsanius, 2004b; Rosberg *et al*., 2014), whilst the known efficacy of biofiltration systems is largely based on the hostility of biofilms to pant pathogens (Brand & Wohanka, 2000; Calvo-Bado *et al*., 2003; Werres & Wohanka, 2014). A good example of this was observed in microcosm studies at HRI Wellesbourne where zoopores of *Phytophthora cryptogea* and *Pythium aphanidermatum* were attracted to, encysted, germinated directly (see Fig 3 in Lifecycles & Sporulation above) and were lysed within 24 h, on biofilms that were built up on glass surfaces maintained under a continuous flow of recycled irrigation water (Pettitt *et al.*, 2004, unpublished). Nevertheless, despite a good deal of research having been carried out on the microbiology of natural disease suppression and antagonism to oomycete pathogens in irrigation systems (Alsanius et al., 2014), biofilms in irrigation systems remain little studied and relatively poorly understood.

Concluding remarks on water treatments:

In addition to the treatment options considered in depth here, there are a number of other techniques that show considerable promise but still need more research on their effective operation. The most prominent of these are UV photo-catalytic oxidation treatments, electrochemically activated- or electrolysed-oxidised-water (ECA or EO) and copper ionisation. Photo-catalytic oxidation operates by exposure of a titanium oxide (TiO_2) catalyst in water to UV light (either sunlight or more often from a UV lamp). This generates hydroxyl radicals which kill micro-organisms by oxidation (Chong *et al*., 2010). Photo-catalytic cells have been developed for treating horticultural irrigation water (Polo-López et al., 2010), but proper efficacy trials assessing the impact of pH, alkalinity, turbidity and other quality parameters within an operational nursery setting still need to be carried out. The ECA/EO treatment technology has been available for some time. Essentially, a salt solution (NaCl or preferably for horticultural operations KCl) is electrolysed in a cell with cathode and anode separated by a dielectric membrane.

At the anode a solution rich in free chlorine (hypochlorous acid) is produced and this is used as a very effective biocide used in the food industry (Al-Haq *et al*., 2005; Huang *et al*., 2008)). Some ECA/EO units have been installed in nurseries in the Netherlands (Wohanka pers. Comm.), but this is a technology that looks to have similar limitations to chlorination and requires more research to determine efficacy and phytotoxicity limits. Copper ionisation systems have been available for some time but until recently the control systems have been poor giving highly variable results.

More recently systems with improved monitoring of solution electro-conductivity and release rates have been developed (Wohanka, 2014) and these have given very effective control of oomycete propagules on ornamentals nurseries, although further

work is needed to define the limits of efficacy and phytotoxicity, as well as assess the potential accumulation of copper residues throughout production systems. Microfiltration is another effective water treatment that showed great promise in large-scale trials at STC (McPherson *et al*., 1995; McPherson, 1996). However, this technology has never really been taken up by nurseries, possibly because of the high costs of equipment as well as some potential operational problems (Schuerger & Hammer, 2009), and it has not been considered in this review.

In **Error! Reference source not found.** the main pros and cons for each of the major ater treatment techniques currently available in the UK are considered. Also, in **Table 7**: *Incidence of interactions between some important parameters of irrigation water quality and the main water treatment techniques available for controlling water-borne oomycete propagules*.

An attempt has been made to collate, where available, information on the possibilities of interactions between water treatments and key parameters of water quality such as pH and turbidity. From this it can be seen that the most 'interactive' treatments are those involving oxidation, and that Pasteurisation and biofiltration suffer the lowest amount of potentially problematic interactions with water chemistry etc. In addition to water parameters, possible reactions pesticides and herbicides need to be taken into consideration when designing a new system and integrating it into an individual nursery's operations. In the words of Professor Walter Wohanka of Geisenheim University, Germany: 'there is no single *right* way to treat water, only the *right* way for you'!

Table 6: *Advantages and disadvantage of the main water treatment technologies currently available for treating irrigation water for the control of oomycete stem and root rot pathogens. (Table adapted and expanded from Pettitt & Hutchinson (2005) with additions from Atwood (2014) and Fisher (2014))*

Water treatment	Advantages	Disadvantages
Pasteurisation	• Known, safe, reliable and robust method for treating water • No chemical inputs – no residues • Water mineralogy & pH largely unchanged	• Expensive to install • High energy consumption and therefore high running costs • Only effective on relatively small-medium sized systems • No residual effect • Oxygenation reduced
Ultra-Violet light (UV)	• Relatively low to medium running costs • UV units occupy comparatively small space • No chemical inputs no residues • pH unchanged – relatively minor chemical changes (degradation of iron chelate)	• Expensive to install • Water must be free from suspended particles or turbidity • Correct flow rate essential for thorough irradiation • High maintenance with cells requiring regular cleaning • Continuous electrical power supply needed • No residual • Lime scale in cell at pH >6
Ozonation	• Strong oxidising agent – effective biocide • Adds oxygen • No noxious products formed • No chemical inputs – no residues	• Not widely used and limited guidelines on efficacy – not readily available • High installation and running costs • No residual • Will oxidise iron manganese and sulphides precipitating them from nutrient solutions
Chlorination	• Relatively simple to install and maintain • Long record of successful use • Creates environment hostile to algal growth	• Most plants are sensitive to chlorine – if injected at high rates may cause phytotoxicity • Chlorine solutions are dangerous to humans and wildlife and must be handled

	• Keeps pipework and irrigation system clean • Economic installation • Residual disinfectant activity	according to COSHH regulations • Risk of organochlorine formation • Chlorine reacts with ammonium, so cannot be used in conjunction with this form of N fertiliser • Reacts with Iron and Manganese, removing them from solution and forming insoluble salts that can cause mineral fouling of irrigation lines • Corrosive • Horticultural grade hypochlorite must be used as other grades contain phytotoxic chlorates • pH must be kept to 6-7 • Depending on concentration, dosed water needs to be stored for a time to allow dissipation of chlorine
Chlorine dioxide	• Strong oxidising agent • Active over wide pH range (pH 4 – 10) • Primarily an oxidant – no chlorination, therefore no organochlorine formation • Low phytotoxicity • Can clean pipework with 'shock treatments' • Does not react with ammonium • Slow to react with organic matter • Single treatment systems economic to install	• Relatively costly chemicals • Chlorine solutions are dangerous to humans and wildlife and must be handled according to COSHH regulations • Although promising, efficacy against oomycetes not fully understood • Can escape solution as chlorine dioxide gas under turbulence (egg sprinkler irrigation nozzles) • Reacts rapidly with Iron and Manganese, removing them from solution and forming insoluble salts that can cause mineral fouling of irrigation lines

