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OPINION PAPER

Getting the best from pot trials with soil-borne Oomycetes

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Abstract Soil-borne Oomycetes are important pathogens of nursery plants, agricultural and horticultural crops, and woody plants in natural ecosystems. They are most damaging when plants are overwatered or growing in poorly drained sites. Poor growth could result from root infection, root damage resulting from the anoxic conditions which develop in saturated soil, or both. This is essential information for devising appropriate management options, as these will differ depending on the primary cause of poor health. Pot experiments are often used to determine whether these soil-borne pathogens cause root infection which is assumed to be by zoospores produced in wet soil. Soil saturation followed by draining, is included as part of the experimental protocol to generate zoospores from the inoculum and facilitate their movement to, and infection of, plant roots. However, if soil saturation persists until the soil becomes anoxic, this may affect the host. In our opinion, this can muddle the interpretation of results, unless there are adequate controls which include root infection in unsaturated soil, and the effect of soil saturation on the host in the absence of the pathogen. Pot experiments are expensive in both time and equipment. They must be conducted to provide clear answers to the postulated hypotheses and ensure experiments are repeatable. We provide guidelines for conducting such pot experiments which will assist in clarifying the roles of these pathogens and soil saturation on plant growth, both separately and in combination.

Keywords Oomycetes · Pot experiments · Anoxia · Repeatability

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Introduction

Soil-borne Oomycetes such as *Phytophthora* and *Pythium* are important pathogens of nursery plants, agricultural and horticultural crops, and woody plants in natural ecosystems. They are most damaging when plants are overwatered, following excessive irrigation or rainfall, or growing in poorly drained sites such as those with duplex soils. In these situations, it may not be possible to determine whether poor growth results from infection, root damage resulting from the anoxic conditions which develop in saturated soil, or both. This information, however, is essential to formulate

appropriate management options, as these will differ depending on the primary cause of poor health.

Pot experiments are often used to determine whether these soil-borne pathogens cause root infection. Infection by Oomycetes is assumed to be by zoospores produced in wet soil. Soil saturation followed by draining, is included as part of the experimental protocol to generate zoospores from the inoculum and facilitate their movement to, and infection of, plant roots. However, if soil saturation persists until the soil becomes anoxic, this may affect the host. In our opinion, this can muddle the interpretation of results, unless there are adequate controls which include root infection in unsaturated soil, and the effect of soil saturation on the host in the absence of the pathogen.

Pot experiments are expensive in both time and equipment. They must be conducted to provide clear answers to the postulated hypotheses and ensure experiments are repeatable. We do not intend to be prescriptive because each plant/pathogen combination will differ, and precise experimental details will be coloured by the experience of the researcher. Here we provide guidelines for conducting such pot experiments. These will, from our experience, assist in clarifying the roles of these pathogens and soil saturation on plant growth, both separately and in combination. Before discussing these, it is important to note that it is critical to ensure the container growing medium has adequate air-filled porosity, water infiltration, and readily available water for healthy plant growth (Handreck and Black 2010).

Guideline 1: Clarify what you want to do and how the experiment will be conducted

This type of experiment addresses a minimum of three hypotheses. The null hypotheses are given below:

Hypothesis 1 The pathogen does not infect the host plant's roots maintained at container capacity. Infection is determined as either incidence (number of plants, or number of roots infected) or severity (number of lesions per plant, or proportion of root length infected).

Hypothesis 2 Soil saturation and subsequent drainage do not affect plant growth at a known soil temperature. Effect on the host plant to be most easily determined as the effect on evapotranspiration.

Hypothesis 3 Soil saturation and subsequent drainage do not affect the infection of plant roots. Infection is determined as either incidence (number of plants, or number of roots infected) or severity (number of lesions per plant, or proportion of root length infected). Although plant death is sometimes used as a measure of infection, this may only occur several weeks after the imposition of the experimental conditions and will not separate root infection (Hypotheses 1 and 3) from root damage resulting from soil saturation (Hypotheses 2 and 3).

Testing these hypotheses means there must be four treatments:

Control: no inoculum, no soil saturation.
Hypothesis 1: inoculum, no soil saturation.
Hypothesis 2: soil saturation and drainage, no inoculum.
Hypothesis 3: inoculum, soil saturation and drainage.

Additional treatments, such as comparing different cultivars or harvest times, can be added as needed in subsequent experiments.

At this stage, it is essential to discuss the experiment with a statistician who will advise on the experimental design, the number of replicates, and the best way to analyse the data.

Guideline 2: Use vigorously growing plants and control soil moisture

Vigorous, healthy plants must be used with the age of the plants depending on whether they are annuals or perennials. Plants that are too old, nutrient-deficient, or growing in pots that are too small (which could cause root-binding), may not give reproducible results. Fortunately, several texts provide advice on how to grow plants in containers, either as general principles for the nursery industry (Handreck and Black 2010) or specifically for pot experiments (Passioura 2006; Poorter et al. 2012).

