RFSR007

UF UNIVERSITY of FLORIDA
IFAS Extension

Sample Submission Guide for Plant Diagnostic Clinics of the Florida Plant Diagnostic Network. [1]

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Florida Extension Plant Diagnostic Clinics

The primary role of the Florida Extension Plant Diagnostic Clinics (FEPDC) is to determine whether symptoms in submitted plant samples involve an infectious causal agent, e.g. fungus, bacterium or virus, or other cultural or environmental factor that causes similar symptoms. The goal of the FEPDC system is to educate clientele by providing plant disease and disorder diagnoses and recommendations for preventative and therapeutic measures. The FEPDC is a fee-based service provided to any Florida resident by the Plant Pathology Department of the Institute of Food and Agricultural Sciences (IFAS), University of Florida, in conjunction with the Cooperative Extension Service. The FEPDC is open from 8:00 AM to 12:00 PM and 1:00 to 5:00 PM Monday through Friday (except for University holidays). The FEPDC consists of five UF/IFAS laboratories:

Gainesville:

Florida Extension Plant Diagnostic Clinic (SPDN hub laboratory)

Box 110830, U.F., Bldg. 78, Mowry Rd.,

University of Florida, Gainesville, FL 32611-0830.

Note: For overnight mail or package delivery service (UPS, FedEx, etc.), be sure to include the physical street address: "UF, Bldg. 78, Mowry Rd."

http://plantpath.ifas.ufl.edu/pdc/

Phone: (352) 392-1795

FAX: (352) 392-3438

Quincy:

Florida Extension Plant Diagnostic Clinic-Quincy.

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http://tmomol.ifas.ufl.edu/pdc.htm

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Immokalee:

Florida Extension Plant Diagnostic Clinic-Immokalee

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Golf Coast REC, 14625 C.R. 672, Wimauma, FL 33598

Phone: (813) 633-4133

FAX (813) 634-0001

E-mail: naperes@ufl.edu

Homestead:

Florida Extension Plant Diagnostic Clinic-Homestead

Tropical REC, 18905 S.W. 280th St., Homestead, FL 33031-3314

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Plant Sample Submission and Diagnoses

Plant disease diagnosis depends in large part upon the quality of the sample received and the information provided by the submitter. For complete, accurate and timely service, please note the following FEPDC policies:

1. All plant samples should originate within the geographical boundaries of the state of Florida or be accompanied by appropriate USDA/DACS plant importation permits. The PDCs in Gainesville and Immokalee hold a permit to receive "unknown" plant samples from states within the contiguous U.S. for diagnostic purposes, in support of their role as the hub for the Southern Plant Diagnostic Network.

2. Plant samples must be of adequate quality and quantity (see below) and be accompanied by a completed Plant Clinic Diagnostic Form or equivalent information. All potential users should first contact their local county extension office (http://solutionsforyourlife.ufl.edu/map/) and consult with a county agent before submitting a plant sample to the FEPDC. Diagnostic forms are available at all county extension offices, directly from FEPDC locations, or online at the clinic'"s website.

Direct links to PDF forms:

Gainesville

http://plantpath.ifas.ufl.edu/pdc/submission_forms/PDC_submission_form.pdf

Quincy (HTML version)

http://tmomol.ifas.ufl.edu/sampleform.htm

Immokalee

http://www.imok.ufl.edu/plant/clinic/plantfrm.pdf

Homestead

http://trecclinic.ifas.ufl.edu/pdf/TREC-CLINIC-FORM-09-07.pdf

3. Plant diagnostic determination through the FEPDC has been a fee-based service since December 1993. Please make remittance payable to the "University of Florida - FEPDC." There is a single base fee per sample of $20.00 at all clinic locations. Some specialized tests and services to confirm certain diagnoses are more expensive and will require a fee beyond the base fee; FEPDC staff will consult with you if this is necessary. If the specific pathogen cannot be identified with "in-house" diagnostic tools, it can be sent to a private or state lab for further testing at the client's request. The cost to the client will be the single base fee plus the fees charged by the private or state lab.

