IPM 2.0
Towards future-proof crop protection in Europe

Real-time PCR diagnostics: a tool for managing soil-borne potato diseases

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Real-time PCR diagnostics; assessing disease risk of soil-borne potato pathogens

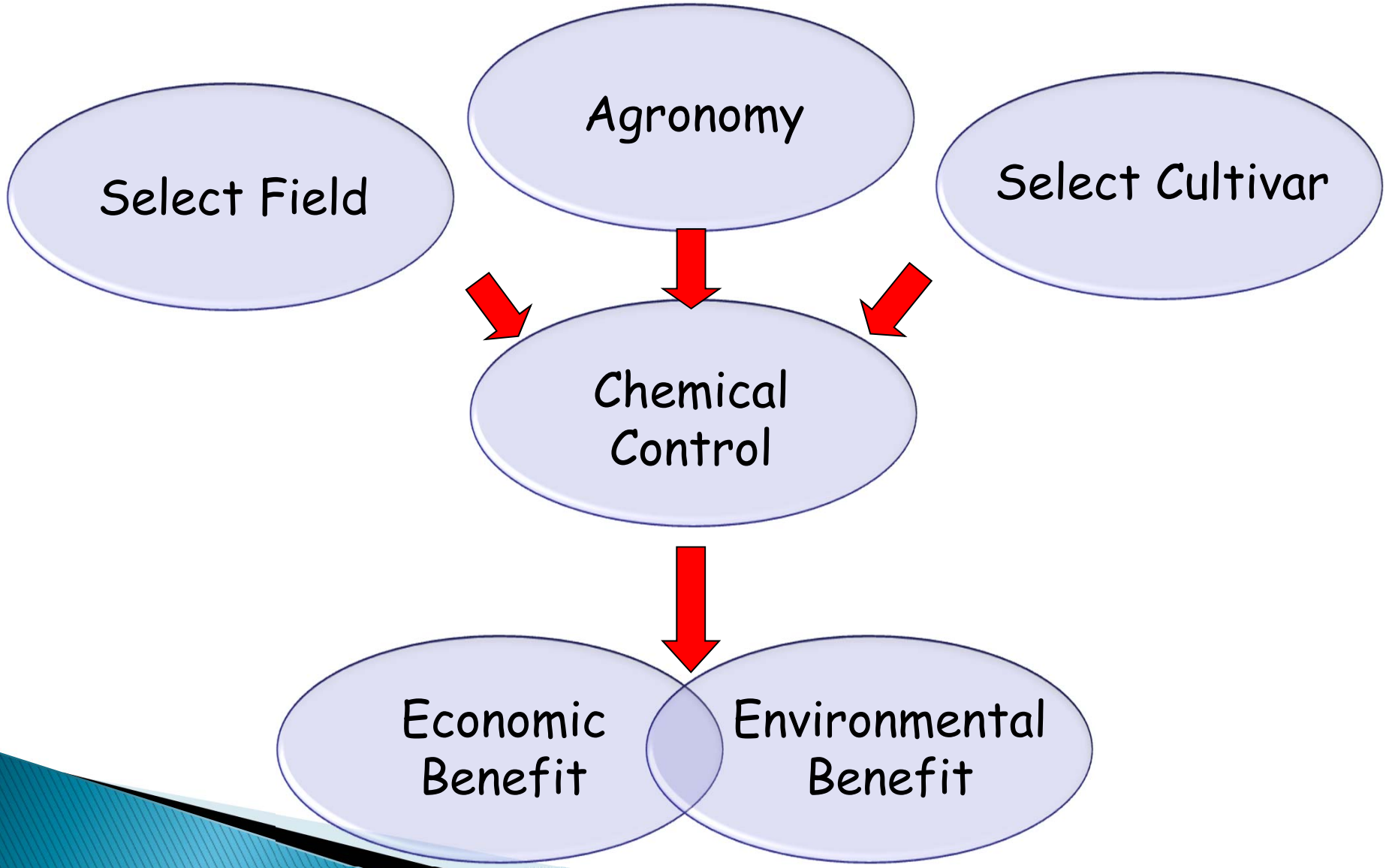
Real-time PCR: a tool for

- studying disease epidemiology
- developing disease risk assessments based on levels of soil inoculum

Soil-borne pathogens

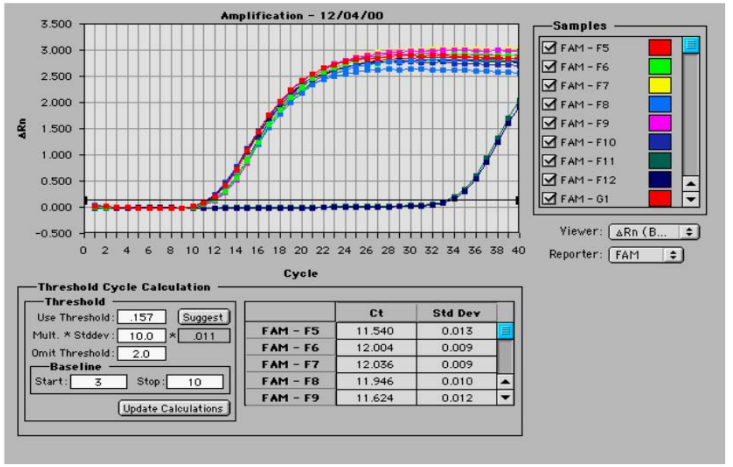
- *Colletotrichum coccodes* – black dot
- *Spongospora subterranea* - powdery scab
- *Rhizoctonia solani* AG3- stem canker/black scurf

