KWAZULU-NATAL PROVINCE
AGRICULTURE AND RURAL DEVELOPMENT
REPUBLIC OF SOUTH AFRICA

Plant Health Diagnostics
(A division of Analytical Services, Cedara)
Private Bag X9059, Pietermaritzburg, 3200
✆ 033-3559248 Michael Relihan
✆ 033-3559160 Thobile Gwala

PLANT HEALTH DIAGNOSTICS

Price list: April 2024 – March 2025

Plant Pathologist in charge: Michael Relihan 084-5119445
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Testing Services	COST per sample
Diseased plant (or insect): By symptoms and basic microscopy	R160
Diseased Plant: Diagnosis by isolation and advanced microscopy	R320
Seed: Isolation test for specific pathogen per 100 seed sample	R180
Seedlings/cuttings Isolation screen: for *Pythium* or *Phytophthora* or *Rhizoctonia* or *Fusarum* spp. (up to 6 pooled per sample)	R120
Seedlings/cuttings PCR screen: *Pythium* or *Phytophthora* or *Rhizoctonia* or *Leptosphaeria maculans* or *Aphanomyces* or *Ralstonia solanacearum* or *Meloidogyne* (4 - 6 pooled per sample)	R150
Supplied baits: Isolation test for *Pythium* or *Phytophthora*	R100
Water: *Pythium* enumeration by MPN bait isolation (need 4L)	R120
Water: *Phytophthora* detection by bait isolation (need 4L)	R120
Water: *Ralstonia solanacearum* by centrifugation & PCR (need 1L)	R130
Water: Viable bacteria count, spread-plate method on R2A (need 20 mL)	R80
Water: Viable fungi count by dilution plating on RBC (need 200ml)	R130
Soil: Bait detection of *Pythium* or *Phytophthora* or *Rhizoctonia*	R140
Soil: Nematode count with ID of parasitic genera (need 200mL soil)	R230

(SAMPLE SUBMISSION INSTRUCTIONS ON NEXT PAGE)

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SAMPLE SUBMISSION INSTRUCTIONS

Diagnosis of a diseased plant sample

Representative sampling: One or more symptomatic entire plants, or representative symptomatic plant parts (if the plant is large such as a tree), can be submitted as one collective sample for diagnosis of the cause of disease symptoms. To be considered one sample all plants should be sampled from close proximity at the same location and have similar symptoms, but supply of examples of early stage symptoms plus late stage symptoms is encouraged in the same sample. Isolations from early stage symptomatic tissue is more likely to recover causal organisms than from late stage symptomatic tissue, but late symptomatic tissue may have superficial fungal sporulation or sufficiently distinct symptoms to be adequately diagnostic to negate the need for isolation. It is plant material that we need to diagnose diseased plants instead of soil, since soil, aside from doing nematode counts where pathogenic nematodes are the cause, is seldom useful for achieving a diagnosis.

Packaging and labelling: Above-ground plant samples (leaves, flowers, fruit, stems, etc.) should be wrapped in newspaper at sampling, especially if it is likely that delivery to Plant Health Diagnostics will take longer than 4 hours. Newspaper absorbs excess moisture on the plant sample while also retarding rapid dehydration of the sample and insulating it from major temperature fluctuations; which consequently retards secondary decay or deterioration of the sample. The newspaper-wrapped sample can then be packed inside a bag or box for transport to us. Label the sample at sampling with a name or code that is meaningful to the client and write this clearly on the packaging (preferably with a water-insoluble koki pen). Roots (with or without attached soil) can be placed directly in a plastic bag, which can then be packaged in another bag or box. Refrigeration (1-10°C) of samples after sampling, or in transit, will also be beneficial to preserve their condition, but may only be necessary if submission of the sample will be several days after sampling.

Completion of the 'PHD Diagnosis of diseased plant form': The information requested in this form is very valuable to appropriate processing of the sample so the form should be completed in full and submitted with the sample, or scanned and emailed by the time the sample is received by us. Management information requested in the bottom of the form may be crucial for us to provide helpful recommendations to resolve the problem in the future.

Pathogen screening tests of water, baits, seedlings or soil

Water samples: Ensure that the container used for sampling has previously been sterilized. We recommend autoclaving (if the container can handle temperatures of 121 °C) or chlorination with a solution containing super-high chlorine levels. We routinely use and recommend 10g/L of HTH calcium hypochlorite for sterilization of plastic bottles before each container to be sterilized is partially filled with this, capped, vigorously shaken for more than 1 minute before draining this into the next container needing sterilization to sterilize it. Just prior to sampling, each pre-sterilized container should be filled 50% or more with its water to be analysed and shaken vigorously >10 seconds before pouring to waste, which is repeated for a total of 3 rinses. After the last rinse the sample bottle should be filled to the top with its sample water and tightly capped. To run all available elective tests on a water sample we need almost 10L, and most clients use two 5L bottles for this. Water samples should be clearly labelled at sampling, such as with a water-insoluble koki pen.