The first decision is whether to use drained or undrained pots. Drained pots are convenient and can be watered by an automatic irrigation system;

however, the growing medium at the base of the freshly watered and drained pot is saturated, and if the pot is squat, most of the growing medium may be hypoxic (Passioura 2006). This can affect both the plant and pathogen because hypoxic conditions damage plant roots and reduce the sporulation of soil-borne Oomycetes (Mitchell and Mitchell 1973; Davison and Tay 1986). Undrained pots allow much greater control of soil moisture by watering to a pre-determined matric potential; recording the weight of water added to maintain this matric potential provides a measure of evapotranspiration. Undrained pots can also be used in root cooling tanks, allowing control of soil temperature (see Guideline 3). If a watering tube is inserted before the pot is filled with the growing medium, the pot can be drained by suction following the soil saturation treatment (Davison and Tay 1985). An example of this type of pot is given by Bennett et al. (1986).

The pot is filled with a growing medium which must provide air and water to the developing plant. Using a coarse potting mix is better than field soil because it has a greater air-filled porosity and is less likely to become hypoxic (Handreck and Black 2010; Passioura 2006). Both Handreck and Black (2010) and Passioura (2006) contain methods for measuring the air-filled porosity, water infiltration, and readily available water in the growing medium.

Guideline 3: Understand how soil saturation affects the plants

When soil is saturated with water, it rapidly becomes anoxic because oxygen in the soil solution is used by the respiration of soil microorganisms and plant roots (Gibbs and Greenway 2003). As oxygen diffuses 10^4 times more slowly through water than through air, it is used more rapidly than it is replaced, and this happens more quickly at higher than lower soil temperatures (Russell 1973). Soil temperature control is therefore essential to ensure the reproducibility of pot experiments, and this can be achieved by using root cooling tanks, necessitating the use of undrained pots.

The oxygen concentration of the soil solution needs to be monitored, which can be done in the following way. An aquarium aeration stone is buried in the pot when the pot is filled with the growing medium. The stone is attached to a long tube which is closed with a three-way tap. A water sample is withdrawn through this tube, with the three-way tap enabling an aliquot to be removed without being contaminated by air. The oxygen concentration of this sample is measured under a nitrogen atmosphere, using an oxygen electrode.

Soil saturation is usually imposed for one or two days, and then the pots are drained. For free-draining pots, this is by gravity, while in undrained pots, this is by suction through the watering tube. The water content of the drained growing medium will be greater than before soil saturation because the micropore spaces within the growing medium will still contain water (Russell 1973). Therefore, the growing medium is likely to be more hypoxic than before saturation was imposed.

If the growing medium becomes anoxic, this will immediately impact the plant. Root respiration will change from aerobic to anaerobic, reducing the rate of energy production and resulting in leakage of ethanol from the roots (Gibbs and Greenway 2003). Stomatal closure is common, resulting in reduced transpiration and photosynthesis (Pallardy and Kozlowski 2007). These changes can be detected by measurement in pot experiments but may not be apparent to the naked eye. The simplest way to determine whether this has changed is to compare the daily evapotranspiration from pots saturated with water with the control pots maintained at a pre-determined matric potential (see Guideline 2). This change from aerobic to anaerobic respiration is important for infection because zoospores are chemotactically attracted to ethanol (Allen and Newhook 1973).

Guideline 4: Inoculation

Although difficult under controlled conditions, inoculation should represent what happens in the field as closely as possible, both in the type of inoculum used and the concentration of propagules.

Inoculum can be introduced to the growing medium in several ways, and care should be taken to avoid damaging roots. Zoospores can be used as an inoculum and inserted into the growing medium at a known depth. Other methods use an inoculum of colonised wooden plugs or grown on various food sources. To minimise the chances of root damage

when introducing the inoculum, dowels or tubes can be placed into the substrate when the plants are placed into the containers. These are gently removed at the time of inoculation, and the inoculum placed into the holes, which are backfilled with the growing medium.

Guideline 5: Harvesting

Plants should be harvested within 14 days after the imposition of the experimental conditions, before infected fine roots disintegrate. The growth medium should be gently washed from the roots, and the roots used to assess infection; washing the roots through a series of sieves is one way to collect as many fine and coarse roots as possible. If the inoculum has been placed at a specific depth in the growing medium, root harvesting need only be done above and below this region (Davison and Tay 1987).

Assessment of infection can be done in several ways, depending on which hypotheses are being tested (Guideline 1). Infection needs to be assessed by plating roots onto selective agar, and there are several Oomycete-selective agars suitable for this. If selective agar is used in combination with root measuring equipment (e.g., APS Assess 2.0 software (The American Phytopathological Society, USA), or WinRhizo software (Regent Instruments Inc.)), this will allow estimates of incidence and/or severity of infection. When using any of the above methods, it is appropriate to number the treatments so that all data collected is unbiased.

Guideline 5: Data analysis and interpretation

Discuss the analysis of the data with your statistician.

By following these guidelines, you can determine whether soil saturation results in hypoxic or anoxic soil under your experimental conditions. You will be able to determine whether this affects water uptake by the host, and whether additional experiments are needed to quantify these effects on host physiology.

These guidelines will allow you to compare the incidence and severity of root infection in the growing medium maintained at container capacity, and in the growing medium that has been saturated for a short time. If there is an increase in the incidence and severity of root infection following soil saturation, does this result from increased sporulation, increased mobility of zoospores, the increased attraction of zoospores to roots, or reduced ability of the host roots to contain infections? Further experiments could include a time course to determine whether infection increases over time.

It will allow you to determine whether poor growth results from infection, saturated soil, or both. Should it be necessary, it will allow you to confidently design additional experiments to further explore these effects.

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Declarations

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