		• Will react with and be neutralised by very high organic matter loads
Hydrogen Peroxide	• Strong oxidising agent • Simple injectors – low installation costs • No noxious products formed • Used widely for animal drinking water disinfection	• Very rapid breakdown in presence of organic matter • Concentrate solution potentially dangerous to humans and wildlife and must be handled according to COSHH regulations • Efficacy against oomycetes not fully understood
Slow sand filtration	• Flexible and simple design • Easy to install and maintain • No dangerous chemical or noxious products • Low running costs • Environmentally friendly	• Filters and storage tanks occupy large area • Can require regular cleaning, although techniques exist to reduce this substantially • Treatment process comparatively slow necessitating storage of treated water

Table 7: *Incidence of interactions between some important parameters of irrigation water quality and the main water treatment techniques available for controlling water-borne oomycete propagules*.

Water parameter	Pasteurisation	Ozone	Peroxide & per-oxygen's	Chlori-nation	Chlorine dioxide	UV	Bio-filtration
pH >7.5	-	+		+	-	-	-
pH <4.5	-					-	-
Organic matter (OM)	-	+	+	+	+	+	-/+
Dissolved OM	-	+	+	+	+	-/+	-
Turbidity	-	-/+	-/+	-/+	-/+	+	-
'Colour'	-	-	-/+	-/+	-/+	+	-
Nitrates	-	-	-	-	-	-	-
Nitrites	-	+	+	+	+	-	-
Fe	-	+	+	+	+	+	+/-
Mn	-	+	+	+	+	+	+/-
Sulphide	-	?	?	?	+	-/+	-
Ammonium	-	+		+	+	-	-
Bicarbonate	-	+		?	+	-/+	-

OTHER APPROACHES TO CONTROL OF STEM & ROOT ROT OOMYCETES

Fungicides

Extensive studies have been undertaken to evaluate novel fungicides for oomycete disease control. Not surprisingly, much of this effort has focused on the control of pathogens of economically dominant crops in the world market (e.g. *Phytophthora infestans* blight on potatoes and downy mildew in various crops e.g. vines, lettuce etc.) as discussed earlier in this review (section 'Oomycetes as pathogens'). Meanwhile, studies on the control of the more complex root-infecting oomycetes have been more limited, especially on the more minor horticultural targets. Nevertheless, and probably due in no small part to the huge economic importance of potato blight around the world, there is a relatively large range of different active ingredients and commercial fungicide products available with reported activity against oomycetes (see **Table 8**).

Table 8 : *Current fungicide groups with reported activity against oomycetes; some examples of products their active ingredients, FRAC group codes and the risk of resistance development in field populations.*

Fungicide Group	**Active ingredient example(s)**	**Target site of action** (Mode of action)	**Products (examples only)***	**FRAC Group**	**Resistance risk**
Phenylamides	Metalaxyl	RNA polymerase I	Fubol Gold	4	High
Isoxazoles	Hymexazol	DNA/RNA synthesis†	Tachigaren	32	Low
Benzamides	Zoxamide	ß-tubulin assembly in mitosis	Electis	22	Low-Medium
Acylpicolides	Fluopicolide	Delocalisation of spectrin-like proteins	Infinito	43	Not Known
Q_oI (Quinone outside inhibitors) {Strobilurins, Oxazolidinediones & Imidazolinones}	Azoxystrobin	Inhibition of Complex III: cytochrome bc1 (ubiquinol oxidase) at Q_o site (cyt b gene)	Amistar	11	High
	Famoxadone		Tanos (mixture)		
	Fenamidone		Sonata		

Fungicide Group	Active ingredient example(s)	Target site of action (Mode of action)	Products (examples only)*	FRAC Group	Resistance risk
Q_iI (Quinone inside inhibitors)	Cyazofamid	Inhibition of Complex III: cytochrome bc1 (ubiquinone reductase) at Q_i site	Ranman	21	Not Known (medium-high)
	Amisulbrom		Shinkon		
Q_xI (Quinone '*x*' inhibitors)	Ametocradin	Complex III: cytochrome bc1 (ubiquinone reductase) at Q_x (unknown) site	Initium	45	Medium-High
Carbamates	Propamocarb-HCl	Cell membrane permeability, fatty acids†	Previcur Energy	28	Low-Medium
Carboxylic Acid Amides (CAA)	Dimethomorph	Cellulose synthase	Paraat	40	Low-Medium
	Benthiavalicarb		Valbon		
	Mandipropamid		Revus		
Ureas	Cymoxanil	Unknown	Option	27	Low-Medium
Phosphonic acids	Phosphonates	Unknown§	Aliette	33	Low
			Plant Trust		
	Phosphonic acid & salts	Unknown§	Various		
Pyridinamines	Fluazinam	Uncoupler of oxidative phosphorylation	Shirlan	29	Low
Dithiocarbamates	Mancozeb	Multi-site contact activity	Dithane	M3	Low

Fungicide Group	Active ingredient example(s)	Target site of action (Mode of action)	Products (examples only)*	FRAC Group	Resistance risk
Chloronitriles	Chlorothalonil	Multi-site contact activity	Bravo 500	M5	Low
Sulfamides	Dichlofluanid	Multi-site contact activity	Elvaron	M6	Low
	Tolyfluanid		Euparen		
Quinones	Dithianon	Multi-site contact activity	Dithianon WG	M9	Low

* Note that not all products listed are necessarily currently approved for use in the UK. It is essential that you take specialist advice on product authorisation prior to use of a particular product to ensure you comply with all current legislation regarding pesticide application.