Reducing disease risk:

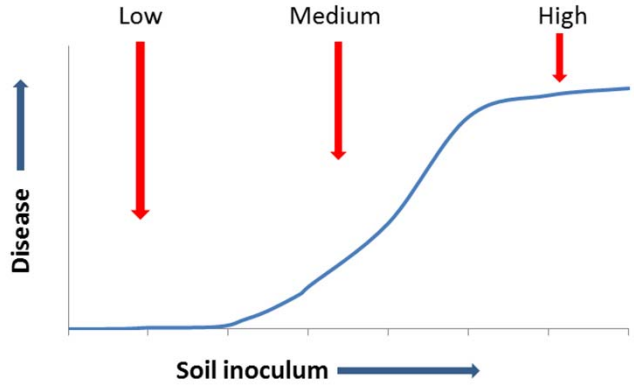


Evaluating disease risk using real-time PCR

Soil sample
(pre-planting)



Quantification of target inoculum



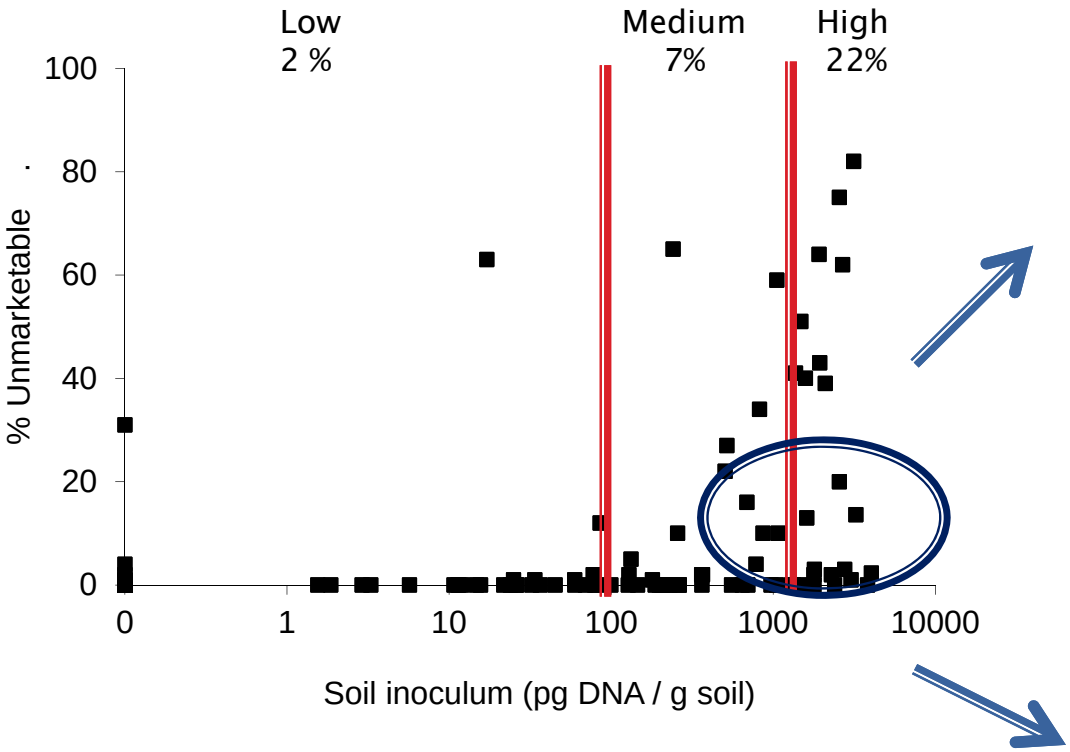
Disease risk assessment

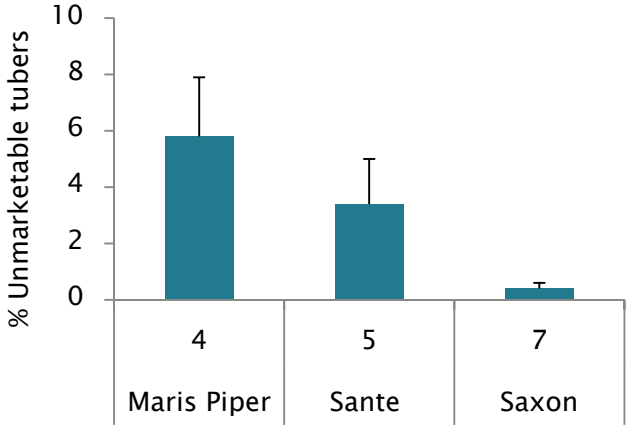
Soil sampling

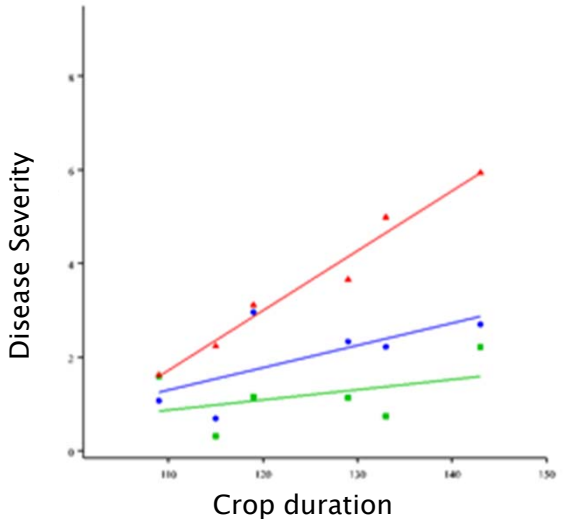
A suitable soil sampling strategy underpins the reliability of soil testing.

- must be representative of field scale
- must be practical (sampling and processing time and cost)
- Based on “old” PCN sampling strategy.
  - Sampling area: 4ha or less. (divide larger fields)
  - Sample size: 1Kg for standard testing