4. Samples can be submitted to the FEPDC via mail or delivered to the FEPDC from the site of origin (e.g. homeowner, farmer, nursery, etc.). Clientele using the FEPDC often (more than 5 times per month) can arrange for monthly invoicing by contacting FEPDC staff. Guidelines for submitting plant disease samples for DDIS (Distance Diagnostic and Identification System) are found in the EDIS publication Guidelines for Submitting Plant Disease Samples Using the Distance Diagnostic and Identification System *(DDIS)* (http://edis.ifas.ufl.edu/DDIS1).

5. As each sample is received at an FEPDC location, it is given a number and recorded. Samples are routinely processed on a "first come--first served" basis. Depending upon the nature of the problem, some diagnostic techniques take longer than others. FEPDC staff will notify clients about diagnostic processes that will require more than 7 days for completion.

6. Plant disease determinations and associated control options are sent to the clientele via mail, email, or fax depending on the contact information submitted with the sample. The results of these samples are electronically mailed or faxed to the extension faculty in the county of sample origin to keep them informed about active local plant diseases.

Appropriate Sample Quality and Quantity

Many of the pathogens that cause plant diseases have the ability to exist both as disease-causing microbes and as saprophytes on plants or in soil. The presence of a particular organism does not always implicate disease. Sample quality will often determine whether clinic staff can interpret the presence of a pathogen in a sample as being disease-related. The local county agent should be cognizant of the other variables that may cause plant dysfunctions that mimic or interact with plant disease. They are often in the best position to make the final diagnosis.

Samples that exhibit early symptom development and have plant parts that are still partially alive (green) offer the best quality samples for accurate disease diagnosis. Samples that are totally necrotic, dry, and long dead are not adequate for an accurate diagnosis. Dead tissue is essentially a food source for many saprophytic microbes, hence determination of the primary pathogen is often impossible. The best samples arriving at the FEPDC represent early stages of disease development (i.e. before the plant part is totally necrotic or turns into mush). Samples should be packaged and shipped carefully so plant symptoms remain relatively unchanged when they arrive at the FEPDC. Samples should be accompanied by a completed data form, which is separated from the tissue and soil. Many times the accuracy of a plant diagnosis depends on the variety of techniques used on any one sample and the frequency with which a particular pathogen is identified by these techniques through replicated processing. For example, a leaf spot on geranium will be processed with the following techniques:

1. Visual examination to decide on parasitic vs. nonparasitic involvement.

2. Low and high power microscopic examination to look for pathogen signs, such as bacterial flow or fungal spores. This will require a minimum of 3 to 4 spots on 3 to 4 leaves.

3. Placement of several leaves in a high humidity chamber to stimulate reproduction of a suspected pathogen. This will use 3 to 8 leaves depending on leaf size and lesion frequency.

4. Culturing of leaf spots on two or more general or selective growth media for pathogen identification. At least 10 to 12 leaf spots from 3 to 6 leaves will be used to replicate these culturing tests.

5. Streaking of macerated tissue onto two or more general bacteriological media. At least 3 leaves and 3 leaf spots will be used for this procedure.

These techniques require adequate amounts of tissue. This sample would require at least a dozen leaves with multiple leaf spots present. If a two-leaf sample were received, two or more of the above techniques could not be performed because there would not be enough tissue. As technique selections decrease, so does accuracy! If the disease problem is important enough to be submitted, it should be accompanied with enough tissue for clinic staff to process it adequately. Remember, the accuracy of the FEPDC diagnosis is directly correlated with obtaining an adequate quantity and quality of sample. Most samples are processed within 7 days, but some must be subjected to more time-consuming procedures so the "turn-around time" may take longer.

