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SAMPLING GUIDELINE FOR:

IDENTIFICATION OF DISEASES AND PESTS

DIAGNOSTIC SERVICES PROVIDED BY THE PLANT PROTECTION DIVISION, ARC INFRUITEC-NIETVOORBIJ:

- Identification of fungal diseases
- Identification of bacterial and phytoplasma diseases
- Nematode identification and counts
- Bud mite analysis
- Bud fertility
- Mealy bug trap counts
- Identification of insect pests
- Other services

INFORMATION REQUIRED WHEN A SAMPLE IS SUBMITTED FOR ANALYSIS

The quality of the diagnosis is dependent on the quality of the sample and submission form.

- **Complete the Diagnostic services form. This form can accompany the sample or can be emailed to the contact person.** Customer information: name, invoice address and contact information of the producer as well as the person submitting the sample.
- Information on the sample submitted: material submitted (soil, roots, whole plant, leaves, etc.), cultivar, rootstock, age of vine/planting date and certification status of plant material.
- A short description of why the sample was sent for analyses including, description of symptoms in the field and the distribution of symptoms in the block/on the farm.

Below find sampling guidelines for the different analyses.

INVESTIGATIVE PROCEDURE FOR ALL SAMPLES

Upon arrival at the diagnostic laboratory, the sample is visually examined to determine which process of analysis should be followed. The information on the attached form, together with the characteristic symptoms observed, will all contribute to a more comprehensive diagnosis being made. In the case of stem diseases, a visual examination is insufficient, and full analyses are strongly recommended.

Please note that if you are uncertain regarding what analyses to request, feel free to inform one of the relevant contact people on this form. We will then investigate the sample and advise you on which analyses to include.

SAMPLING FOR DIAGNOSIS OF FUNGAL PATHOGENS (Soil-borne

(Contact Dr Minette Havenga, 021 809 3161/HavengaM@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

- Where possible, the entire vine/tree/plant with roots and a soil sample should be submitted.
- If impractical, large vines/trees can be divided into pieces. Keep graft union and crown intact and include roots.
- Send a representative sample that of the observed problem the vineyard/orchard.
- Please do not submit dead plants.
- To prevent drying out, do not leave samples in the sun, place the root system of the sample in a bag with soil.
- Soil sample should be taken where most root growth occurs, approximately 30 cm from ground level.
- Soil samples with fine roots (approximately 1 kg) should be placed in a plastic bag to prevent drying out.
- *Deliver samples to the laboratory on the Nietvoorbij campus (R44/Adam Tas Road, Stellenbosch) for analysis as soon as possible.*

SAMPLING FOR DIAGNOSIS OF FUNGAL DISEASES (foliar diseases including symptoms observed on shoots and clusters)

(Contact Dr Minette Havenga, 021 809 3161/HavengaM@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

- Includes identification of powdery mildew, downy mildew, stripe spot, Botrytis, Anthracnose, etc.
- Wrap leaves, shoots or bunches in newspaper or paper towel. Place in a plastic or paper bag to prevent sample drying out.
- *Deliver samples to the laboratory on the Nietvoorbij campus (R44/Adam Tas Road, Stellenbosch) for analysis as soon as possible.*

SAMPLING FOR DIAGNOSIS OF BACTERIAL DISEASES

(Contact Dr Yolanda Petersen, 021 809 3479/PetersenY@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

- Collect plant parts displaying symptoms.
- Do not submit dead plants. Send a plant that is in the process of dying, i.e. living and dead tissue is present on the sample.
- Prevent samples from drying out by placing them in a plastic bag and keep them cool at 4^{o}C until the sample can be dispatched (preferably within 24 - 48hours).
- *Deliver samples to the laboratory on the Infruitec campus (Lelie Street, Stellenbosch) for analysis as soon as possible.*

NEMATODE ANALYSIS AND COUNTS

(Contact Dr Rinus Knoetze, 021 809 3461/KnoetzeR@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