  - Sampling points: 100 x 10g samples (0-15 cm depth)
  - Sampling pattern: W pattern.

Example 1: *Colletotrichum coccodes* – black dot



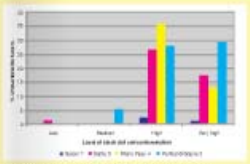
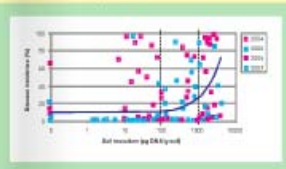
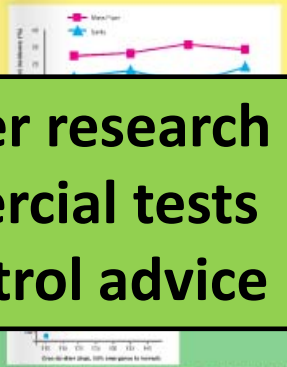
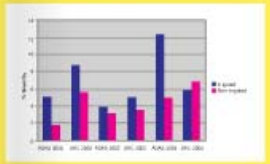
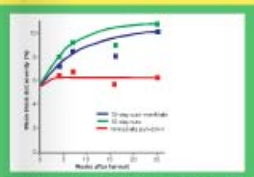




Black dot disease risk assessment

- Soil test result → risk
- Variety grown → Reduce/increase risk
- Likely crop duration → Reduce/increase risk
- Irrigation → Increased risk
- Chemical control → Reduce risk (may depend on level of inoculum)
- Weather conditions ?