General Sample Submission Guidelines

1. Take samples before applying pesticides; otherwise the ability to recover pathogens may be limited.

2. Submit generous amounts of plant material representing a range of symptoms. (See below for specific scenarios).

3. Don't add water or pack a sample that is wet.

4. Keep samples refrigerated after collection until they are submitted. After collecting good samples, do not allow them to bake in the sun or on the back seat of a car prior to submission because doing so will ruin them.

5. Do not mix different samples in the same submission bag. Moisture from root samples will contribute to the decay of foliage samples if they are mixed together.

6. Plant disease identification procedures do not utilize soil. Excess soil can be hand shaken from root systems but leave enough soil to keep roots at field moisture levels.

7. Please mark sample packages with a "Warning" if sample has thorns or spines.

8. All samples must be accompanied with a completed Plant Clinic Diagnostic Form. These are available at all county Extension offices or on-line. Give complete information on the form and keep it separate from the sample. Complete a separate form for each sample and plant problem. You are encouraged to include any pertinent information under the remarks area of the form. The more information available the more likely a problem can be associated with possible causes.

9. Remember to note recent pesticide history on the Plant Diagnostic Form accompanying the sample.

10. Samples arriving from sites in Florida that are 2 days or less mailing time from a clinic, can be sealed in plastic bags for shipping.

11. Samples arriving from distances greater than 2 days mailing time from a clinic should be packed tightly in a box with dry paper. Do not seal in plastic because of the likelihood of sample deterioration during the mailing period. Do not add moisture.

12. Mail samples early in the week to avoid the weekend layover in the post office.

13. For emergency samples, use overnight courier services or US overnight mail.

14. See guidelines for specific type of plant dysfunction.

Note: FEPDC staff reserve the right to immediately discard any sample not meeting the above submission criteria. Submitters will be notified when such action is taken. Please obtain the appropriate sample before submission. This will save both time and expense.

Specific Collection and Submission Guidelines

Suspected High-risk Plant Pathogens

1. Determine that the sample in question is possibly a high-impact disease by consulting available Extension literature and notices.
2. Notify appropriate county or laboratory personnel that the sample will be coming to them and request collection, packaging and mailing instructions specific to that disease, as needed. General guidelines for sample collection and packaging appear below.
3. Wrap the sample in dry paper towels and place in a zip-top bag.
4. Disinfest the outside of the bag (an alcohol hand gel works well for this purpose) and place inside a second plastic bag, along with a completed Sample Submission form.
5. Place the bagged sample inside a sturdy, crush-proof box and seal the edges with packing tape.
6. Use a mailing or delivery service with appropriate delivery timing; quick delivery may be crucial for accurate and timely diagnoses and response.

Cankers and Galls

1. Prune out swollen or galled tissue several inches below symptoms. Collect several galls for a minimum sample size. Avoid old, weathered, long dead samples.
2. Submit dry samples for Clinic processing.

Diebacks

1. Prune dieback tissue from the affected plant; include some living tissue.
2. Wrap prunings in dry paper towels or newsprint and place in a box or mailer.
3. Collect root samples in case the dieback is a secondary symptom of a root rot disease.
4. Submit the roots (not soil) in strong, sealable plastic bags. Include these bags in the box with the prunings.

Fruit Diseases

Fruit samples are often high in water content. Therefore, they decay rapidly in the mail and are subject to considerable bruising.

1. Please accurately describe fruit symptoms on the Plant Diagnostic Form prior to submission because fruit tissue may change color and begin to decay in transit.
2. Do not enclose fruit in plastic bags, plastic wrap, aluminum foil or other moisture-holding coverings that encourage decay.
3. Wrap fruit in dry toweling or paper and pack firmly in a non-crushable container to prevent bruising in transit.
4. Place the Sample Submission Form in a plastic bag; avoid packing the form with the fruit so as to preserve the form.