† proposed target site of fungicide action

§ possibly inhibition of calcium-dependent ATPases (Stasikowski *et al*., 2014), although also known to strongly influence the salicylic acid resistance pathway (Groves *et al*., 2015) and directly inhibit *Phytophthora* cytoskeleton and cell wall synthesis (King *et al*., 2010).

The majority of these appear to work most effectively as protectants, helping prevent initial infection and secondary spread rather than eradicating ('curative' action) established disease. This necessitates their use either in advance of i.e. precautionary or very early i.e. at the first signs of disease outbreaks to be fully effective. Unfortunately, some of the older effective products with multi-site inhibitor activity have either been revoked or are under threat of revocation (e.g. mancozeb and the copper formulations of such importance to organic growers) as a result of UK & EU legislative change. Thus, there is an increased reliance on the use of single-site inhibitors such as metalaxyl-M and the opportunities for alternation between different products have greatly diminished.

This has increased the risk of fungicide resistance developing in pathogen populations and in the horticultural sector there have been some prominent examples of this. One of the most recent of these examples has been the development of metalaxyl resistance in *Plasmopara obducens* (the cause of downy mildew in *Impatiens walleriana*) which has been spread around the globe on vegetative cuttings and now the lack of effective alternative fungicides for control has effectively 'persuaded' the industry to halt production of *Impatiens walleriana* in many parts of the world.

Delivering active ingredient to the parts of host plants where it is needed is another potential problem when considering root-infecting oomycete diseases, possibly necessitating the use of systemic chemicals and thereby reducing choice and consequentially increasing the selection pressure for the development of resistance against these products. However, the situation regarding pathogen resistance amongst root-infecting oomycetes is not clear-cut and if anything there are fewer examples of resistance in oomycete root pathogens than there are in foliar-infecting oomycetes. For example, in cavity spot of carrot caused by *P. violae* the risk resistance developing appears to be much lower than for the downy mildews or *Phytophthora infestans,* as even after some 20-30 years of repeated use at high levels on carrots resistance to metalaxyl-M has not been detected.

This is made more remarkable by the fact that for treating carrots single active ingredient formulations of Metalaxyl and then Metalaxyl M have been used since the research of Geoff White's group at HRI Wellesbourne (White, Wakeham & Petch, 1992) showed that metalaxyl formulations containing mancozeb were deleterious to field populations of *Pythium oligandrum*, one of several mycophagous species of *Pythium* mentioned earlier in this review (see 'Oomycetes as pathogens') that was demonstrated to provide some degree of natural disease suppression in carrot fields.

The lack of resistance development in root-infecting oomycetes is not necessarily true for all pathogenic species, for example in tests of 72 isolates from carrots from around the UK no metalaxyl resistance was found in *P. violae* isolates whilst all *P. sulcatum* isolates showed some degree of tolerance and one showed resistance (AHDB Horticulture FV5f, Hiltunen *et al*., 2002) though without validated baseline data we cannot be certain whether the *P.sulcatum* population was inherently more tolerant to this particular fungicide.

This observation of a possible differential resistance risk may be linked to the increased opportunities for mutations and selections that result from the rapid turnover of generations associated with asexual sporulation. In the case of the airborne oomycetes many generations of spores are produced over very short periods, and whilst *P. sulcatum* produces zoopsores, *P. violae* is considered non-zoosporic (van der Plaats-Niterink, 1981; Robideau *et al*., 2014).

It is of course important to recognise that novel products continue to be developed by the agrochemical & bio-control industries so the situation is not static. Product availability will change over time both in terms of revocation of existing active ingredients through regulatory reviews etc and through authorisation of new substances & products. It is therefore important to keep abreast of developments in this area to capture the full benefit of their use.

The particular problem at the moment is that most commercial approvals in the horticultural sector relate to use against foliar pathogens, particularly downy mildew. There does need to be greater emphasis on efficacy & crop safety studies against the

important root-infecting oomycete pathogens in future with a special focus on rates of use in the soil, substrates and hydroponic growing systems to ensure that appropriate data is available to assist with the approval process.

Biological control

Despite many decades of research into the possibilities of biological control of soil-borne oomycete plant diseases (Van Luijik 1938), there are still very few commercially successful examples against diseases caused by *Pythium, Aphanomyces or Phytophthora*. One example is Prestop (*Gliocladium catenulatum* strain J1446), which is approved for use in the UK and has a label recommendation for the control of damping-off and root diseases caused by *Pythium* spp. and has been shown to be effective against *Pythium ultimum* and *Rhizoctonia solani* damping off both when mixed with new growing medium or applied as a drench (McQuilken et al., 2001).

The biological control of *Pythium* was reviewed by Whipps & Lumsden (1991), since then a great deal more work has been done although many of the barriers to successful commercial uptake still remain. Inconsistency in comparison with equivalent agrochemical preparations is the main problem with the use of biocontrol agents (BCAs) for the control of oomycete stem and root rots. Partly this higher level variation is driven by the increased level of complexity of a system involving the activities and environmental responses of host organisms, pathogens and BCAs and crucially there is still insufficient knowledge on the mechanisms of action of pathogens, as well as biocontrol agents (Timmusk *et al.,* 2009). Unfortunately as indicated above, to date, there are very few effective biological control agent products with activity against oomycetes on the market in the UK.

However, after many years of largely uncoordinated effort, the HortLink 'SCEPTRE' programme was set up. Working closely with the agrochemical and bio-control industries, this programme has started to evaluate low risk conventional chemicals & bio-pesticides in efficacy & crop safety studies in a more structured and coordinated manner; though importantly the focus in this project is on edible crops only and ornamentals have been excluded. To address this, another AHDB Horticulture project has been commissioned to fund a parallel ornamentals study (MOPS - Managing Ornamental Plants Sustainably) adding to the valuable database of efficacy and crops safety knowledge in SCEPTRE. The data from these important projects, together with other *ad hoc* studies in the UK and elsewhere will help identify gaps in current knowledge on the control of oomycete root pathogens.