Managing the risk of black dot

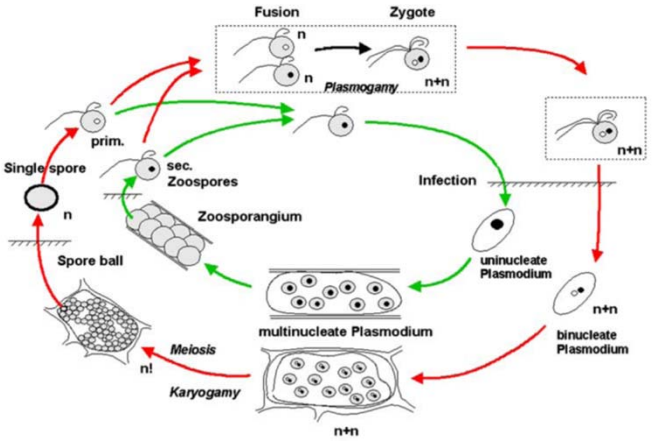
HIGH RISK	FACTOR	ACTION	TECHNICAL
Highly susceptible varieties, e.g. Estima, King Edward, Pentland Squire	Variety	**Check varietal resistance to black dot at www.potato.org.uk/varieties, in the NIAB pocket guide, or contact your supplier**  Although the majority of pre-pack varieties are susceptible to black dot, there are a number of moderately resistant varieties. Resistance ratings vary from 2 (very susceptible) to 8 (resistant). The most susceptible varieties should not be planted in fields known to be contaminated with black dot inoculum at high risk levels. Susceptible varieties include Estima, King Edward, Maris Piper and Pentland Squire. More resistant varieties include Cabaret, Saxon, and Sante.	 Figure 1. Impact of varietal resistance on black dot development Varietal resistance can help reduce the incidence of black dot. In Potato Council-funded trials investigating control measures against black dot, changing a more susceptible variety to a more resistant variety resulted in a significant reduction in black dot. In field trials performed at sites with different levels of soil contamination with *C. coccodes*, growing a variety with a high resistance (resistance rating 7 or higher), significantly reduced the percentage of unmarketable tubers at sites with high or very high levels of contamination. At sites with medium contamination, changing the variety from resistance rating 3 (e.g. Pentland Squire) to one rated 4 (e.g. Maris Piper) reduced the % unmarketable tubers.
Planting a crop where soil is contaminated with black dot	Field selection	**Perform a black dot soil test (this is particularly valuable on rented land with unknown field history). Otherwise, if field history is known, take this into account e.g. crops grown in short rotations with a history of black dot, presence of host weed populations and high volunteer numbers may be high risk** Soil-borne contamination is the most important source of inoculum for causing disease. A soil test can help determine the risk of black dot developing. Where a number of potential fields are tested, those with the highest level of soil contamination can be avoided.	 Figure 2. Effect of increasing soil inoculum (pg DNA / g soil) on the incidence of black dot in progeny crops monitored in 2004, 2005, 2006 and 2007. *Source: Potato Council projects R249 and R253.* Using the soil test, the risk of disease can be split into three categories depending on level of soil inoculum: low risk (0-100 pg DNA/g soil), medium risk (101-1000 pg DNA/g soil) and high risk (>1000 pg DNA/g soil). As part of a Potato Council-funded study, 130 fields were assessed for soil contamination. 83% of soils were found to be contaminated, with 55% having a moderate or high risk. *
Infected seed, particularly when planted in uncontaminated land	Seed health	**Avoid contaminated seed of more susceptible varieties and avoid planting contaminated seed in soils not contaminated with black dot** The relationship between the amount of black dot on seed and disease on daughter tubers is complex. It is widely accepted that most black dot infection originates from	 In Figure 3, in each of four trials, contaminated seed stocks of Maris Piper and Sante were graded by hand according to the extent of black dot symptoms covering the tuber surface. Tubers from each of the four seed categories were planted in trials at sites where little or no soil contamination was detected. …e of disease in Sante crops was significantly less than in Maris …e resistance ratings 5 and 4 respectively). There was little difference …four seed categories in black dot incidence in the daughter crop. …visually free from black dot symptoms can cause disease in …ps.
Harvest date after early October or where crops are grown for a long duration (120-130 days from 50% emergence to harvest date)	Crop duration	**Harvest by early October or limit duration in the ground according to susceptibility and maturity group** …grown for more than 130 days duration (from 50% emergence to harvest). For second earlies that are susceptible, such as Estima, there is a higher risk of economic loss from black dot when crops are grown for more than 130 days duration.	…data are means of 2005 and 2006 seasons. Black dot values …surface area accumulated over a 20-week storage period (crop held …rapid pull-down). …n the vertical axis can be roughly translated into incidence of tubers …an 10% surface area. Therefore, at 1,000 the percentage of …bers reaches 10%. At or above this level, crops would be at risk …of being downgraded. Figure 4. Relationship between crop duration and black dot surface area in a susceptible maincrop cultivar, Maris Piper. *Source: Potato Council project R249*
Wet soil conditions, particularly late in the season Over irrigation	Soil conditions	**Avoid over irrigation especially of susceptible varieties and long-duration crops** Black dot is a disease favoured by wet and warm soil conditions. Where soil inoculum is present, irrigation or high levels of summer rainfall will increase black dot, particularly where a susceptible variety or a long-duration crop is grown. Action should be taken to reduce risk in other ways such as reducing initial inoculum and harvesting as early as possible.	 Figure 5. Effect of irrigation on severity of black dot in six separate field trials *Source: Potato Council Project R249* Results show that where soil inoculum is present irrigation can encourage black dot to develop. In field trials irrigation significantly increased black dot severity in three out of six trials performed between 2004 and 2006 compared with a non-irrigated control (Figure 5). In trials, seasonal variations in incidence of disease in association with soil inoculum were found. In the above average summer rainfall seasons of 2004 and 2007, crops had higher incidences of disease than in the lower than average rainfall seasons of 2005 and 2006 at lower soil inoculum levels (i.e. <100 pg DNA/g soil).
Slow pull-down to holding temperature	Storage	**Rapid pull-down to holding temperature**  Provided skins have adequately set, it is advisable to reduce the crop temperature as quickly as possible after harvest. Aim for a 0.5°C pull-down per day to holding temperature. Adequate ventilation is required during pull-down in order to remove surface moisture. Further details can be found in the Potato Council Store Managers' Guide.	 Figure 6. Effect of early storage conditions on black dot development. *Source: Potato Council project R249* Figure 6 illustrates how disease levels can be held throughout storage by rapidly pulling down temperatures after store loading.

Bringing together research findings, commercial tests and disease control advice

'pg – picograms or trillionth of a gram.

