Gladiolus Rust (*Uromyces transversalis*): A National Management Plan for Exclusion and Eradication



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INTRODUCTION:

Pathogen and Disease Information:

Systematic Placement

Domain: Eukaryota Kingdom: Fungi

Phylum: Basidiomycota
Class: Urediniomycetes
Liveline

Order: Uredinales Family: Pucciniaceae

Biology:

Uromyces transversalis Thum., the causal agent of gladiolus rust (GR), is an obligate parasite that only grows and reproduces on members of the family Iridaceae, including *Gladiolus, Tritonia, Crocosmia* and *Watsonia* spp. Spore dimensions and diagnostic features have been described, enabling microscopic confirmation of this pathogen (Garibaldi 1980; Hernández 2004; McKenzie 2000; Rodríguez-Alvarado 2006). Urediniospores germinate at 5-20°C, with an optimum germination temperature of 15-20°C. Infection of gladiolus plants is most severe when temperatures are between 10 and 20°C and leaves remain wet for at least 12 hours. The incubation period appears to be temperature dependent, requiring a period of 22-23 days at 10°C and only 8-10 days at 25°C (Garibaldi 1980).

Host range:

Uromyces transversalis is found on tropical members of the Iridaceae (Hernández 2004). Gladiolus hybrids (sword lily) are the primary/major hosts, and *Crocosmia* spp. are described as secondary/minor hosts (CABI 2005). Identified hosts are *Anomatheca laxa* (false freesia), *Crocosmia aurea*, *C. aurea* var. aurea, *Gladiolus dalenii* (sword-lily), *Gladiolus x hortulanus*, *G. psittacinus* (dragon's-head-lily), *G. saundersii*, *Gladiolus* spp., *Melasphaerula ramosa*, *Tritonia lineata*, *T. securigera*, *T. squalida*, *Tritonia* spp., *Watsonia* spp., *W. angusta*, *W. densiflora*, and *W. meriana* (bulbil *Watsonia*) (Farr 2006; McKenzie 2000; OPIS 2004).

Disease Symptoms and Signs:

Once symptoms manifest, GR is readily identifiable in the field. *U. transversalis* is named for its characteristic transverse sori which develop horizontally across the veins of the infected leaf, as compared to most rusts on monocots whose sori burst through longitudinally along the veins of the leaf (Hernández 2004). After the production of uredinia (also called uredia), telia are produced as small black pustules surrounding

uredinia. The first symptoms of GR are small yellowish spots that later break the wall of the leaf, forming pustules measuring 1mm x 1cm. The pustules are full of yellowish-orange spores and coalesce to form large lesions. As damage increases, lesions turn dark brown to black. Symptoms are typical for a rust disease, with yellowish-brown (uredinia) or blackish-brown (telia) pustules on the leaves, either solitary or aggregated. Uredinia develop first and produce urediniospores, followed by development of the telia, which produce teliospores (CABI 2005). Spermagonia and aecia are unknown from this fungus (Hernández 2004).

Distribution:

Uromyces transversalis is indigenous to eastern and southern Africa (Hernández 2004) and is present in Kenya, Malawi, Mauritius, South Africa (localized), Tanzania, Uganda, Zambia, and Zimbabwe (CABI 2005; OPIS 2004). In Europe, *U. transversalis* is present with a limited distribution in France, Italy, Malta, Portugal, and Spain (CABI 2005; Hernández 2004). Hernández states that reports of gladiolus rust in France and Spain are questionable (2004). The fungus has been reported from Martinique in the Caribbean and from Argentina, Brazil (localized), and Colombia in South America (CABI 2005; Guidicipietro 2006b; Hernández 2004). *Uromyces transversalis* is present in Australia and established in New Zealand (McKenzie, 2000; Hernández, 2004; CABI, 2005). In North America, *U. transversalis* is present in Mexico.

Pathways and spread:

Gladiolus rust infects the leaves and stems of host plants (NAPPO 2005) and under heavy inoculum pressure, may also infect flowers (Ferreira 1989). Spores can contaminate corms, rhizomes, and flowers and are carried long distances by wind (NAPPO 2005). Windblown urediniospores constitute a significant pathway for entry of *U. transversalis* from Mexico and provides a means of dispersal from sites in California and Florida in which the fungus has already been detected. Movement of infected or contaminated plant parts by trade or in passenger baggage is another pathway for entry.