It is also important to note that the Sustainable Use Directive (http://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides/index_en.htm) already (since 1st January 2014) requires growers in the EU, including the UK, to develop an IPM plan on the nursery prior to any decision to use a pesticide product for pest or disease control. For the SUD to be implemented successfully it is imperative

that growers have a wider range of efficacious bio-control products, that ideally have been independently evaluated to demonstrate robust efficacy & crop safety. It will also be important to investigate opportunities for integrated use of conventional chemical products with bio-control products as, in some cases; such mixed use could be counter-productive.

Activity of potential micro-organism based plant protection products (PPP) against oomycete phytopathogens

Although the number of approved micro-organism products available with label activity against oomycetes is small and there can be problems with variability in performance, there are still a number of potentially useful micro-organism both those PPPs approved for other plant pathogens that may be effective against oomycetes and some micro-organisms yet to be developed and approved[1] For example certain preparations of *Bacillus amyloliquefaciens (*formerly *B. subtilis)* which are also known to be able to induce systemic resistance in host plants to a range diseases/pathogens including *Pythium* damping off and *Phytophthora* late blight in tomatoes (Yan *et al*., 2002; Kloepper *et al*., 2004), and even act as antagonists by producing anti-fungal volatiles (Fiddaman & Rossall, 1993). However, disease suppression by *B. subtilis* is inconsistent (Gilardi, *et al*., 2014), and populations can fail to establish as found in hydroponic tomato systems at HRI despite repeat inoculations (Pettitt *et al*., 2002b).

Maintaining stable populations of mcro-organism PPPs above the threshold of their efficacy is key to obtaining effective disease control (Pagliaccia et al., 2007), although the actual value for such thresholds and how they might be expressed in terms of distributions on plant surfaces and within the rhizosphere is not clearly understood. With the plant growth-promoting rhizobacterium *Paenibacillus polymyxa*, antagonism against *Pythium* and *Phytophthora* zoospores and consequent protection to host plants, was best maintained by isolates that were capable of establishing rhizosphere biofilms by the active secretion of a mucoidal substance, regardless of the production of actively antagonistic compounds (Timmussk *et al*., 2009). Other well-known groups of bacteria with oomycete disease-suppressive activity, including *Bacillus* spp. and *Pseudomonas* spp., are also important biofilm producers/colonisers (Bais *et al*., 2004; Couillerot *et al*., 2009). In both of these genera the production of lipopeptides is vital for the attachment of biofilms to surfaces, the initiation of biofilm layers and the maintenance of the liquid-filled channels that facilitate the distribution of nutrients and oxygen needed for an effective film structure (Ron & Rosenberg, 2001; Raaijmakers *et al*., 2010). These are not the only functions of these compounds which are also classed as biosurfactants, some with quite potent anti-oomycete activity (van de Mortel *et al*., 2009).

Biosurfactants are one of a number of categories of compounds produced by micro-organisms that are classed as allelochemicals (Saraf *et al*., 2014). Allelochemicals are compounds produced by living organisms that exert a detrimental physiological effect

[1] Micro-organisms used for plant protection must be registered.

on individuals of another species when released into the environment and **Table 9** illustrates the main categories that have activity against oomycetes with examples of each. A wide range of allelochemical interactions has been recognised and the earlier literature has been summarised by Martin and Loper (1999). These authors also listed the bacteria *Burkholderia cepacia* and *Enterobacter cloacae,* in addition to *Bacillus* spp. and *Pseudomonas* spp., as having anti-oomycete activity as well as the fungal genera *Trichoderma* spp. (e.g. Benítez *et al*., 2004), and the already mentioned *Gliocladium* spp. In addition to these groups, the Gram-negative bacterium *Serratia marcescens* has been assessed for suppression of *Pythium ultimum* damping-off in cucumber (Roberts *et al*., 2007), whilst colonisation of the roots by glomeromycotan (Arbuscular mycorrhizal fungi (AMF)) species of mycorrhiza can protect plants from infection by oomycetes, although only fully-established and compatible mycorrhizal associations are likely to be effective (e.g. Slezack *et al*., 2000; Monaghan *et al*., 2002). In addition to the production of allelochemicals, oomycetes can be controlled to a certain extent by competition for resources in the rhizosphere as well as having their chemotactic 'prompts' quenched by metabolites from 'antogonists' (Islam & Tahara, 2001). And, as mentioned above (see 'Oomycetes as Pathogens' section), some fungi and oomycetes are mycophagous (or even oomycetophagous?), most prominently *Trichoderma* spp. (Papavizas, 1985) and *Pythium oligandrum* (Martin & Hancok, 1987). *P. oligandrum* has also been found to protect its hosts by eliciting host resistance by secreting tryptamine (an auxin-like compound) and a glycoprotein – oligandrin (Vallance *et al*., 2009).

Table 9: *The main categories of allelochemicals with activity against oomcete root and stem rot pathogens illustrated with references to selected examples.*

Allelochemical category	**Putative biocontrol agent & process**	**Oomycete target**	**References**
Siderophores	*Pseudomonas aeruginosa* 7NSK2 Pyoverdin, pyochelin and salicylic acid	*Pythium splendens*	Buysens *et al*. (1996)
	Trichoderma Iron availability	*Pythium*	Benítez *et al.* (2004)
	Burkholderia cepacia AMMDR1	*Pythium aphanidermatum* but NOT *Aphanomyces euteiches*	Heungens & Parke (2000)
Antibiotics	Fluorescent	*Phytophthora capsici*	Arora *et al.*

Allelochemical category	Putative biocontrol agent & process	Oomycete target	References
	Pseudomonas PGC2		(2008)
	Bacillus and *Pseudomonas* spp. Lipopeptides with antibiotic activity – also involved in motility and biofilm establishment	*Oomycetes et al.*	Raaijmakers *et al*. (2010)
	Pseudomonas fkuorescens SBW25 Cyclic lipopeptides with antibiotic acvtivity	*Phytophthora infestans*	De Bruijn *et al*. (2007)
	Pseudomonas fluorescens; P. chlororaphis; P.aeruginosa Phenazine-1-carboxylic acid (PCA)	*Pythium sp.*	Perneel *et al*. (2008)
Lytic enzymes	*Trichoderma harzianum* T28 Over-expression of BGN13.1 gene for β-1,3-glucanase activity	*Phytophthora citrophthora*	Benítez *et al*. (2004)
Volatile metabolites	*Trichoderma harzianum* and to lesser extent *T. viride* and *Bacillus thracis*	*Pythium aphanidermatum*	Christy Jeyaseelan *et al*. (2012)
Biosurfactants	*Bacillus* and *Pseudomonas* spp. Lipopeptides disrupting zoospore membranes as well as acting as	*Oomycetes et al.*	Raaijmakers *et al*. (2010)