Plant baits previously incubated in water: Several years ago, some clients were incubating leaf baits in their irrigation water in special containers for several days for detection of *Phytophthora* species and then submitting only the incubated baits to us for testing. This system has not recently been used, but we endorse this since it enables very sensitive pathogen detection as baits can be exposed at source in volumes much greater than the 4L we use for testing, while transport of the exposed baits instead of the bulky water samples drastically reduces the hassle and cost of submission. We are using a

combination of leaf baits of rhododendron leaves + camellia leaf disks + lemon leaf disks for our bait detection of *Phytophthora* species, and popcorn for detection of *Pythium* species.

Plant seedlings, cuttings or prunings: For the specific detection of certain target pathogens, especially in visually non-symptomatic plant tissue, small plant parts can be submitted to us in these forms. Up to 6 pooled plant parts (e.g. 6 seedlings or cuttings) can be combined for submission as one sample. We employ baiting, isolation or enrichment protocols for most of these tests, and for tests that might be positive according to initial results we often run PCR often followed by DNA sequencing of PCR amplicons to confirm. Plant samples are best wrapped in newspaper, that is in turn packaged in another bag or box. Refrigeration after sampling or in transit may be recommended if submission will be long after sampling. The packaging of each sample should be clearly labelled such as with a water-insoluble koki pen.

Soil or growing media: Soil is naturally home to millions of different types of micro-organisms and the detection of target plant pathogens that are often at very low levels in soil is very challenging. Currently we only offer tests for the detection of *Pythium*, *Phytopthora* and *Rhizoctonia* species in soil, since we can apply effective baiting strategies to detect them. We require at least 200 mL of soil for these tests. Soil should be put into a plastic bag which is in turn packaged in another bag or box. The packaging of each sample should be clearly labelled such as with a water-insoluble koki pen.

Completion of the 'PHD_Pathogens in water, baits, seedlings or soil_form': Only the relevant sections of the form need to be completed that correspond with the sample type and the elected tests.

Pathogen screening of plants by direct PCR.

Representative sampling: We have developed some tests that reliably detect certain pathogens in plant tissue by DNA extraction directly from plant tissue followed by PCR with target primers, and if positive with expected melt curve data then DNA sequencing of amplicons (outsourced). Small quantity plant samples of seedlings, cuttings or prunings can be tested for the listed pathogens. The listed PCR tests do not involve prior isolation or baiting of samples, and since we are limited to extracting DNA of about 0.1g plant tissue per sample this in turn limits the test sensitivity of target pathogens that are non-systemic in the tissue. For bulk tissue screening of pathogens unlikely to be systemic in the sample we therefore recommend submission via the 'PHD_Pathogens in water, baits, seedlings or soil_form' since most of those tests employ preliminary baiting or isolation from larger quantities. Plant samples are best submitted wrapped in newspaper that is packaged into a bag or box. Each sample's packaging should be clearly labelled such as with a water-insoluble koki pen.

Completion of the 'PHD_Pathogens in plants by PCR detection_form': Only the relevant sections of the form need to be completed specifying the sample type and the elected tests.

Soil and plant tissue nematode counts

Representative sampling: We process 200mL of representative soil for a soil nematode count which is a quantity typically processed by most reputable labs. We recommend sampling more soil than this from several nearby sampling positions and pooling this, mixing thoroughly and then submitting more than the required 200 mL of soil. Soil sampling is best done with a soil auger to a depth of 15 to 20 cm from several locations and then pooling and mixing the cores. We recommend submitting about 500 mL soil since we will again thoroughly mix the sample before sub-sampling 200 mL for processing. Cedara's Soil Science labs provide soil sample boxes of 500 mL volume which are also quite suitable for submission of soil nematode counts to us, though putting the soil in a plastic bag that is packaged in another box or bag is fine. Processing of plant tissue for nematode counts is sometimes relevant such as for root tissue with galls, and the form accommodates submission of plant samples too. Root material should be put into a plastic bag while above-ground plant tissue should be wrapped in newspaper that is packaged into another bag or box. The packaging of each sample should be clearly labelled such as with a water-insoluble koki pen.

Completion of the 'PHD_Nematode analysis_form': The form is simple to complete and one line for each soil sample or plant sample is allocated. Recording the current crop and the next likely crop to be planted there is very valuable, since the nematode count results are presented according to the nematode pathogenicity by common crops. We also strongly advise recording the GPS longitude and latitude of soil samples for nematode counts as results will be highly relevant to that location.