General

- Samples can be taken throughout the year.
- Avoid sampling wet soil with lumps or fine, dry or powdery soil.
- Samples should also not be taken close to dead trees or in “dead” spots.
- Samples taken from barren or brackish soil will also give inaccurate results.
- Samples should be collected systematically, not randomly. This could either be in a zig-zag, W or V pattern at equally spaced points that cover the whole sampling unit.
- The sample area should be smaller than 3 hectares.
- Soil samples should be collected at a 10 - 40 cm depth. The top 5 cm of soil must be removed to ensure that roots from weeds or other herbaceous plants are not sampled.
- It is important to include fine hair roots in the sample to detect the presence of endoparasitic nematodes.
- A representative sample must consist of 10 - 20 thoroughly mixed sub-samples, adding up to at least 2 kg of soil and 10 g of fine roots.
- The soil containing roots must be placed in a plastic bag and properly sealed. Each bag must be marked on the outside. Information should never be placed inside the plastic bag.
- Samples should be delivered as soon as possible and can be stored for short periods but must never be kept in the back of delivery vehicles or the boot of a car.
- Samples must always be taken in the root zone, where new, active root growth takes place.
- *Deliver samples to the laboratory on the Infruitec Northern Campus (Lelie Street, Stellenbosch).*

Annual crops

- Samples must at all times be taken when a crop is still present.
- Samples must always be taken in the root zone, where new, active root growth takes place.
- In the case of tuberous crops (potato, sweet potato, bulbs etc.), tubers should also be included in the sample.

Perennial crops

- Samples can be taken throughout the year. However, counts are highest during the warmer summer months.
- If you want to compare nematode numbers over time, the samples must be taken at the same time every year.
- Samples from different rootstocks and plant ages must be separated.
- In the case of trees/vines being replanted, it is important that the samples are taken before soil preparation.

BUD MITE ANALYSIS

(Contact Dr Abongile Mbande, 021 809 3357/MbandeA@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

- Collect samples after leaf drop, but before or during pruning.
- Sample 12 canes per hectare.
- Each cane must contain 6 basal buds (closest to the cordon). Only buds 1,3 and 5 will be analysed.
- The canes must be selected to represent a 1 hectare area.
- Each sample must be clearly labelled with the farm name, block number and cultivar information.
- As soon as the canes are cut, do not allow the canes to lie in the sun or dry out. Store canes in a fridge or cold room, but never in a freezer. Package the sample by wrapping the canes in cling wrap or place them into a bag and ensure to deliver to the ARC as soon as possible.
- A bud mite report will reflect the level of infestation of mites inside the buds.
- *Deliver samples to the laboratory on the Infruitec Northern Campus (Lelie Street, Stellenbosch).*

BUD FERTILITY (AND BUD MITE) ANALYSIS

(Contact Dr Abongile Mbande, 021 809 3357/MbandeA@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

- Collect samples after leaf drop, but before or during pruning.
- Table grape cultivars: sample 10 canes per hectare. Each cane must contain the first 10 basal buds (closest to the cordon).
- Wine grape cultivars: sample at least 10 canes per hectare. Each cane must contain the first 6 basal buds (closest to the cordon).
- Raisin grape cultivars: sample 10 canes per hectare. Each cane must contain the first 12 basal buds (closest to the cordon).
- Select canes to represent 1 hectare area.
- Each sample must be clearly labelled with the farm name, block number and cultivar information.

