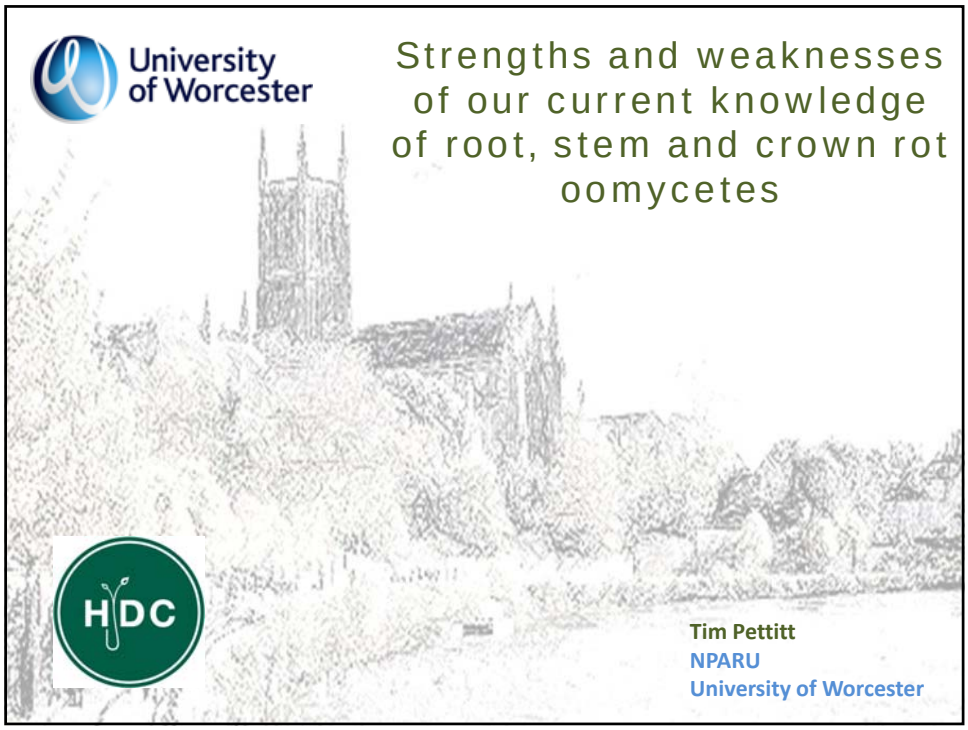
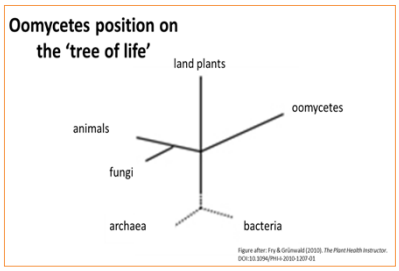


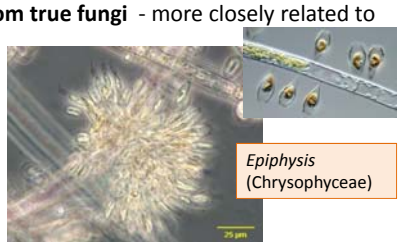
Strengths and weaknesses of our current knowledge of root, stem and crown rot oomycetes

- What are oomycetes?
- Oomycete root and stem diseases in horticulture
- Life-cycles & Ecology
  - Inoculum: spore types & behaviour
  - Dispersal, Survival & Infection
- Disease Risks - predisposition, disease thresholds & inoculum potential
- Diagnostics
- Management & Control

What are oomycetes?