International trade and smuggling of corms and cut flowers are the primary pathways for the introduction of GR. The local spread of GR occurs by aerial distribution of urediospores, which are produced in prolific quantities on above ground portions of the plant and disperse easily in wind or if lightly brushed. Spores are transported long distances by wind or by movement of infected cut flowers. Urediniospores can also be spread on contaminated corms, rhizomes, and flowers that have been contaminated with rust spores. Clothing and equipment coming in contact with heavily infected plants may also carry spores of *U. transversalis*. Interception records indicate that cut flowers are a major pathway (Brown 2005; Enright 2005; Guidicipietro 2006b).

Current Status of Gladiolus Rust in the United States:

The pathogen was intercepted by APHIS/PPQ in August 2004 in shipments of cut flowers from Mexico to ports in California and Texas. GR was confirmed to be present in Florida in April, 2006. In May 2006, GR was confirmed present in California. In countries where it is known to occur, GR is a serious disease in nurseries. It may cause a complete loss of commercial gladiolus crops in the absence of fungicides in all but the

coldest and hottest areas of climate zones 7-12 (Brown 2006). Other states outside of CA and FL known to grow commercial quantities of *Gladiolus* spp. are: NY, NJ, IL, MI, MN, and AZ.

GR is not established in the United States, but has been introduced, detected, and is in the process of eradication in California and Florida. If GR were to become established within the United States, it would significantly damage the commercial horticulture and floral industries. Currently, gladiolus rust surveys are being conducted in California and Florida where *Gladiolus* spp. are sold as cut flowers or corms and are grown in residential sites near production areas. At selected sites, all *Gladiolus* spp. are visually surveyed for GR. Literature describing the signs and symptoms of the disease has been prepared and will be distributed throughout the course of the survey. In addition to these surveys, California and Florida will need to survey in areas that are currently not known to be infected with *U. transversalis*.

Gladiolus - Host Information:

Systematic Placement

Domain: Eukaryota Kingdom: Plantae

Division: Magnioliophyta

Class: Liliopsida Order: Lilliales Family: Iridaceae

Gladiolus is a cormous plant and forms one of the largest genera of the family Iridaceae, consisting of over 200 species. Gladiolus species are widely distributed in Africa and are found in parts of the Middle East, the whole Mediterranean region, and many parts of Europe. The southwestern region of South Africa is considered the center of origin of gladiolus, as well as being the main source of species used in the development of commercial cultivars. Gladiolus is a very popular flower all over the world because of its attractive forms and colors. Gladiolus plants are suitable for gardens, as an ornamental plant, and are mainly successful as cut flowers. In addition to Gladiolus, several other related genera and species that serve as hosts for gladiolus rust are considered within the context of this document. A current host list is provided above for Uromyces transversalis. In addition, a host pictorial is included as a supplement to this document.

Intended Use of this National Management Plan:

GR detections in Florida and California catalyzed the need for a standard protocol for GR exclusion and eradication to be used by state and federal regulators to respond to new finds of GR in nurseries in the United States. To ensure that there is consistency in responding to new detections of GR, this eradication protocol describes the official activities performed within affected nurseries by USDA-APHIS in cooperation with state and county agriculture regulatory officials.

The goal of this protocol is to ensure that any GR infections are effectively and consistently addressed and eradicated. Early detection and reporting of potential GR infections is critical to ensure that spread is contained and the disease eradicated. Implementation of a well formulated survey will assure that any future outbreaks are caught early enough to be eradicated. Furthermore, negative survey data will support effectiveness of eradication efforts and facilitate the interstate and international movement of gladiolus. The current document is intended for use as a standardized early detection survey methodology for use in states that have no known occurrence of GR.

The eradication of GR is feasible due to the following:

- * At present, GR has a very limited distribution only known to be present in localized areas in California and Florida.
- * GR curative fungicides are available one fungicide group discussed for GR management is the triazoles. Triazoles are products containing myclobutanil, propiconazole, tebuconazole, or tetraconazole. These products provide rapid systemic activity with the ability to kill the rust fungus once it infects plant tissue. The second group of fungicides is the strobilurins which include azoxystrobin, pyraclostrobin and trifloxystrobin. This group has some systemic activity, but the strobilurins are not as rapidly absorbed as the triazoles. This group is also not as systemic as the triazoles and does not appear to be as effective in killing the fungus once it is established in the plant.
- * Complete and full GR growers understanding and cooperation to combat the disease.
- * Only foliar tissue and not the corms become infected.
- * GR pathogen can survive only in green host tissue.