- As soon as the canes for a block are cut, do not allow the canes to lie in the sun or dry out. Store canes in a fridge or cold room, but never in a freezer. Package the sample by wrapping the canes in cling wrap or place them into a bag and ensure to deliver to the ARC as soon as possible.
- A bud fertility report will reflect whether the bud is reproductive, vegetative or dead as well as the level of infestation of mites inside the buds.
- *Deliver samples to the laboratory on the Infruitec Northern Campus (Lelie Street, Stellenbosch).*

MEALYBUG TRAP COUNTS

(Contact Mr Levocia Williams, 021 809 3425/WilliamsL@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

Pheromone trap protocol

- Use commercially available Grape Vine Mealybug (GVM) pheromone capsules, yellow delta trap & sticky pads.
- Replace the pheromone capsule every three months.
- Trapping starts in October up to harvest.
- After harvest, pheromone trapping in commercial blocks should continue on a monthly basis if high infestation levels were recorded.
- Out of season pheromone trapping should continue throughout the year in propagation and quarantine units.
- Collect and change the sticky pads bi-weekly.
- When collecting the sticky pads, they should either be covered with cling wrap or put into a PVC sleeve to prevent them from getting stuck to one another and submitted for counting.

Pheromone trap position

- Traps can be hung on or above the cordon, attached to the trellis wire.
- The trap's open ends should be exposed so that GVM males can easily fly into the triangular centre.
- Traps should be hung at a rate of 1 trap every 1 hectare.
- Place traps in areas of high human movement i.e. roads, end of rows, near to packhouses, etc.
- A bi-weekly trap count of 65 or more should be followed by physical monitoring (see below).

Physical monitoring

- This monitoring is essential, but only necessary when the above threshold has been reached.
- Draft a vineyard plan of a specific block, indicating number of rows and number of sections per row.
- Select 20 sections with 5 grapevines each, proportionally throughout the block.
- Monitor each of the 5 grapevines in each section, in the areas of new growth.
- Record the presence/absence of GVM on each vine.
- The total number of infested vines will indicate the percentage GVM infestation for that block.

- Threshold: stem infestation rates of above 2% warrants control action or begin release of commercial natural enemies.
- Physical monitoring is necessary before control action can follow.

Deliver samples to the laboratory on the Infruitec Northern Campus (Lelie Street, Stellenbosch).

IDENTIFICATION OF INSECT PESTS

(Contact Dr Abongile Mbande, 021 809 3357/MbandeA@arc.agric.za or or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

- Send a sample of the symptoms/damage. If no fresh material (shoots/leaves/fruit) is available, a clear colour photograph can be sent.
- Send a sample of the insect (or other organism), if possible. Mites and insects (especially soft types such as mealybugs or insect larvae) can be preserved and shipped in a 70% solution of ethanol (ethyl alcohol) in water. Ethanol can be obtained from a pharmacy.
- Larger insects can be killed using household insecticide and then carefully packed in a container. The container should either not be sealed tightly or be perforated with a few small holes to prevent the sample from rotting before it can be identified.
- *Deliver samples to the laboratory on the Infruitec Northern Campus (Lelie Street, Stellenbosch).*

OTHER SERVICES

SUPPLY OF LESION AND ROOT-KNOT NEMATODE INOCULUM

(Contact Dr Rinus Knoetze, 021 809 3461/KnoetzeR@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

At our facility, we maintain cultures of lesion nematodes (*Pratylenchus* spp.) and root-knot nematodes (*Meloidogyne* spp.) which are all species-verified using molecular methods.

On request, we can multiply and deliver nematode inoculum tailored to your research needs:

- Lesion nematodes: Grown *in vitro* on carrot discs for high purity.
- Root-knot nematodes: Mass-reared *in vivo* on tomato plants for vigorous populations.

Available lesion nematode species include:

- *P. hippeastri*
- *P. penetrans*
- *P. vulnus*
- *P. thornei*
- *P. vanderbergae*
- *P. bolivianus*

Available root-knot nematode species include:

- *M. incognita*
- *M. javanica*

SUPPLY OF FUNGAL INOCULUM

(Contact Dr Minette Havenga, 021 809 3161/HavengaM@arc.agric.za or Ms Alison Moody, 021 809 3484/MoodyA@arc.agric.za for further information)

At our facility, we maintain fungal cultures that can be used in inoculation trials. Spore suspension will be prepared for trials. Fungal isolates include but are not limited to:

- *Botrytis cinerea*
- *Colletotrichum gloeosporioides*
- *Alternaria alternata*