- Large group of **'Fungus-like micro-organisms'** – resemblance is superficial
- Representatives in virtually all terrestrial, marine and freshwater habitats
- Many spp. are saprophytes but significant proportion are pathogens of wide range of plants and animals including humans
- **DNA studies show they are quite distant from true fungi** - more closely related to golden algae (Chrysophyceae) and diatoms

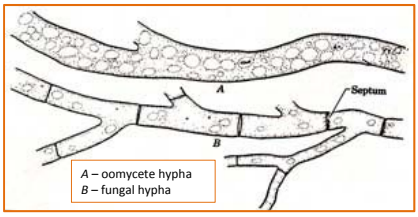


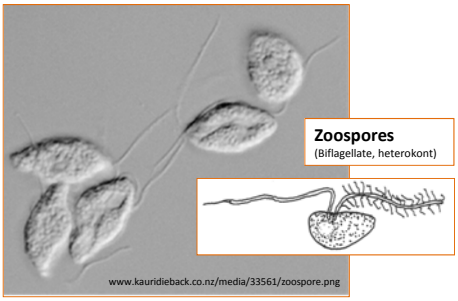


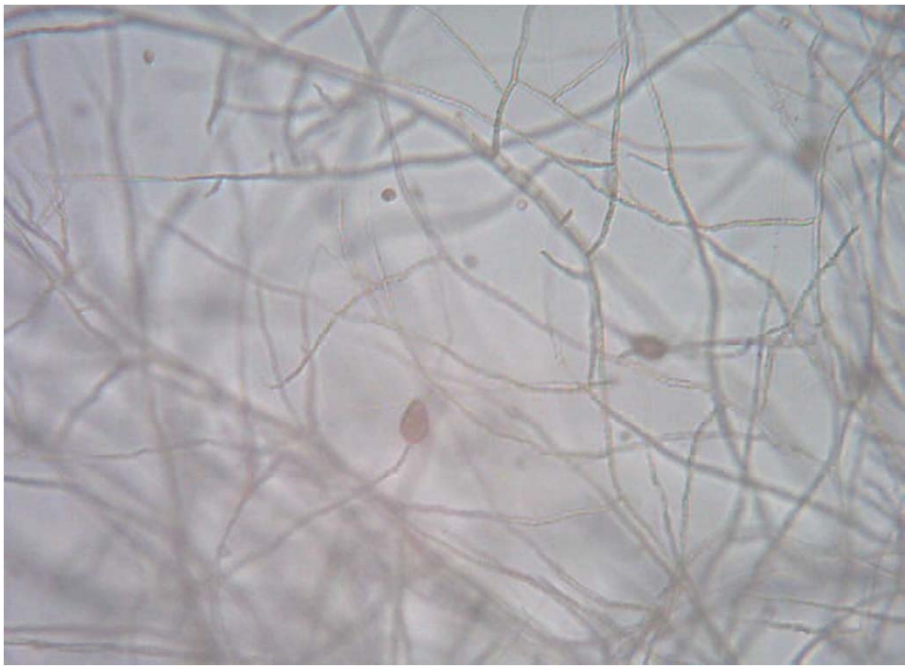
Epiphysis (Chrysophyceae)

What are oomycetes?

- **Oomycetes are NOT fungi**
- There are a number of fundamental biochemical differences - **probably the most important is that oomycete cell walls contain cellulose and β- glucans whilst fungal walls are chitin**
- **Oomycete hyphae are non-septate – lacking cross walls most fungi have abundant septa –** this helps with recognition under microscope
- The majority of oomycetes produce a very special motile swimming spore – **the zoospore** – which has important characteristics shared with closely related groups but NOT the fungi







