THE DIAGNOSTIC CENTRE

1 INTRODUCTION

The Diagnostic Centre, better known among South African citrus growers as the "DC", is a diagnostic facility created to assist growers with management decisions regarding plant protection. The DC laboratories are situated at the CRI in Nelspruit. Approximately 10 000 samples are analysed annually.

The DC concentrates mainly on soil-borne pathogens such as *Phytophthora* and *Pythium* and the citrus nematode *Tylenchulus semipenetrans*. These pathogens form part of the citrus root rot complex and are responsible, in combination with other factors such as soil compaction and ineffective irrigation, for the deterioration of a large number of the citrus trees currently established in South Africa. The DC is also responsible for testing red scale for resistance to organophosphates and the black spot fungus, *Guignardia citricarpa*, for resistance to benzimidazole fungicides.

The DC not only renders a service to commercial citrus growers, but also does analyses for the Cultivar Improvement Programme (CIP) to ensure that only disease-free trees are certified. In addition, samples are analysed for the various research workers at CRI and for chemical companies in the process of developing new plant protection products. Packhouse water is monitored at critical control points for fungal counts, total plate count and Coliform.

2 ANALYSES

Analyses conducted at the DC can be classified into three groups, *viz*. mycological or fungal, nematological and entomological.

2.1 Mycological analyses

2.1.1 *Phytophthora* and *Pythium*

Phytophthora is one of the most harmful pre-harvest fungal pathogens found on citrus. It is the most important pathogenic problem in the citrus nursery industry and is responsible for collar rot and root rot in established orchards.

To isolate *Phytophthora*, the DC uses the citrus leaf disc baiting technique. This method is qualitative and is based on the attraction of fungal zoospores by plant exudates from the leaf discs.
Like *Phytophthora, Pythium* is also a water-borne fungus. Its effect on seedlings can be as devastating as that of *Phytophthora*. In older orchards this fungus is often associated with over-irrigation. The same basic technique is used to isolate *Pythium* and *Phytophthora*, the only difference being the selective medium used to culture the fungus.

By only slightly changing the citrus leaf disc baiting technique, the DC has developed a spore baiting method to test irrigation water for the presence of *Pythium* and *Phytophthora*.

2.1.2 Other soil-borne fungi

The DC is also able to isolate a wide range of other soil-borne pathogens such as *Fusarium solani* and *F. oxysporum* which are also responsible for root decay under certain conditions. These analyses are usually made for research purposes and are at present of little commercial value.

2.1.3 Above-ground fungal diseases

There are a number of fungal pathogens that attack the fruit, leaves and branches of trees. The most common are *Guignardia citricarpa* (Black spot), *Alternaria, Diplodia, Colletotrichum* (anthracnose) and *Diaporthe citri* (melanose).

Since the lesions caused by some of these pathogens are often very similar, the causal pathogen needs to be isolated and identified before recommendations can be made.

Black spot resistance to the benzimidazole group of fungicides has increased in recent years. It is therefore extremely important that growers submit fruit samples to the DC whenever poor control of black spot is experienced, in order to determine whether this is due to resistance to the benzimidazole fungicides or to incorrect application procedures. If required, the fungi can also be screened to determine its pathogenicity, using the oatmeal agar technique as described by Baayen *et al*.

2.2 Nematological Analyses

2.2.1 Citrus nematode

More than 4 000 samples are analysed for citrus nematode annually. Analysis for commercial purposes is based on female counts in the roots; these being subject to the least variation during the year. In samples analysed for research purposes, larval counts in both the soil and roots are also taken into account.

2.2.2 Other nematodes

The DC is also able to undertake analysis of non-citrus samples for nematodes. Numerous tobacco, banana, litchi, paw-paw and sugar-cane samples are processed annually.

2.3 Entomological Analyses

Samples from citrus growers are analysed by the DC for red scale resistance to organophosphate insecticides. Resistance was identified in the Western and Northern Cape for the first time in 1996.

3 SAMPLING PROCEDURES

3.1 Irrigation water

A spore trap has been designed to monitor the presence of *Phytophthora* and *Pythium* spores in irrigation water (Figure 5.2). This method is mainly applicable to the nursery industry. The trap can be prepared and installed as follows:

- Use a plastic container, approximately 100 mm in height and 80 mm in diameter, with a tight-fitting, screw-on lid. The container should be thoroughly cleaned before use. Metal containers are not suitable, as these may release ions capable of destroying the fungal zoospores.

- Drill about 10 holes of 3 mm diameter around, and approximately 20 mm below, the top edge of the container. Insert a dripper, connected to the main irrigation line, into the container by passing it through a hole in the lid and allow the container to fill with water. (Drippers capable of delivering two litres water/hour at a pressure of 50 to 500 kPa are commercially available.)