Example 2: *Spongospora subterranea* – powdery scab

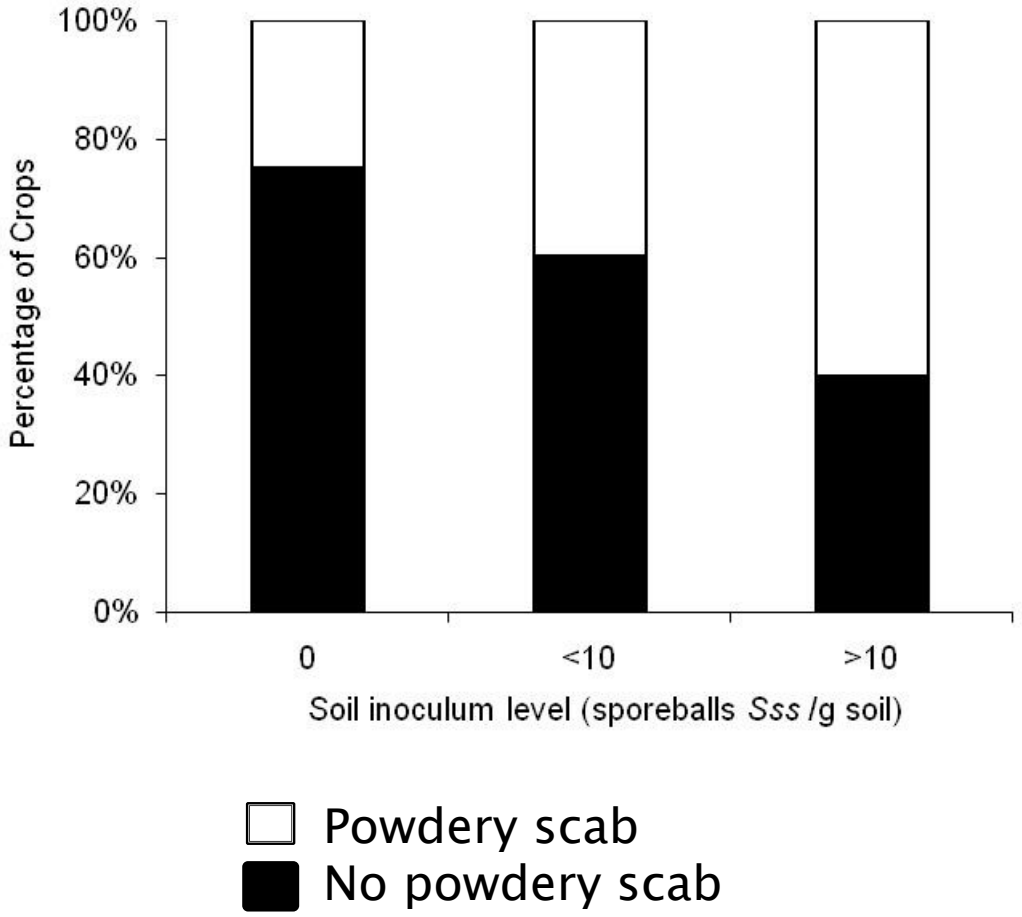
- Biphasic life cycle:
- Resting spores which persist in soil for many years.
- Rapid multiplication through the formation of secondary zoospores.



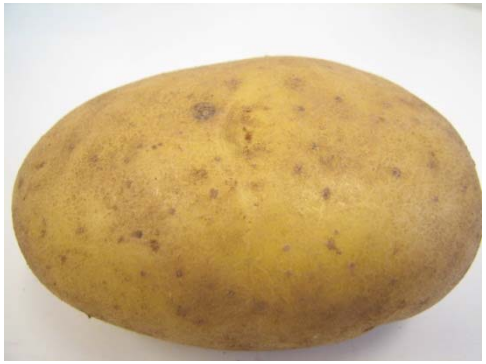
- We set out to determine if soil inoculum levels of *Spongospora subterranea* are related to disease risk.