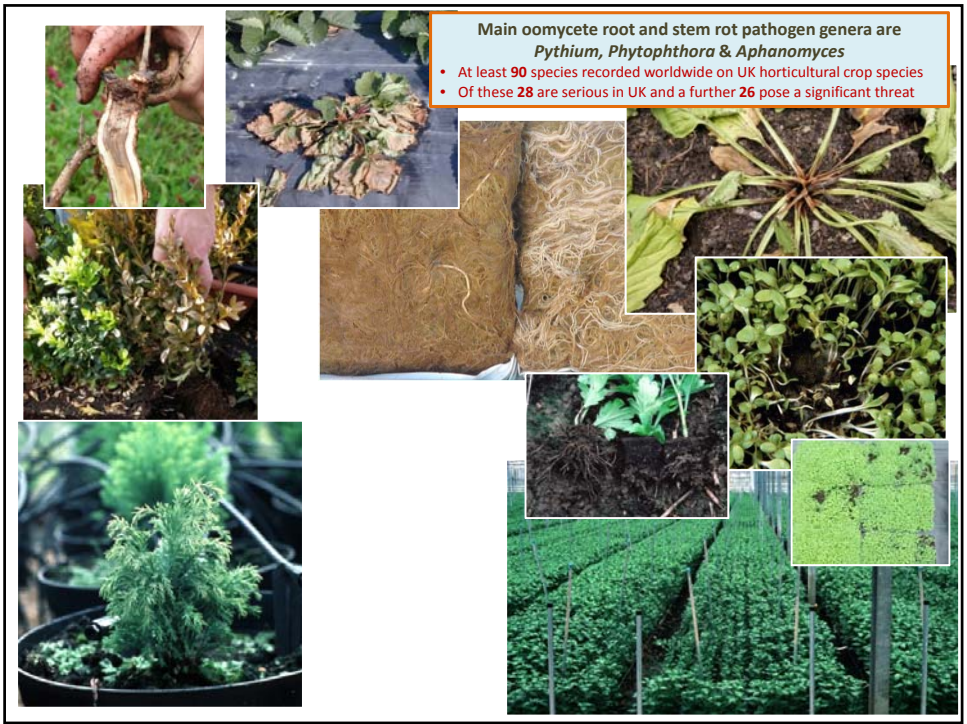
Strengths and weaknesses

Research on understanding the origins etc. of oomycetes has been strong.

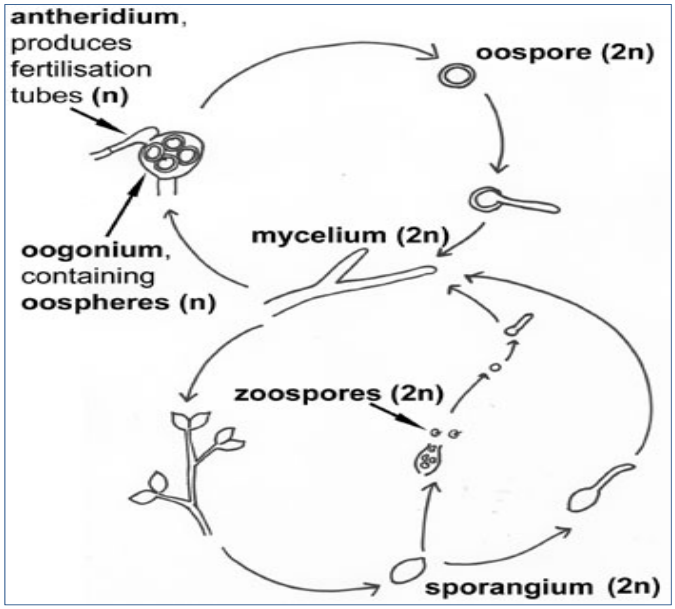
- Much excellent work carried out on phylogeny (understanding their place on the tree of life) and molecular diagnostics over last 15 years
- Many new species have been discovered and a good basis for understanding their complex interrelationships
- Most research has focussed (justifiably) on the 'threats' (novel pathogens – implication = 'all germs are bad'). Not enough study of ecosystem services and what keeps endemic species 'in balance'

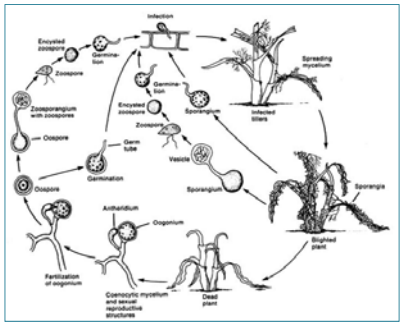
Why is this important?