Allelochemical category	Putative biocontrol agent & process	Oomycete target	References
	antibiotics		
	General review in relation to irrigation water treatment	Zoosporic oomycete plant pathogens *et al.*	Hultberg & Alsanius (2014)
	Pseudomonas fkuorescens Cyclic lipopeptide massetolide A – disrupting cells but also eliciting plant resistance responses	*Phytophthora infestans*	Tran *et al*., (2007)

A key area where great advances are being made is in the science of chemical signalling for example the elicitation of disease resistance (Zhang, Dick & Hoitink, 1996; Kloepper, Ryu & Zhang, 2004; Bakker *et al*., 2013; Saraf, Pandya & Thakkar, 2014) and more intriguingly co-operative signalling between plants and microbes (Droque *et al.,* 2013) almost deciding who lives where! Indeed, it is now becoming apparent that plants engage in a far more active role in in the dynamics of rhizosphere microbial communities; actively and selectively excreting large quantities of sugars organic acids and amino acids into the soil (Doornbos *et al*., 2012). Up to 40% of a plant photosynthetically fixed carbon is secreted into the rhizosphere (Bais et al., 2006) in a set of processes that are now considered not to be passive (Loyola-Vargas *et al*., 2007) and in compositions that vary with species, growth stage, growing substrate and levels of stress (Uren, 2000) and is also influenced to some degree by 'feed back' from the rhizosphere microflora (Kamilova *et al*., 2006). Certain groups of compounds, for example the strigolactones appear to play a role in very specific interactions between plants and specialised groups of micro-organisms. In this case the secretion of strigolactones by plants stimulates AMF fungi to form associations with their roots (López-Ráez *et al*., 2012); AMF in turn can induce systemic resistance mechanisms in plants (Vos *et al*., 2012). The comparatively large quantities of organic compounds exiting plant roots and present in the rhizosphere also have a spectacular impact on the population densities and the biodiversity of the microflora (Whipps, 2001; Raaijmakers

et al., 2009), the composition also appears to be 'regulated' by the host plant to some degree and remains remarkably stable (Postma *et al*., 2005; Calvo-Bado *et al*., 2006; Hunter *et al*., 2006; Rosberg *et al*., 2014). Nevertheless, changes in the dynamics of rhizosphere communities can be achieved by agronomic activities such as adding composts, and sometimes by the addition of new micro-organisms (Kowalchuk, *et al*., 2003; He *et al*., 2012; Vallance *et al*., 2012)

Cultural control

Under this heading a number of different practices are considered, which together provide a basis for 'best practice', and many of which might be considered plain 'common sense'. These include simple considerations of water management, treatments to encourage natural disease suppression, using soil and media additives, pathogen avoidance and the use of barriers, and the use of hygiene and physical eradication measures.

As oomycetes require free water to successfully infect their hosts, it is best to set up production systems that reduce exposure to free water to a minimum. Situations where roots remain in contact with water, especially still water, provide ideal conditions for infection by zoosporic species and should be avoided. Plants need to be well drained, and if growing in soils,hard layers and pans need to be broken up to avoid standing water or waterlogging in the soil as these conditions can encourage the release and geotactic swimming of zoospores from inoculum dispersed within the soil profile. Persistent puddles need to be eliminated as these often harbour and maintain infectious propagules (White *et al*., 1998). Flood irrigations need to be optimised and set to avoid waterlogging, taking extra caution when beds contain a mixture of container sizes. As stated above ('Treating water to control oomycete disease spread' section), irrigation water itself is a serious potential source of oomycete pathogen inoculum and should be tested for pathogen presence, although this source of infection is readily eliminated.

Cultural practices that might encourage or maintain natural disease suppression are still not well understood, causing some to advocate maintaining 'active' sterilisation treatments wherever feasible within the production cycle. Unfortunately, even the impacts of this approach, which potentially destabilises the microflora of the rhizosphere, are fascinating but not well understood. Practices designed to encourage or establish biofilm function or stability under some circumstances could greatly improve the reproducibility of biological control (Timmusk *et al*., 2009) or natural disease suppression (e.g. McPherson *et al*., 1995). For example the use of capillary beds like the 'Efford sand bed' (Scott, 1984), biofiltration of irrigation water or the use of certain media additives (e.g. biochar) will all encourage the development of rhizosphere populations of beneficial micro-organisms. However, our knowledge of biofilm types is still limited and certainly in some circumstances, such as irrigation lines, the type of biofilm and its build up is highly undesirable (Pachepsky *et al*., 2011)

Making additions to growing media, such as composted hardwood bark (Kuter *et al*., 1983), are known to reduce *Phytophthora* infection and disease by encouraging natural antagonists like *Trichoderma* spp., as well as releasing natural inhibitors and improving the air-filled porosity and favouring young root growth. Additions of various types of composted materials have given highly variable results; vermicomposts apparently have oomycete disease suppressive effects (Jack, 2010) as do certain types of composted waste (e.g. Mandelbaum & Hadar, 1990; Noble & Roberts, 2004; Chen & Nelson, 2008), although some inconsistency in suppression from composted green wastes has been recorded – a problem possibly linked to inconsistent composting process parameters (Vestberg *et al.*, 2014). Many composts are known to contain populations of micro-organisms that are naturally disease suppressive and/or that stimulate host plant resistance mechanisms (Zhang *et al*., 1996). Additions of organic matter to soils do improve the general soil 'health' parameters (Janvier *et al*., 2007) and often give reductions in disease expression.