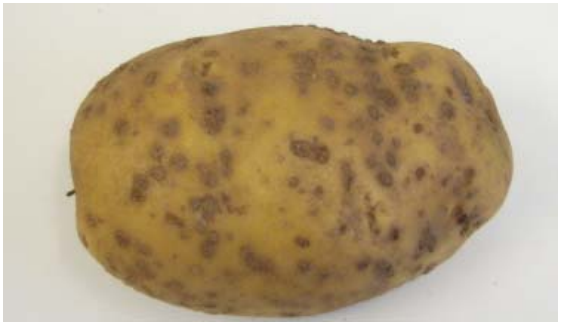
Monitoring of commercial potato fields

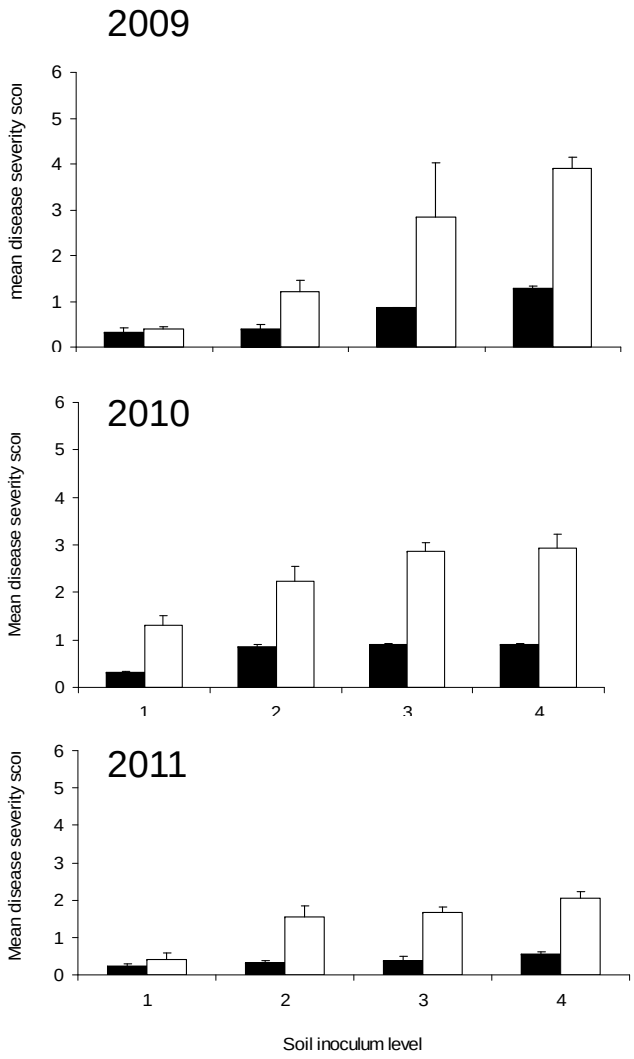
In commercial crops there was an increased risk of the progeny tubers having powdery scab from 25% to 65% based on pre-plant levels of soil inoculum



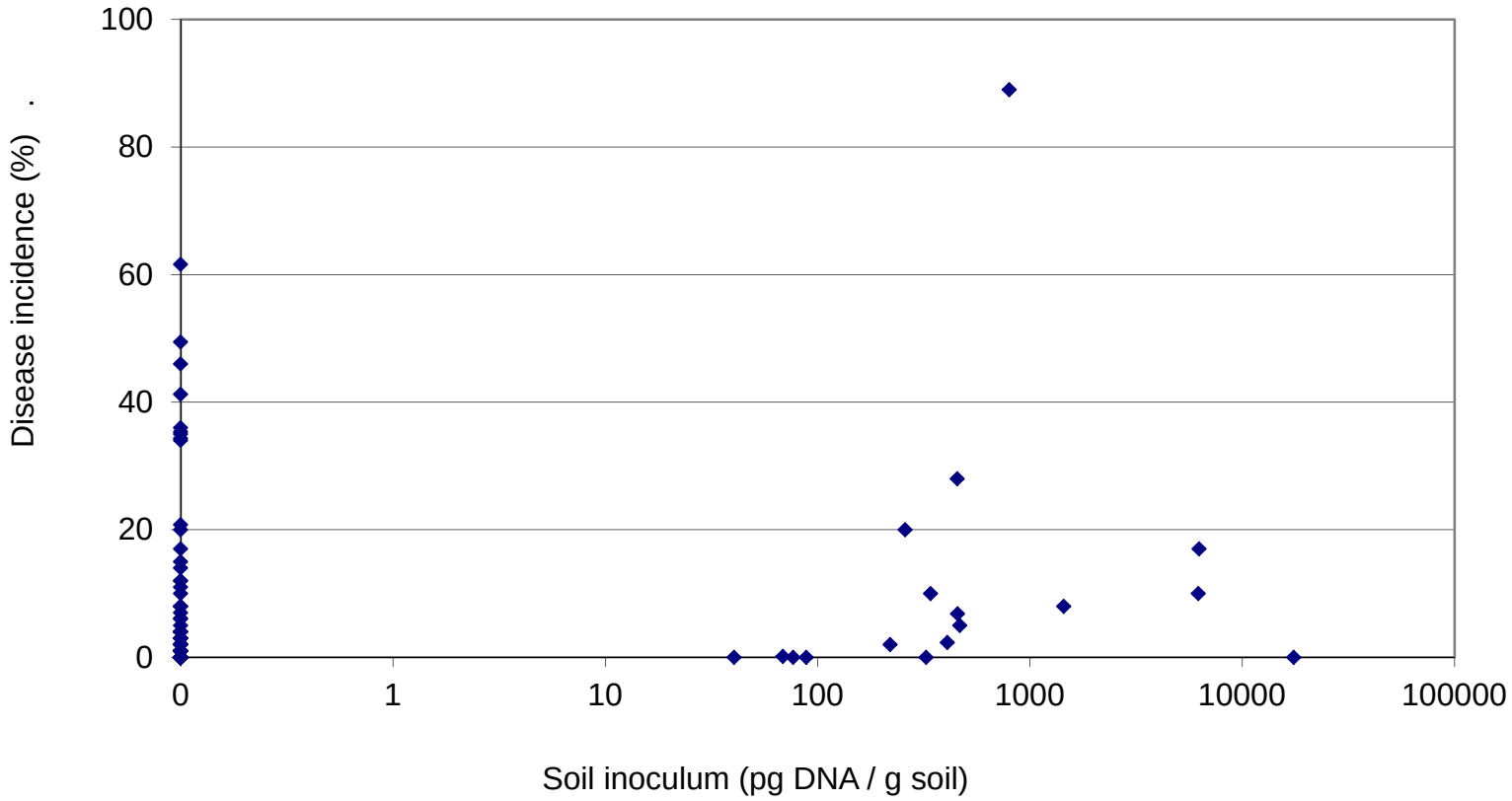
Effect of soil inoculum levels on powdery scab: field trials