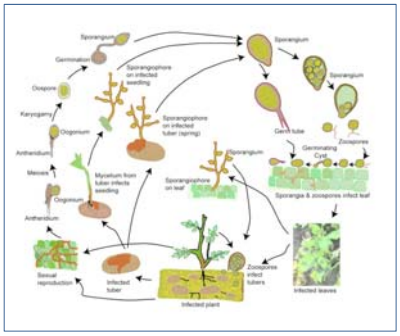
- Effective management strategies reliant on understanding pathogen biology/ecology – there are big differences between fungi and oomycetes
- Many chemicals that give control of fungi do not work on oomycetes and *vice versa*
- **Accurate and timely diagnosis is vital**



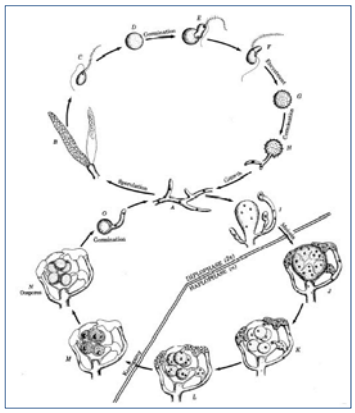
Generalised oomycete life cycle

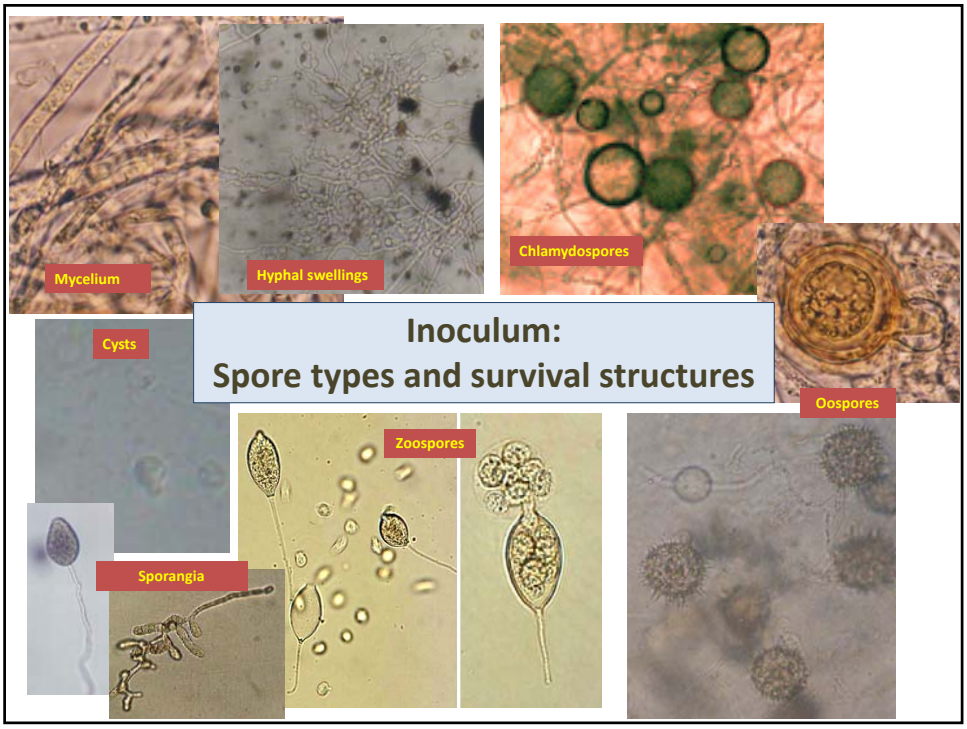






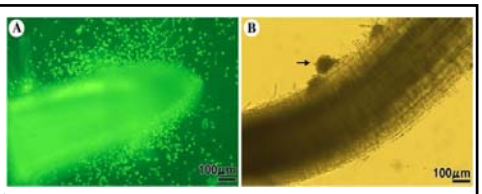
Variations on the theme



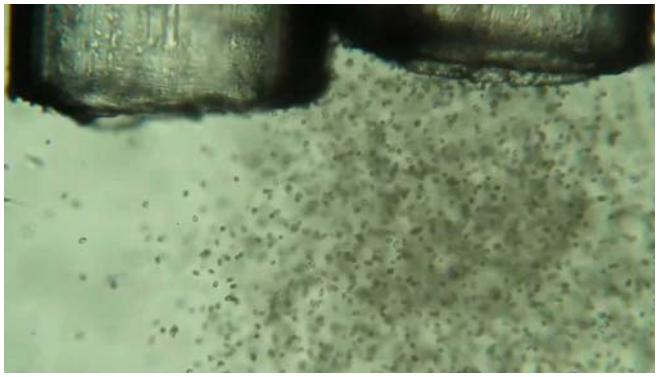


Zoospores:

- Motile and free-swimming
- Can swarm together (auto aggregation)
- Naturally swim upwards
- Are attracted by chemicals and electric fields of host root systems



A) *Aphanomyces cochlioides* zoospores Chemotactically Attracted to Sugar Beet Root Tip.
(B) *Aphanomyces cochlioides zoospores* Aggregated on Root Tip (arrow).
(Tofazzal and Taharat, 2001).