- Place approximately 20 citrus leaf discs, about 4 mm in diameter, on the water in the container. These discs are obtained by punching holes in fully grown citrus leaves collected from trees that have neither been treated with systemic fungicides such as Ridomil, Aliette, Phytophos or Phytex for the control of root rot, nor sprayed with fungicides such as copper.

- The top part of the container should be covered to exclude all light. The container should preferably be installed in a pumphouse and should be absolutely level so that water will leak simultaneously from all the holes.

- Allow water to pass slowly through the trap for three to four days. Excess water will escape through the holes around the top. If the container is not level or if the dripper is set too fast, the zoospores, if any, will simply be carried away, as it will not be possible for them to be attracted chemostatically to the citrus leaf discs.

- The discs are then removed from the container, blotted dry with tissue paper and transferred to petri-dishes containing a selective medium supplied by the DC. Seven leaf discs are placed in each of two petri-dishes. The petri-dishes are then sealed with masking tape and speed-mailed to the DC for incubation and identification of the pathogens present.

3.2 Soil

In established orchards, soil can be tested for the presence of *Phytophthora.* The following sampling method should be used:

- The soil in the orchard to be sampled should be moist.

- In the case of routine sampling, select 20 trees at random in an orchard block comprising a maximum of 1 000 trees. Larger orchards should be subdivided. If different rootstocks or different soil types are present, these should be demarcated and sampled separately.

- If isolated trees in an orchard show signs of deterioration, they should specifically be sampled. Do not postpone sampling until such deterioration has reached an advanced stage, as secondary pathogens will by then have taken over from the primary pathogen, thus making it difficult to diagnose the problem correctly.

- One subsample should be taken at each of the 20 selected trees.

- The subsample should be taken halfway between the trunk and the drip line of the tree.

  Remove any weeds as well as the top 2 cm of soil in the area to be sampled. Use a spade to collect a sample from the top 15 cm of soil. Each sub-sample should consist of about 20 g of soil and feeder roots (approximately one handful).

- One composite sample from an orchard should consist of approximately 400 g soil.

- Place the sample in a plastic bag, press out any excess air, seal and store in a cool place (at not less than 4°C) until dispatched to the DC. Use a cardboard box if a coolbag is not available and store away from direct sunlight.

3.3 Roots

Isolating *Phytophthora* successfully from the roots is often difficult. However, the sampling procedure is the same as for soil, except that roots of pencil thickness and thinner are also collected and placed in the moist soil.

3.4 Bark

Phytophthora is the main cause of collar rot. However, not all collar rots are caused by *Phytophthora*. In most cases lesions occur at ground level. To identify the causal organism a soil sample should therefore be taken at soil level where the trunk and the soil meet.

3.5 Nematodes

The same procedure as described for *Phytophthora* roots and soil should be used for nematodes. For nematode analysis, 40 g feeder roots (1 large handful) should be included in the sample. Do not separate the roots from the soil. Since the feeder roots, on which nematodes feed, are only about 1 to 2 mm in thickness, roots no thicker than this should be collected.

3.6 Above-ground fungi

Use pruning shears to cut a 20 cm piece from the infected branch, 10 cm of which should consist of healthy tissue and the remainder of infected tissue. Place in a paper bag and send to the DC as soon as possible. Drying out or secondary infections will complicate analysis of this type of material. Ideally therefore, samples should be delivered personally to the DC as soon as possible to eliminate the possibility of contamination during dispatch.

3.7 Red scale resistance tests

Fruit collected for red scale resistance tests should be infested with live, crawler-producing female red scale. The fruit should be collected at random from different trees in the orchard.

The sample should comprise 25 fruit, each with 100 to 300 live, mature female red scale. If the female count per fruit is less, correspondingly more fruit should be collected. The fruit should be placed in a paper bag or a carton box and should not be wrapped in plastic.

3.8 Black spot resistance to the benzimidazole fungicides

Collect 20 fruit with virulent-spot blemishes from different trees in the orchard. Place them in a paper bag or a box.

4 DISPATCH OF SAMPLES

Samples should be clearly marked. Do not write on plastic bags, as the ink rubs off easily. Use stickers or adhesive tape to label each bag. Do not put numbers inside the bag of soil as the paper will disintegrate.

Number the samples with the orchard numbers given on the application for analysis form.

4.1 Analysis documents

Application for analysis forms must be completed in full. The following information required on the standard form is of critical importance:

- grower's name and address
- orchard number
- scion/rootstock combination (replant or not)
- previous fungicide/nematicide applications in the case of soilborne pathogens
- type of analysis required

Analysis forms are obtainable from the DC (ph. 013 759 8000).

4.2 Dispatch

Samples should be dispatched as soon as possible after being taken. Moist soil and roots, if stored at room temperature, can be analysed up to six weeks after sampling.