Biofumigation, largely concentrating on either growing certain brassica species and then ploughing them in or by incorporating brassica seed meal, operates by the hydrolysis of glucosinolates in the brassica tissues to release biocidal isothiocyanates (Sarwar *et al*., 1998). Other green manure approaches have been considered and some have shown promise (e.g. incorporation of Acacia foliage in *Faba* bean field soils in Uganda does reduce the severity of *Pythium* bean root rot), but brassica manures seem to give the best results in terms of biofumigation (Wiggins & Kinkel, 2005). Brassica seed meal, a byproduct of the food and oil extraction industries with a high glucosinolate content and stable shelf-life, appears to be the most effective material for this process (Mazzola & Zhao, 2010). Treatments with this material have given good control of oomycete diseases in a range of crops, for example in strawberries (Porras *et al*., 2009), and seed meal of *Brassica juncea* gave good inhibition of a range of *Phytophthora* species (*P. cactorum, P. cinnamomi, P. citricola, P.cryptogea* & *P. megasperma*) in comparative experiments by Dunne *et al*. (2003). In addition to the shorter-term impact of isothiocyanates, Muditha et al. (2012) noted a longer-term effect of using *Brassica juncea* seed meal amendments for control of *Pythium abappressorium* when antagonistic fungi (predominantly *Trichoderma* spp.) recolonized the treated soil after the isothiocyanate effect had worn off. On-going research work at Wellesbourne in the UK using green manuring of mustard has shown promise for the control of *Pythium violae* carrot cavity spot (Clarkson, 2014), but it has also revealed many practical problems and variables in the operation of biofumigation, especially by this technique which relies on cropping conditions to obtain sufficiently high concentrations of glucosinolates at the right time.

High temperatures can eliminate oomycetes (e.g. see **Table 4** above). Steam treatments are very effective at treating production beds and even outdoor soils (White, 1999; Pettitt, 2001; Linderman & Davis, 2008). The steam acts as both a biocide and a soil improver by opening up the soil structure, and it can often give new plantings a boost of thermal time by raising the soil temperature by a small fraction resulting in

stronger initial root establishment. Solarisation can be a very effective treatment in warmer and sunnier parts of the world (Porras *et al*., 2009) but is generally less effective in the UK due to insufficient sunshine of sufficient intensity!

Pathogen avoidance and cultural eradication are processes that can make sense on paper, but components of which are often difficult and potentially costly to implement, as they require constant vigilance to maintain. Avoidance can be achieved at several different levels. At the highest level this involves restrictions on the movement and quarantining of potentially diseased planting materials and seeds. Wherever possible, it is best to use certified and passported planting materials and even with these, it is advisable to carry out checks/tests and far as is feasible (often not at all!) to keep new planting material separate or even 'quarantined' from the rest of the nursery. This is because even high grade planting material schemes can be (admittedly rarely) compromised at times by cryptic oomycete pathogens (e.g. *Phytophthora cactorum* crown rot had to be eliminated from the super-elite virus-free strawberry stocks in the early 1980s; Harris & Stickels, 1981). New containers of growing media are normally clear of oomycete propagules, but it is vital to keep opened containers covered and if in doubt, to test suspect materials before deploying them widely. Obviously, where possible, contaminated ground should be avoided and measures to avoid contact between roots and contaminated ground should be taken. Other common routes of entry for oomycete root rots are stressed and pot-bound root systems – even small delays in potting-on can greatly increase *Pythium* root rot. Top-heavy plants should not be allowed to fall over, especially if there are areas of standing water and puddles.

Text book definitions break cultural eradication into three components: rotation, use of sterilants (and/or steaming) between crops, and general hygiene. Rotation is rarely feasible and will achieve only partial removal of oomycete pathogens at best. Rotations are best achieved with annual plantings that can be rotated with non-susceptible crops, and are more likely to work with *Phytophthora* spp., as these have poor saprophytic capability compared to other oomycete species and are thus less able survive long in large numbers without a host.

The use of sterilant chemicals to keep equipment, beds, tools, containers and irrigation lines clean between crops is a highly effective measure for reducing and even eliminating oomycete disease problems. Many effective sterilant chemical products have been withdrawn recently, although two highly effective materials (PAA {Jet 5} and hypochlorite bleach – White *et al*., 1998) remain available. This is a subject outside the scope of this review as it is being well covered by the SEPTRE and MOPS programmes of work, where some interesting new materials and processes (e.g. use of foams) are being investigated (Hough & Wedgwood, 2015).

Good routine hygiene is facilitated by the use of effective sterilants and still remains the backbone of any effective oomycete disease control campaign. *Pythium* and *Phytophthora* propagules are readily spread on and between nurseries on footwear, media & plants, tools, Danish trollies and vehicle tyres (Al-Sa'di *et al*., 2007; White *et*

al., 1998). It is essential to keep equipment, beds/benches and containers clean throughout cropping cycles and where possible, to eliminate standing water and puddles – a very common cause of *Pythium* spread is *via* hose lines contaminated by having been left on the floor, lying in contaminated standing water. In an AHDB Horticulture funded project, the incidence of *Pythium* infection on protected ornamentals nurseries was reduced by >70% in 12 months by the implementation of a simple programme of cleaning benches, potting machines and Danish trollies and executing a policy of 'zero tolerance of puddles' (Pettitt *et al*., 2001).

RESEARCH GAPS

A great deal is known about oomycete pathogens of horticultural crops, and there are many options currently available to growers for their detection, avoidance, management and control. Nevertheless, there remain key areas where current knowledge is lacking, either fundamentally or in all-important detail. These gaps in our understanding have been grouped here under general headings; Diagnostics, Inoculum and disease risks, Control Strategies and Costs of water treatment, although the key themes of diagnostics and inoculum recur across all of these groups - most notably the need for accurate, economic, and reliable high throughput identification and quantification of oomycete pathogens in plants, media and water.

Diagnostics

Water, soil, growing media and plant tests for viable oomycete inoculum still need to be improved for sensitivity, specificity, reliability, speed and cost.