Garrison (2008) http://www.cals.ncsu.edu/course/pp728/Aphanomycescochlioides/Aphanomycescochlioides.html



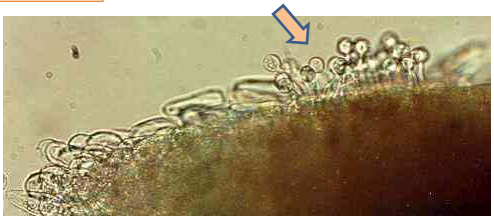
Phytophthora attracted to root extract in a capillary tube next to a tube containing water
(**Ronaldo Dalio** https://www.youtube.com/watch?v=F4slTLkhwuY)

Infection: zoospore cysts

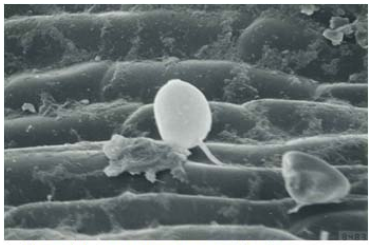
- **Often aggregate – especially on roots**
- **Align themselves – germinate directly towards host**

Not all infection *is* by zoospore cysts-

- some pathogen spp. do not produce zoospores
- Hyphal-tip infection not so readily measured (maybe not as photogenic!)



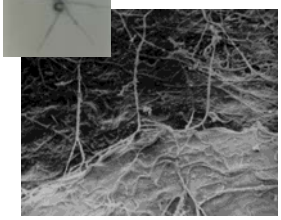
Pythium aphanidermatum cysts infecting a root tip
Deacon - http://archive.bio.ed.ac.uk/jdeacon/microbes/zoospore.htm

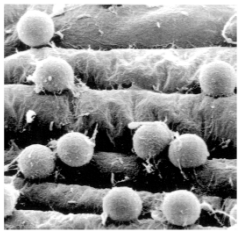


Phytophthora cactorum cysts infecting strawberry petiole base

Pythium violae cavity spot lesion on carrot (no zoospores)