Spots and Blights of Leaves and Flowers

Leaf diseases probably represent the largest category of plant diseases. There are many types, shapes, and colors of leaf spots and blights that occur on plant material in Florida

1. Describe leaf disease symptoms because leaf tissue will change color and begin to decay in transit.
2. Collect at least 12 leaves with diseased tissue for a minimal sample size. All tests are replicated in

the clinic, thereby requiring 10 to 20 leaf spots per sample.

3. Obtain a range of disease stages from early infection through older infected areas. Press leaf tissue between cardboard, paper, or dry toweling so that the tissue does not become brittle and fracture in the mail. Avoid sealing succulent foliage in airtight plastic bags. Dry tissue can be rehydrated but rotted tissue is useless for Clinic processing.

Mushroom Identification

Many mushrooms submitted for identification are forwarded to Dr. James Kimbrough of the Plant Pathology Department for assistance. When these samples are identified, the results are sent back to the clinic and the answer forwarded to the appropriate county.

1. Note the collecting date, collection location, and/or associated plant material.
2. Do not add water or soil to mushrooms prior to mailing.
3. Do not seal samples in a moisture proof bag; pack them dry.
4. Do not seal the Plant Diagnosis Form in with the samples; place the form in a plastic bag.

Systemic Symptoms

Plants exhibiting such systemic symptoms as general yellowing, browning, wilting, stunting etc., may reflect complex disease problems, and can be in conjunction with cultural disorders such as drought, flooding, nutrient levels, etc. In these cases, the symptomatic tissue may not be the primary site of colonization by a plant pathogen. Symptoms such as wilting, chlorosis, leaf drop, dieback, and decline often are the result of root damage or root disease. For accurate diagnosis of these problems, the entire plant is needed for processing through the clinic. Send whole plants showing a range of symptoms with roots and adjacent soil intact.

1. Dig the plant from the growing site if plant size allows.
2. Shake off the excess soil and seal the root ball in a plastic bag and tie the bag around the stem at soil level. Detach the plant top from the roots if this facilitates packing the sample.
3. Do not wet the tops or add moisture to the root ball prior to submission.
4. Appropriate sub samples of foliage, branches, stems, and roots must be collected to define the cause of systemic symptoms of plants too large to be removed from the growing site.
5. Always collect a minimum of a sandwich-sized plastic bag of feeder roots from the plant side exhibiting decline or dieback symptoms.
6. Collect as much information concerning the sample as possible and submit the completed form to the clinic.

Turf Samples

Rapid Turfgrass Diagnostic Service (http://turf.ufl.edu): Turfgrass samples that require a rapid turn-around time and specific fungicide management options may be sent for the Rapid Turfgrass Diagnostic Service at the Gainesville lab. The sample fee for this service is currently $75.00. Additional information pertaining to this service is available on http://turf.ufl.edu by clicking on Rapid Turf Diagnostics.

Virus Verification

Commercial producers of plants in Florida have some control measures for viral diseases; hence the identity of the virus can be important. Conversely, for most urban dwellers, the identification of a particular virus is an academic pursuit since control measures are few and poorly effective. If an individual desires the identification of a specific virus problem, special care must be given to sample collection and submission. Please observe the following rules for suspected virus samples:

1. Collect fresh, symptomatic and unwilted tissue for submission. Wilted or necrotic samples cannot be used for virus identification.

2. Collect apparently healthy plant tissue as a control sample at the same time.

3. If whole plants cannot be sent, wrap the cut ends in wet toweling and seal the sample in a plastic bag.

4. Note the insects on the samples, if present, as many viruses are spread by insect vectors.

5. Additional tests for specific virus identification will result in additional costs beyond the basic sample fee. . . The diagnostician will contact the submitter for further consultation if it is determined that additional testing is necessary. . .