Challenges to eradication are:

- * GR spore survivability need more information/research.
- * GR latent period is not diagnosable.
- * Volunteer plants and hosts located in residential communities.
- * Wind dispersal of pathogen.
- * Possibility of reintroduction.

Definitions:

Containment Facility: A structure whose purpose is to prevent escape of material held within into the environment.

Emergency Action Notification (EAN): A federal document providing notice of emergency action, which will be taken to eliminate or mitigate pest risk; stipulations are binding until notice is rescinded by issuing authorities. Penalties may apply for violations of stipulations of the EAN. An EAN is issued under Federal authorities.

Exposed plants: All gladiolus or other hosts that have an association (*e.g.*, physical proximity or trade relationship) with the positive location and that are held due to a regulatory response (Emergency Action Notification or Hold Notice).

Find Free: To inspect a consignment, field, or place of production and consider it to be free from a specific pest [FAO, 1990].

Hold Order: A signed official document or verbal order given by state regulatory officials requiring that plant material suspected to be infected with GR be held on-site. Additional stipulation(s) may apply to a Hold Order. This type of order is usually used while awaiting confirmation of a suspect GR sample and/or the issuance of an EAN.

Host Free: A place of production such as single plot, single green house, or an entire nursery that does not contain host material is a host free area. Host material includes all host plants, leaves, roots, stems, flowers, host tissue debris, and sprouts of host plants.

Infected plants: Plants infected with gladiolus rust (*Uromyces transversalis*).

Nursery: Any location where host plants and flowers are grown, propagated, stored, or sold.

Nursery/facility quarantine: Official confinement of regulated articles for observation and research or for further inspection, testing and/or treatment (FAO, 1990; revised FAO, 1995; CEPM, 1999). During this period, the movement of host plants is regulated to eliminate or contain the movement of the pathogen. The quarantine period begins with the confirmation of GR and continues until inspection, sampling, and testing reveals no further GR within the regulated block or area. The quarantine may be communicated by an official Hold Order or EAN. A facility is not released from quarantine until all orders have been satisfied and officially rescinded.

Parallel Regulation: A parallel regulation is one which is imposed by a state or local plant regulatory authority and is substantially the same as a federal regulation.

Regulated Area: An area, county, state, or portion of a state in which nurseries produce and move gladiolus plants, plant parts, or cut flowers of gladiolus plants and that has been placed under specific regulations governing movement of said plants or plant parts due to GR.

Suspected infected plants: Plants with visible symptoms resembling GR that have not yet been confirmed positive for GR by APHIS or state officials

Trigger Events for Use of Protocol:

This protocol should be implemented by APHIS-PPQ and/or its state plant regulatory cooperators when the presence of GR has been detected.

Regulatory Authority:

GR is not established in the United States, and is a pest of quarantine significance (Title 7, Code of Federal Regulations (CFR) 319.37-2 (a), 319.37-5(c)), requiring the implementation of appropriate regulatory measures when found. The regulations described in 7 CFR part 319 prohibit or restrict the importation of plants, plant parts, and related materials to prevent the introduction of plant pests and noxious weeds into the United States. The regulations in "Subpart–Nursery Stock, Plants, Roots, Bulbs, Seeds, and Other Plant Products" (§§ 319.37 through 319.37-14; referred to below as the nursery stock regulations) restrict, among other things, the importation of living plants, plant parts, and seeds for propagation. Conditions governing the importation of cut flowers into the United States are contained in "Subpart-Cut Flowers" (§§ 319.74-1 through 319.74-4). Section 319.37-2 of the nursery stock regulations prohibits the importation of GR susceptible plant species from countries where the disease is established.