Example 3. *Rhizoctonia solani* (AG3) – black scurf



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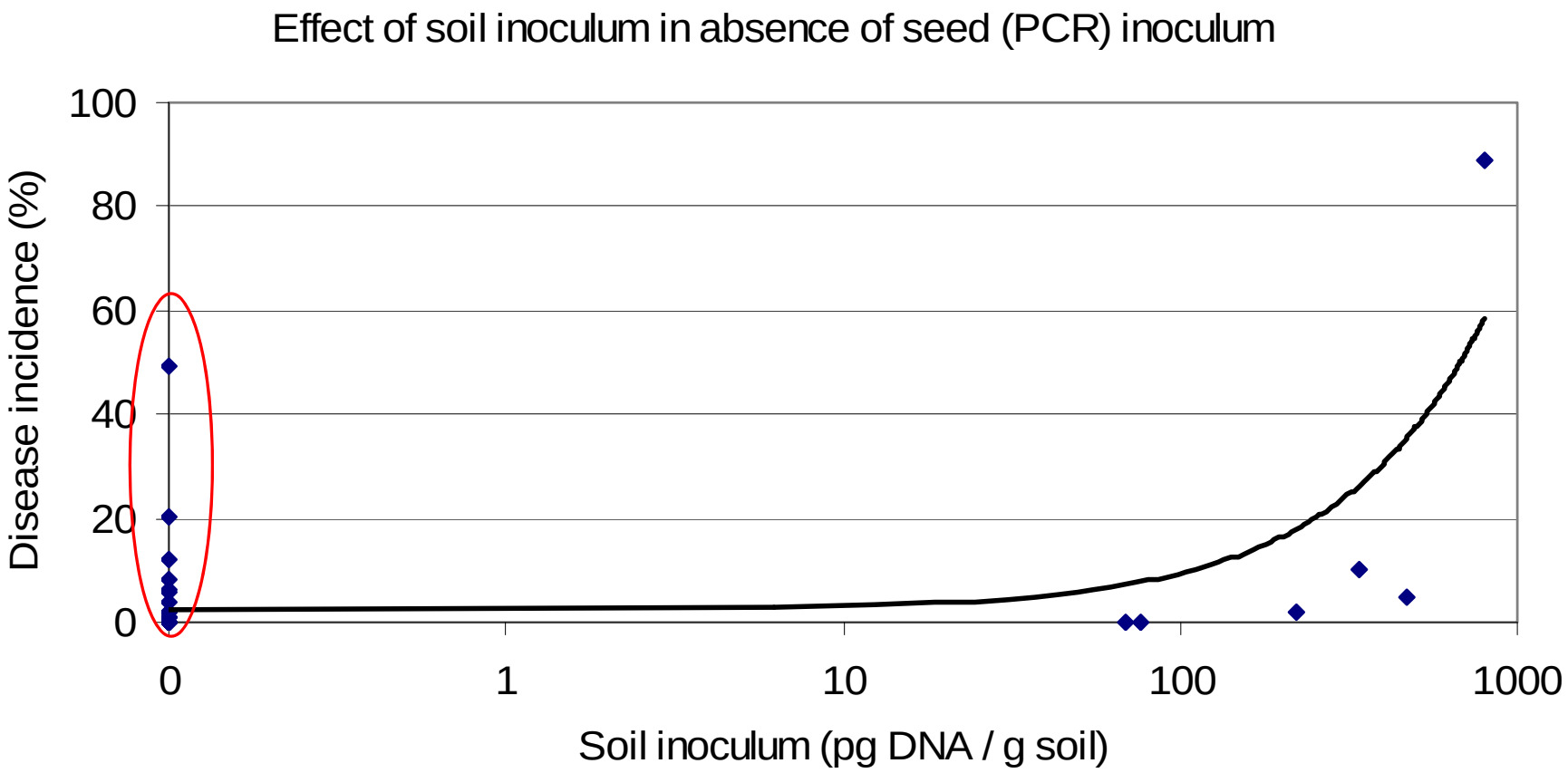
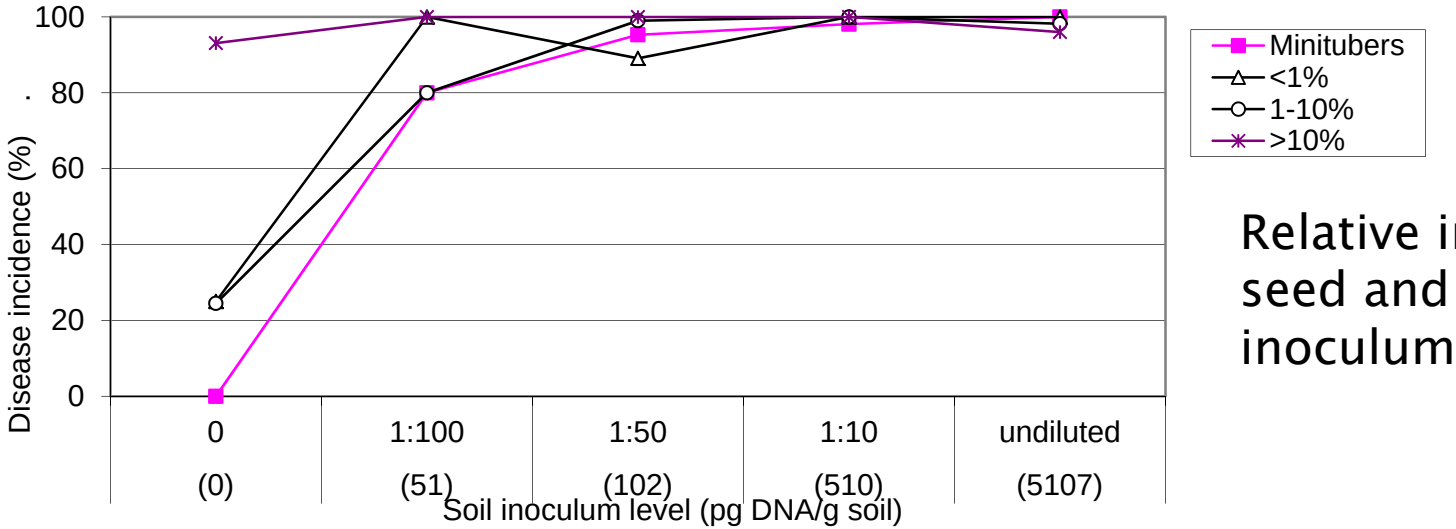
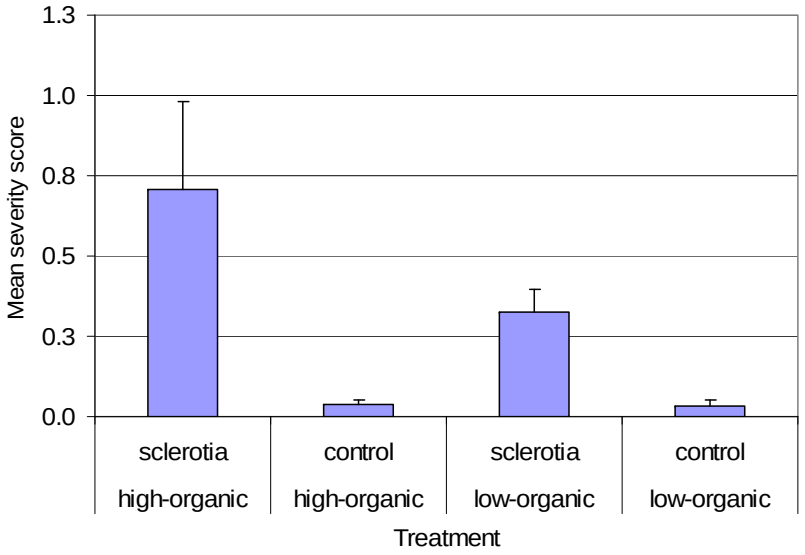


Rhizoctonia solani AG3



Relative importance of seed and soil borne inoculum.

Interaction between soil-borne inoculum and organic matter in the soil



Examples of real-time PCR assays for potato pathogens

Disease	Pathogen
Dry rot	*Fusarium* spp.
Black dot	*Colletotrichum coccodes*
Powdery scab	*Spongospora subterranea*
Black scurf	*Rhizoctonia solani* AG3
Silver scurf	*Helminthosporium solani*
Common scab	*Streptomyces scabies*
Gangrene	*Phoma foveata*
Watery wound rot	*Pythium ultimum*
Pink rot	*Phytophthora erythroseptica*
Late Blight	*Phytophthora infestans*
Skinspot	*Polyscytalum pustulans*

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