6. Submit the samples by one of the following options:

    • Overnight U.S. mail

    • Overnight special courier

    • Deliver it in person to the nearest FEPDC facility

Digital Diagnosis

Access to the Digital Diagnostics Information System is available at:
http://ddis.ifas.ufl.edu/ddisx/jsp/html/guidelines.jsp
This system enables users to complete an online sample submission form and attach digital images of plant problems. . . The user can then choose which experts to send the sample to, and multiple experts can be notified with one sample submission. . . This system can be used in conjunction with sending a physical sample to a laboratory, giving the diagnostician a head start on the symptoms and sample information. . . Some diagnoses are not possible with a digital sample alone and the diagnostician may request a physical sample. As with physical samples, the better the sample and accompanying information, the better the diagnosis.

1. Register an account with the system, or log into the system with your existing account.

2. Fill out the sample form as completely as possible.

3. Select appropriate experts from within the UF system, or add experts outside the system who may be appropriate.

4. Attach multiple images of the sample, including images of the surrounding area, close-up (macro) images of the affected plant parts, and clear images of the symptoms. Familiarity with the macro and lighting options of the digital camera or scanner will produce better results.

5. A minimum of 300 dpi is recommended for image resolution to allow for best viewing by the diagnostician.

Interpretation

FEPDC staff will carry out laboratory tests to attempt to demonstrate an association of plant symptoms and signs with a pathogen-caused disease. However, due to the nature of plant diseases and the many environmental factors that affect plant health, it may not be possible to positively correlate a plant problem with one particular pathogen. Recipients of diagnostic results should be aware of other variables that might be associated with the plant dysfunction observed.

Whenever possible, the response you receive about a sample will include disease management information. Often, disease management can be lengthy and complex, and may involve cultural management as well as chemical controls. These cultural recommendations are important and may make the management strategies more effective, even if the client chooses to apply a chemical treatment. Some cultural recommendations will be applicable to the next season's crop. Many Plant Pathology Plant Protection Pointers, Fact Sheets, and Plant Disease Control Guides contain other necessary disease management information, and these may be referenced in the diagnostic report. These guides are available at http://edis.ifas.ufl.edu

and http://plantpath.ifas.ufl.edu/takextpub.

Services Provided Upon Special Request

The following procedures may be recommended after discussion with FEPDC staff. These procedures are both time-consuming and more costly than the base fee charge for the sample.

1. Laboratory verification of the palm phytoplasmas responsible for lethal yellowing and Texas Phoenix palm decline by PCR methods. . . Here is additional information on how to properly sample for phytoplasmas affecting palms: http://flrec.ifas.ufl.edu/pdfs/LY-TPPD-Trunk-Sampling.pdf

2. Accurate diagnosis of the subspecies of the fungus responsible for Fusarium wilt of Phoenix palms (*Fusarium oxysporum* f.sp. *canariensis*) by PCR methods.

3. Accurate diagnosis of the subspecies of the fungus responsible for Fusarium decline of queen palms and Mexican fan palms by DNA sequencing.

4. Identification of bacterial species and subspecies when necessary by fatty acid analysis techniques.

The following services are time-consuming and are available only upon specific request and after consultation with FEPDC staff; please call before sending samples:

1. Pathogen determinations from water sources.

2. Pathogen determinations from soil or growing media by baiting or culturing methods.

3. Race determination for specific pathogens. . .

Service Provided Upon Special Request (Quincy Clinic)

1. PCR-based diagnostic services: Standard rate plus $20.00 (at client request, available for some bacterial diseases such as crown gall, bacterial wilt and Pierce"'s disease).

2. MIDI-based diagnostic services: Standard rate plus $10.00 (at client request for bacterial pathogens).

Services Not Provided by the FEPDC

The FEPDC does not routinely provide the following services to clientele:

1. Pesticide residue determinations in or on plants and soil.

2. Soil nutrient levels, soluble salts, soil pH, or plant tissue analysis for macro or minor elements.

3. Speciation of all pathogens isolated from plant disease samples.

4. Microbe identification from non-plant samples.

5. Toxic plant identifications and mycotoxin analysis.