Despite massive and on-going improvements and reductions in costs in recent years, especially in molecular diagnostics, accurate identification of oomycetes to species is still relatively expensive and time-consuming. The number of recognised species and/or 'phylotypes' continues to increase rapidly, although the proportion of these that will be of immediate importance to commercial horticulture is debatable. The development of the capacity for reliable rapid multiplex testing to species level is important. This level of precision may never be needed for the purposes of effective oomycete disease management, but is vital to help researchers answer one of the key questions currently asked by growers: '*Phytophthora* or *Pythium* has been detected, what disease management steps should be taken?'

Current practice would be to recommend a pathogenicity test be carried out if the putative pathogen has been isolated, otherwise application of immediate control measures would likely be recommended in the event of detecting *Phytophthora* sp., whereas the immediate response to a *Pythium* sp. positive test would be more ambivalent unless this was linked to plants showing unequivocal symptoms. This is because most *Phytophthora* species currently known are plant pathogens, and whilst different species have different host preferences and host ranges, it is assumed that mere presence of detectable inoculum is an indication of potential trouble.

On the other hand, a large proportion of the 200 or so species of *Pythium* are saprophytic or certainly not known to be pathogenic to any horticultural crop, and at least four species are even mycophagous, some with the capacity to elicit disease resistance mechanisms in plants (Vallance *et al*., 2009) and therefore even potentially beneficial. Rapid *in situ* diagnosis to genus level is currently possible using commercially-available ELISA-based LFD test kits (e.g. Alert LF™ kits, Adgen Phytodiagnostics and Pocket Diagnostic® kits, Forsite Diagnostics). Whilst of some help, these tests are unfortunately limited by their lack of specificity and the potential

cross-reaction of the antibodies used with some non-target species of closely related oomycete genera leading to some 'false-positives'. A new AHDB Horticulture funded project (CP136) is aimed at improving this situation by developing new monoclonal antibodies for LFD kits, raised to selected specific species of *Phytophthora* and *Pythium*. Most currently known *Phytophthora* species and many *Pythium* species can be identified using well-defined PCR methods (e.g. Cooke *et al*., 2000, see 'Oomycetes detection & diagnosis' section above). Multiplexed real-time PCR can be used to identify and quantify several species in one sample (Schena *et al*., 2006), although this procedure is still somewhat limited in the number of species testable in one sample by the number of dyes that can be deployed together (Cooke *et al*., 2007).

In some situations, especially the testing of raw water sources such as reservoirs and rivers and of new field soils, the capacity for a wider multiplex testing capability would be highly desirable. In addition to these ideals for test improvements, it is clear that a definition/statement of the 'hierarchy of testing' and of the 'right' questions to ask and interpretation of results is needed – possibly in the form of a factsheet as part of AHDB Horticulture project CP128.

Inoculum and Disease Risks

As outlined above, accurate, reliable, specific, fast and economic quantification of oomycete pathogen inoculum used together with good measurements of plant infection and symptom development is vital in generating the type and amount of data needed to gain a proper understanding of disease dynamics and assessing the impacts of fungicide, biological and cultural control treatments. Whilst there is a large amount of information available on airborne oomycete diseases, such data is very scarce when it comes to soil borne oomycete pathogens and virtually non-existent for waterborne inoculum.

Despite a few exceptions (e.g. *Phytophthora* in citrus orchards and *Pythium* in AYR chrysanthemum beds), there are very few examples of frequent assessments (in time and space) of pathogen inoculum in soil or in irrigation water collected in direct association with accurate and related measurements of infection and symptom severity. More importantly, studies assessing the distribution of propagule type are virtually non-existent. This is not surprising as it is difficult and time consuming to carry out.

However it is potentially very important as different propagule types have potentially different inoculum potentials and different potential for spread and survival. It is also important that good records of host physiological factors are recorded as these can have a big influence on disease outcomes, for example in the case of *Pythium sylvaticum* in chrysanthemum where a visible disease inoculum threshold was determined at 2000 cfu g^{-1} but symptoms where only seen at this threshold in autumn and spring (Pettitt et al., 2011), similarly in hydroponic tomatoes infected with *Pythium aphanidermatum* and *P. helicoides* (Li *et al*., 2014) reported root rot symptoms only when zoospore inoculum pressure coincided with the plants experiencing certain

environmental factors. The linking of inoculum concentration with host physiological and environmental factors is crucial to being able to develop the concept of disease thresholds and to help take us past the current situation of remedial action being based simply on presence/absence of pathogen inoculum.

Control Strategies

Biofilms

Biofilms play an important role in disease-suppressive systems, both within specially-constructed water treatment apparatus like slow sand filters and in the rhizosphere of crops. And yet, biofilm formation can be highly problematic in irrigation rigs, causing blockages and harbouring potential human pathogens and allegedly even *oomycete pathogen propagules*.

Whilst a good deal of research effort has be applied to the microbiology of irrigation systems (e.g. Postma *et al*., 2005; Calvo-Bado *et al*., 2006; Vallance *et al*., 2010), there has been very little attention paid to the study of the biofilms within these systems from reservoirs to pipework, drippers and nozzles. The composition, deposition and succession of species of such biofilms and how much this varies with environmental conditions and their capacity (or otherwise) for harbouring plant pathogens such as oomycetes is not understood and warrants in depth study; a) to identify whether there are there different types of biofilm ('good' or 'bad'?), b) to determine the potential disease risks and c) investigate potential for, and impacts of sustainable biofilm management including the possible use of strong oxidising agents such as chlorine dioxide or hydrogen peroxide. On species composition, one of the key factors determining biofilm development is the availability of nutrients and it has been suggested that biofilm heterogeneity is inversely related to the nutrient concentration (Møller *et al.,* 1997; Heydorn *et al*., 2000), which might explain the relatively high biodiversity in normally nutrient-sparse slow sand filter biofilms.

Novel control strategies for water

There are a number of what appear to be effective approaches to treating irrigation water that utilise aspects of natural disease suppression. These include SSF, Iris beds and various 'improved' biofilters and capillary mat systems. Observations and tests of such systems on nurseries show great promise, but many of these systems (except SSF) have not been exposed to fully quantifiable pathogen challenges and therefore the limits of their efficacy are not understood and they cannot be fully optimised.