Crocosmia and Watsonia spp. (except bulbs in commercial shipments) are prohibited from Argentina, Uruguay, Africa, Brazil, France, Italy, Malta, Mauritius, and Portugal. Gladiolus spp. (except bulbs in commercial shipments) are prohibited from Africa, Argentina, Brazil, France, Italy, Malta, Mauritius, Portugal, and Uruguay (§§ 319.37-2). Any restricted article of Gladiolus, Watsonia, or Crocosmia spp. from Luxembourg or Spain shall, at the time of arrival at the port of first arrival in the United States, be accompanied by a phytosanitary certificate of inspection, containing accurate additional declarations that: (1) The plants were grown in a disease free environment in a greenhouse; (2) The plants were subjected to 12 hours of continuous misting per day with water at 15–20 degrees Celsius on 2 consecutive days; and (3) The plants were inspected by a plant quarantine official of the country where grown 20 days after the completion of the misting and were found free of gladiolus rust (§§ 319.37-5(I)). All countries except those meeting the conditions for importation in §§ 319.37-5(I) and §§ 319.37-2 and Canada are subject to postentry restrictions under §§ 319.37-7. Bulbs in commercial shipments are not prohibited and are excluded from a postentry requirement; however, bulbs are restricted as per §§ 319.37-3(a) and require a written permit. According to §§ 319.37-3(a): "the restricted articles other than articles for food, analytical, medicinal, or manufacturing purposes) in any of the following categories may be imported or offered for importation into the United States only after issuance of a written permit by the Plant Protection and Quarantine Programs: (1) Articles subject to treatment and other requirements of §§ 319.37-6; (2) Articles subject to the postentry quarantine conditions of §§ 319.37-7; and (3) Bulbs of *Allium sativum* (garlic), *Crocosmia* spp. (montebretia), Gladiolus spp. (gladiolus), and Watsonia spp. (bugle lily) from New Zealand." Any restricted article of *Gladiolus*, *Watsonia* or *Crocosmia* spp. from Columbia or Mexico shall, at the time of arrival at the port of first arrival in the United States, be accompanied by a phytosanitary certificate of inspection, containing accurate additional declarations that, "the shipment has been inspected and found free of *Uromyces transversalis*"

Survey Procedures:

Nursery Hosts:

All nurseries handling gladiolus and associated host material should be periodically surveyed for gladiolus rust. A nursery is defined as any location where host plants and flowers are grown, propagated, stored, or sold. In areas where gladiolus rust has been known to occur, two surveys should be conducted per year by trained personnel during periods of symptom expression. The goal of the survey is to find suspected infected plants or those plants with visible symptoms, but not yet confirmed positive for gladiolus rust by APHIS or state officials. Survey results must be recorded in the National Agricultural Pest Information System (NAPIS) database.

Sanitation Precautions for Inspectors, Vehicles and Equipment:

When visiting destination nurseries to conduct surveys or to take samples, personnel are to take strict measures to prevent contamination by plant pathogens and movement of pathogens between nurseries during inspections.

Regulatory personnel involved in surveys should wear disposable cover alls and decontaminate hands before and after touching host material. Shoes should also be decontaminated before entering and after leaving property. Acceptable methods of protection include the use of disposable gloves and booties changed between visits or the use of an effective antimicrobial soap, lotion, or antifungal disinfectant (70% ethyl alcohol or 10% bleach (sodium hypochlorite) solutions). Vehicles and equipment should also be decontaminated with an antifungal disinfectant.

Disposal of Infected Plant Material:

If infected host material is found, the material must be double bagged and transported immediately to an approved landfill or steam sterilized (212° F or 100° C for 30 minutes followed by burial at landfill). All double bagged contaminated material must be buried with 2 to 6 inches of top soil within a day of disposal.

General Detection Survey:

The purpose of a general detection survey is to determine if a pest exists in an area. This survey can be extremely broad in scope, as when assessing the presence of a disease in a wide area, or it may be restricted to discovering if a specific pest is present in a certain limited area. It is not valid to claim that a pest does not exist in an area based strictly on a negative result in a detection survey.

General detection surveys for gladiolus and related hosts infected by *U. transversalis* should be conducted by state inspectors in conjunction with inspection of nurseries by federal PPQ inspectors. PPQ inspectors may inspect nurseries without a state inspector if they have the permission of the nursery owner/manager and have, in advance, advised the state of their visit.

Materials and Methods:

Each year, during the course of regular nursery inspections, registered nurseries and nursery dealers are inspected for the presence of diseases on the stock on hand and in production. Plants are examined visually by trained inspectors, and signs and symptoms of infection are noted. When warranted, samples are transported to a diagnostic laboratory for further examination by a trained and experienced plant pathologist. If necessary, plant samples will be incubated to encourage growth and sporulation of the causal agent, thereby aiding in disease identification.