Germinating oopspore

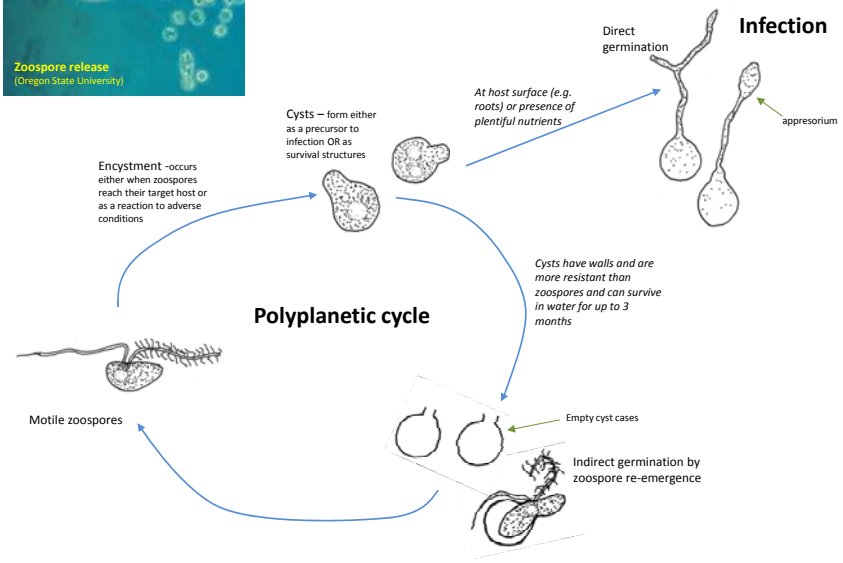




Phytophthora cinnamomi cysts on onion root
Hardham (2001) Aust. Plant Path 30:91-98

Polyplanetism or repetitional-diplanetism

A short-term survival mechanism in adverse conditions that permits zoospore movement in 'stages'



Zoospore cyst formation and cyst survival on exposure to different concentrations of peroxy-acetic acid

PAA concentration (mg/l)	Water source		
	Cyst formation & (% viable)†		
	Efford	Nursery A	Nursery B
0.2	32 (82)	100 (98)	85 (98)
2.0	54 (5)	100 (30)	95 (15)
20.0	96* (0)	100* (0)	100* (0)

* Cysts malformed with 'wrinkled' walls
† Viability determinations on ¼ strength PDA and are probably under-estimates.

Dispersal

- Unlike S.O.D. and blight, the majority of root and stem rot oomycetes are not airborne (although they are still spread by wind-driven rain!)
- Spread depends on
  - Scattering infested soil
  - Scattering infected plant fragments & debris
  - Dust & dirt containing above
  - Contaminated water

Zoospores – dispersed in water
- Surface films & water-logging
- Runoff & irrigation water & PUDDLES

Oospores/Chlamydospores/Mycelium/ Swellings & stromata
- Released from decaying plant matter and soil OM
- Can adhere to benches, floors, equipment, boots & tyres, trays/containers, Danish trolleys

Survival

Structure		Estimated survival/longevity		
		In soil	In water	Dry
	**Mycelium**	Hours-Days	Hours-Days	-
	**Zoospores**	Hours-Days	Hours-Days	-
	**Zoospore cysts**	Days-weeks	>3 months	-
	**Chlamydospores**	Years	?	+
	**Oospores**	Years	?	+
	**Hyphal swellings**	Days-months	?	?

Strengths and weaknesses

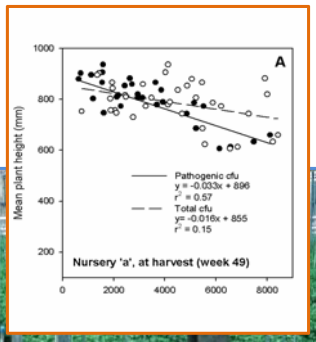
This is generally a well-established area, although there have been some improvements in our understanding of zoospore behaviour.

- Considerable bank of knowledge built up on release, survival, taxis, attachment & germination of zoospores
- For many oomycetes, zoospores are the most important dispersal/infection spores but there is a bit of a 'zoospore fixation', and not enough work on survival structures
- Nevertheless, there is still much to learn about zoospores – what induces 'survival encystment'? Can auto-aggregation and taxis be exploited for control?

Why is this important?