Phytophthora as well as citrus nematode and red scale are sensitive to solarisation. Soil in a plastic bag left for only one hour in a hot vehicle will not yield meaningful results when analysed. Although a coolbag is ideal for packing samples, a cardboard box lined with newspaper will also do.
Samples should be sent to:

Postal address:	Street address:
Diagnostic Centre	Diagnostic Centre
CRI	CRI
P O Box 28	2 Baker Street
NELSPRUIT	NELSPRUIT
1200	1201

4.3 Results

Results are available approximately 10 days from the date the samples are received. Nematode results can be made available sooner, as no incubation is required. If requested, results can be emailed or faxed through as soon as they become available. Results are always posted to the client as well. To test for black spot resistance to benzimidazole fungicides takes 3-4 weeks.

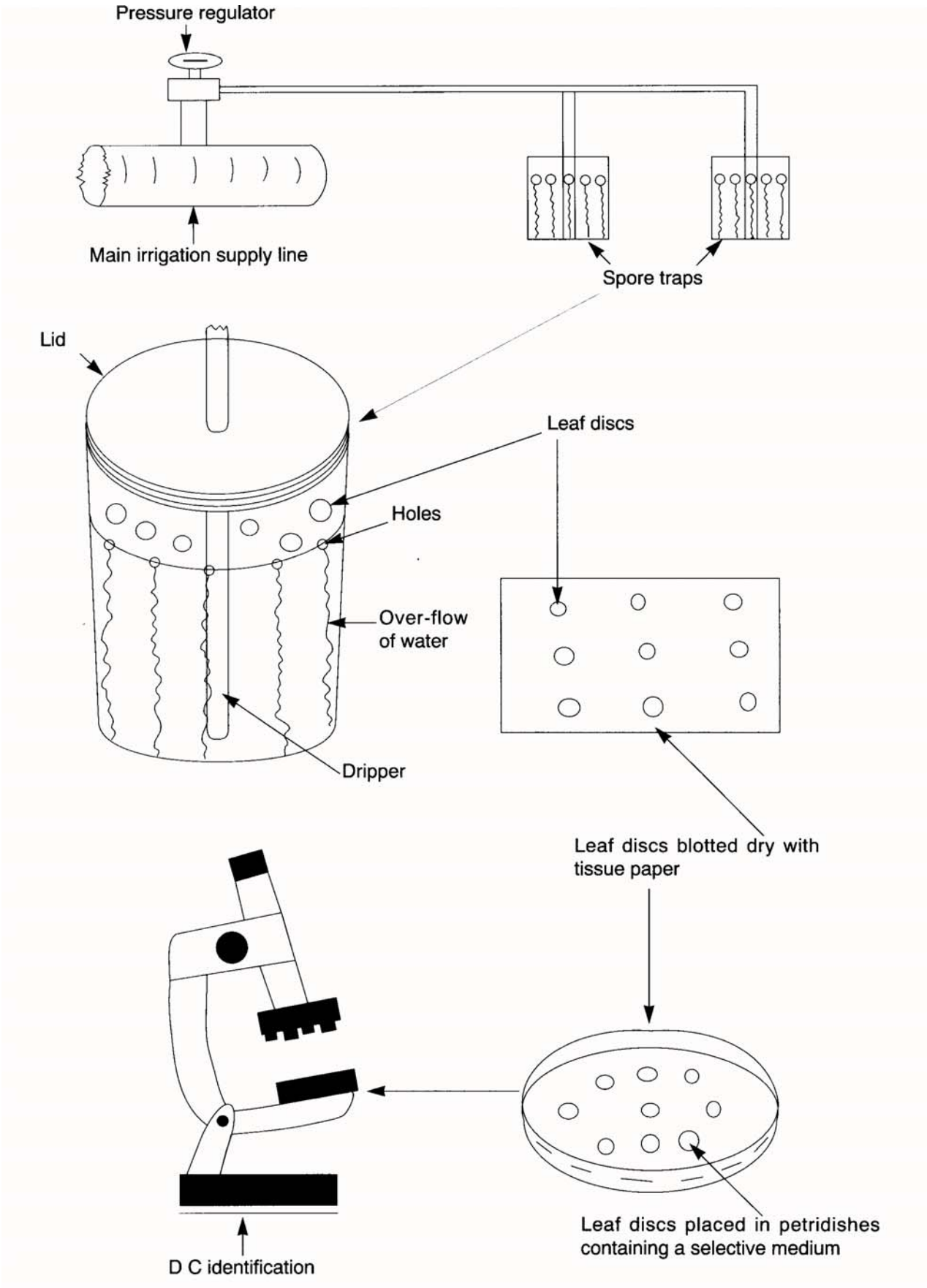

Figure 5.2. *Phytophthora / Pythium* spore trap used to analyse irrigation water.