With many bio-filtration-type systems, flow rates (slow) and water quality parameters and the perception of potentially heavy maintenance commitments are limiting factors to wider uptake by the industry. Recent exciting developments with SSF using coarser, sub angular grits, and the interesting chemistry of china-clay-waste sands have led to possibly increased flow rates and significantly reduced filter blockage. Further work is needed to properly understand and optimise the deposition of biofilm on this material which appears to support much more microbial activity per unit surface area than a,

'conventional', quartz SSF sand. Flow rates through constructed wetland and Iris bed systems are not at all understood and neither is the potential function/role of the weirs normally incorporated in these installations. Nevertheless, they appear to remove oomycete plant pathogen propagules although the mechanism(s) for this removal are not known – is it a similar process to that seen in SSF and capillary systems? Or possibly inoculum reduction is a function of increasing the distance through which pathogen propagules have to pass as seen in runoff collection ponds (Hong *et al*., 2003; Ghimire *et al*., 2011) and attributed to a *natural* decline in zoospore numbers in such environments (Kong & Hong, 2014).

Natural disease suppression

This area overlaps with the biofilms and novel control strategies for water areas. There is still much to be gained from the use of naturally suppressive components to growing media (e.g. composted bark) as well as ingredients that might support increased disease suppression (e.g. biochar). The literature on this subject is vast but mostly rather serendipitous, although some researchers e.g. Harry Hoitink (Ohio State University) have tried to standardise their findings to make them practically applicable.

There is a strong need to develop reliable measures that can be easily applied to growing substrates, as well as possibly components of growing systems that can give an indication of potential for natural disease suppression (e.g. like using the FDA assay (Hoitink & Boehm, 1999)) and soil/growing medium 'health' (Janvier *et al*., 2007). To become truly reliable for practical purposes, work in this area is strongly reliant on successful diagnostics to give a clearer understanding of the behaviour of inoculum under suppressive conditions and therefore consequent disease risks. More research is needed on the development of conditions conducive to the development of natural BCA populations and integrating this with the possibility of introductions of commercially-available organisms.

Independent assessments of limits of efficacy and phytotoxicity

Considerable interest has been generated recently in chlorine dioxide and in hydrogen peroxide treatments for irrigation water.

However, there is limited scientific data on both efficacy and phytotoxicity for both of these products and claims made by commercial chlorine dioxide producers need to be independently verified for safety of both personnel and crops. 'Is all chlorine dioxide created equal?' (Gordon, 2001). Proper CT data for chlorine dioxide and for both silver chelate- and formic acid-stabilised forms of hydrogen peroxide, to independently determine the limits of efficacy and phytotoxicity using approaches similar to Corradini and Peleg (2003). One of the factors that make the use of these strong oxidising agents attractive is their capacity to remove biofilm from irrigation lines. However, this needs to be treated with caution as this same property runs the risk of eliminating or reducing the natural disease suppression or disease-buffering that might be present as well as introduced bio-control agents in production systems and growing media.

Progress in this area needs to be tempered by the findings of biofilm, natural disease suppression and novel control strategy research.

Fungicides, Disinfectants & BCA formulations

The main problems in this area in the medium and short term, is not a lack of promising candidates but their availability and registration for use. This issue is the province of other AHDB Horticulture research & development programmes; SCEPTRE, MOPS and IMPRESS and is outside the scope of this review.

Costs of water treatment

No full comparisons have been made between all water treatments for potential energy consumption and operating costs.

Some water treatment approaches can carry potentially hidden costs in terms of things like extra pumping or possibly increased staff time spent monitoring or carrying out maintenance. There is increased awareness of energy consumption in the water sector (Rothausen & Conway, 2011), whilst some limited comparisons have been carried out by Pettitt & Hutchinson (2005), Atwood (2014) and Fisher (2014).

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WEBSITES

The EU Sustainable Use Directive (SUD)
http://ec.europa.eu/food/plant/pesticides/sustainable_use_pesticides/index_en.htm

Diagnostics equipment, assays, kits & advice:

Lateral-flow test strip readers
www.charm.com/instruments/instruments-rosa-reader

www.vicam.com/vertu-lateral-flow-reader

www.quiagen.com (ESE-Quant Lateral Flow System – Quiagen)

http://www.skannex.com

LFD Test kits
www.neogen.com

http://www.envirologix.com

www.pocketdiagnostic.com

http://www.envirologix.com

DNA- based verification of LFD diagnoses
www.fera.defra.gov.uk/plants/publications/documents/factsheets/pramparks.pdf

Components for improving field immunodiagnostic test efficacy
www.millipore.com/diagnostics

www.whatman.com/DiagnosticComponents

Nucleotide sequence databases
www.phytophthoradb.org

www.phythophthora-id.org

www.q-bank.eu

www.boldsystems.org

Bio-art bvba (Belgium) multiplex DNA diagnostics system - DNA MultiScan®, http://www.bio-art.org

Water treatment companies:

Clearwater	www.clearwater.eu.com	ClO_2 *Legionella* control	Irrigation
Drenntag Clorious 2	www.clorious2.de	Irrigation	
Dupont	www2.dupont.com/Chlorine_Dioxide_Solutions		
GlobalEx	www.globalex-world.com	ClO_2 drinking water – general disinfection	
Hydromax Chlorine Dioxide	www.green-tech.co.uk	Irrigation	
Lenntech BV	www.lenntech.com	Water purification , *Legionella* control	
ProMinent	www.prominent.com	ClO_2 drinking water	
ProWater Ltd	www.prowater.co.uk/WaterTreatment	*Legionella* control	
Siemens	www.usfilter.com	THM control	
Ximax	www.ximaxes.com	ClO_2 for irrigation Drinking water approved	
Certis (Jet 5)	www.certiseurope.co.uk/	Factsheet Jet 5 tech update 0612.pdf	

Flowering Plants Ltd Manchester Filter System (biofiltration) http://www.fpl.irrigation.com/mf01.htm

Education Resources:

APS Introduction to Oomycetes: http://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/IntroOomycetes.aspx

Greenhouse and Nursery Water Treatment Information System, University of Guelph, Canada. http://www.ces.uoguelph.ca/water/

Water Education Alliance for Horticulture, University of Florida, USA. http://watereducationalliance.org/education.asp