- Understanding the subtleties of pathogen life-cycles identifies potential for effective disease management and avoidance
- It is important to remember that oomycetes have a range of propagules and survival strategies – not just zoospores (*important to consider the entire offensive team not just the strikers*)
- Control measures aimed solely at zoospores could be ineffective against other propagules

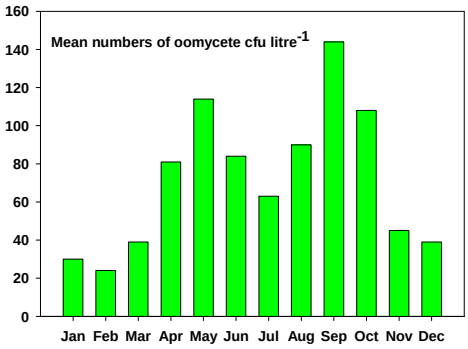
Quantifying soil inoculum – Disease risk thresholds?

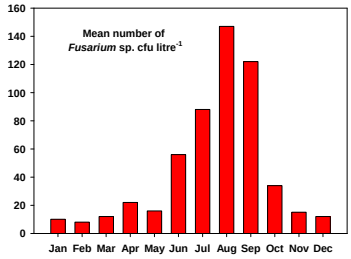


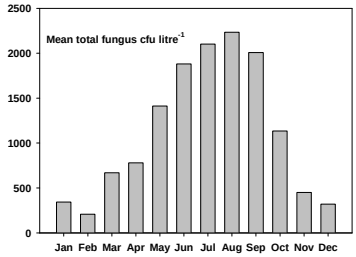
Seasonality of inoculum production:

are there optimum times to test?

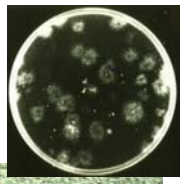
Pooled results from 15 years' irrigation water tests throughout UK



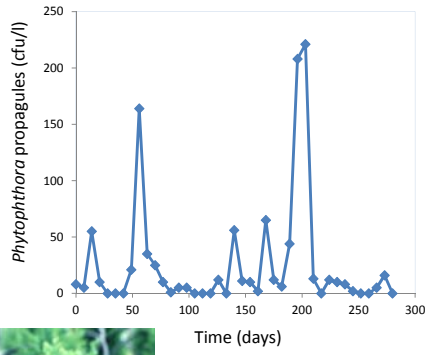




Phytophthora inoculum production in a recirculating outdoors HNS bed



Short-lived 'spikes' of inoculum = discrete high risk periods
- infrequent sampling could miss these



- Going by symptoms alone not sufficient. It can take 3 months to a year for shoot symptoms to appear from a single infection event
- Testing pot effluents can reveal early infections

Inoculum density & infection

Pathogen	Host	% infection	Inoculum concentration		Ref.
Pythium aphanidermatum	Tomato	50	250	Zoospores/ml	Mitchell, 1978
Phytophthora cryptogea	Watercress	50	276	Zoospores/ml	Mitchell, 1978
Phytopythium ostracodes (was *Pythium*)	Cotton	50	281	Zoospores/ml	Mitchell, 1978
Phytophthora ramorum	Range of tree hosts	50	36-750	Sporangia/ml	Tooley *et al.*, 2013
			100-250 with wounding		
Phytophthoa cryptogea	Tomato (Hydroponic)	50	400	Zoospores/ml	Pettitt *et al.*, 2001
Phytophthora cactorum	Strawberry (var. Tamella)	**Zero!**	10 000	Zoospores/ml	Pettitt 1989
		50	1000		
	Cold-stored ditto	100	25		

Crop susceptibility affected by growth stage, season and environment

Periods of high susceptibility likely to coincide with 'inoculum spikes' - little data about this

***Phytophthora cactorum* strawberry crown rot**
- Cold-stored runners = ultra suscetible
- Traditionally a 'spring wilt'

***Pythium* damping-off**
- Seedlings and very young high nutrient plants susceptible

***Pythium sylvaticum* xanths root rot**
- A disease problem that used to plague late winter and early spring crops

Strengths and weaknesses

Research on inoculum and infection potential in soil and water on a field-scale trails far behind that on airborne pathogens and is somewhat hampered still by techniques for detection and quantification

- Excellent techniques such as Q-PCR are now becoming more readily available although all approaches have weaknesses
- Current understanding of the dynamics of inoculum production and disease is still very poor and estimates of disease risks often very elementary (i.e. +/-!)
- A major weakness of many studies is the focus on single 'pathogen' species – next generation sequencing and new nested immunodiagnostics techniques may help address this
- Detection of 'latent' infection still a challenge

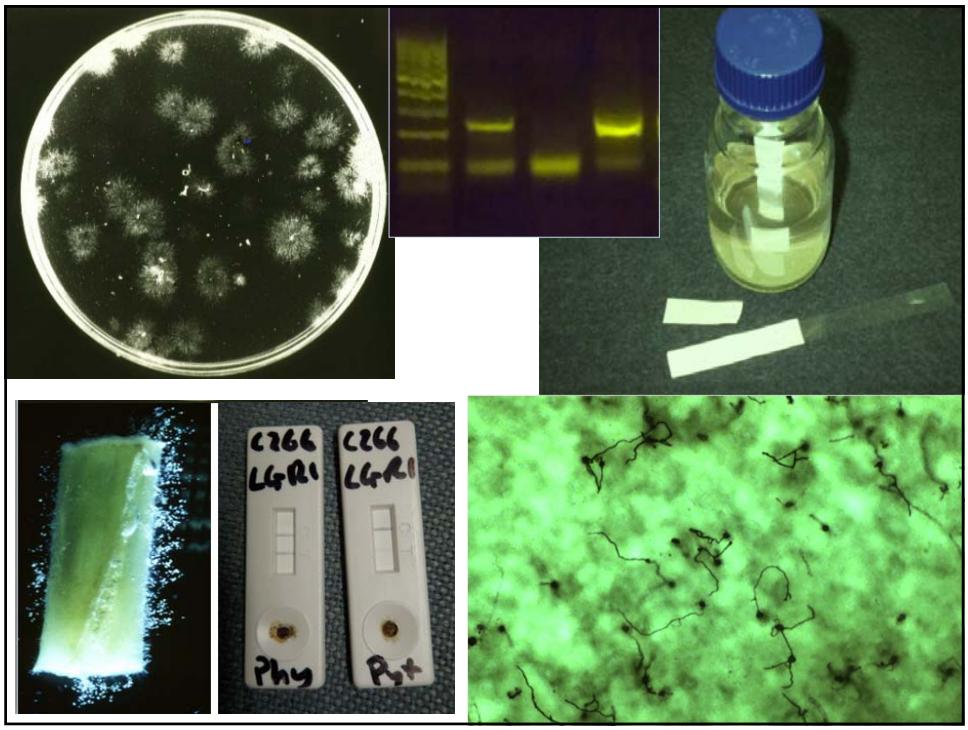
Why is this important?

- Proper understanding of disease risks is the basis for disease management – currently there is the danger of being overly cautious, or worse still, not even trying to determine disease risks
- **Again, accurate and timely diagnosis is vital**

Detection, diagnostics and quantification

Mixed populations:
- Oomycetes/Non-oomycetes
- Pathogens/Non-pathogens

Inoculum:
- Seasonal (seasonal susceptibility)
- 'Spikes'
- Density v. infection (thresholds?)
- Latent (silent) infection (inc. seeds?)



Disease management & control

Recap on dispersal:

- ❖ Infested soil & media
- ❖ Infested plants (& seeds)
- ❖ Decaying infected material
- ❖ Dust, debris , 'dirt'
- ❖ **WATER**

Management and control:

- ✓ Fungicides & biocontrol agents
- ✓ Sterilants/disinfectants
- ✓ Avoidance, certification & HYGIENE
- ✓ Water management

Avoidance and hygiene

Quarantine

Barriers to inoculum spread e.g. SpinOut-treated matting

Clean equipment and containers etc.

Simple hygiene

Zero tolerance of puddles and debris

In HDC PC97 – reduced incidence of *Pythium* infection by 70%

Thank you !

Dr Tim Pettitt
University of Worcester
t.pettitt@worc.ac.uk