Survey records: Survey data will include the following: date of nursery inspection, location of nursery, name and address of the owner, names and cultivars of the host plants, and pests or diseases detected. For samples from the public, similar information will be collected. Records of GR finds and/or no finds will be submitted to the NAPIS database.

Timetable:

The survey will take place when regular nursery inspections occur, usually beginning in mid summer. Inspections will continue until completed.

Data analysis and dissemination of results: Data will be submitted to the National Agricultural Pest Information System (NAPIS) database at the completion of the survey. Distribution data may be mapped by NAPIS staff and made available on the CAPS web site.

Pathogen Signs and Symptoms Used for Inspections during Detection Surveys:

The first symptoms of GR are small yellowish spots on leaves. Surveys in nursery facilities are conducted visually by looking for gladiolus plants with "typical rust" signs with orange pustules (Fig. 1) up to 3 mm on both sides of the leaves. The pustules tend to be elongated across the width of the leaves and contain many uredia. The absence of these signs, however, does not necessarily mean *U. transversalis* is not present in the facility. Plants may be infected by *U. transversalis* and not show symptoms for a period of 8 to 20 days depending upon environmental conditions.

Visual surveys of other hosts in nurseries should be conducted during gladiolus inspections. Signs observed on other infected hosts, such as *Tritonia, Crocosmia* and *Watsonia* spp. are quite similar to those on gladiolus.

In addition, nursery waste areas should be inspected for discarded plants. Ask the nursery owner/manager to identify cull piles. Examine them for the presence of recently discarded or dead gladiolus plants showing rust symptoms. Discarded host plants that are not completely dried up can still be prepared for sample submission. Document where the sample was taken.

Detection Survey Subsequent to a Find:

Detection surveys at nurseries after a confirmed identification in the United States consist of inspections of suspect gladiolus shipments on hold and any other hosts in nurseries suspected of having material at risk for *U. transversalis*. Inspections are conducted by

the affected state and/or PPQ in concurrence with state inspectors. Suspect gladiolus shipments are those held because of their association with a positive detection from another country or nursery. Be sure to follow sanitation procedures during nursery visits.

Detection survey inspections are to be conducted visually by examining gladiolus and other host plants for rust signs, taking samples of symptomatic plants, and having them submitted for testing.

Nursery owners/managers who note the presence of plants with rust-like signs in the absence of inspection officials should immediately remove the plants from the bench or rack, mark the location from which the plants were taken and notify a State or PPQ inspector. The whole plant should be bagged and sealed in double zip-lock bags and labeled with the date, name of person responsible, and location where plant was taken. These plants need to be kept under refrigeration or in cool conditions until the inspector arrives.

Note: Random sampling of gladiolus plants not showing symptoms or signs is not recommended at this time because of a lack of sampling methodology and processing capability.

Delimiting Surveys after Detection:

First tier survey:

All host plants within a one-mile radius of the initial find area should be surveyed visually. If additional positive plants are identified, the one-mile survey area will be extended from the outer most find, with the focus on areas with the greatest prevailing wind exposure. For eradication with the highest probability of success, it is suggested that all green host material (for example: Gladiolus, Crocosmia, Tritonia, and Watsonia spp.) within this one mile radius be removed whether symptomatic or asymptomatic including leaves, stems, and flowering parts. All residential positive finds need to be removed immediately. Educational and outreach material should be provided to nurseries and home owners within one mile of the positive finds. Nursery owner/operators and home owners need to be asked where the host material (corms and cut flowers) was purchased and trace backs should be conducted. Additional questions concerning whether the host material was traded or sold to others must be asked and trace forwards need to be conducted. Other commercial production areas near positive finds should be surveyed on a quarterly basis at a minimum. Retail outlets that sell gladiolus within a three mile radius of an original positive must be surveyed at least three times, beginning before Mother's Day.

Cut flower production:

All sanitation precautions for inspectors must be followed, including decontamination of vehicle and covering (tarp) and all equipment used in the removal process prior to leaving an approved landfill site. The area needs to be monitored for volunteer plants at least twice per month for 120 days from completion of removal date. If volunteer plants are found, they plants must be removed immediately and the 120 day host free period restarted. Fields that were infested in the previous growing season must not be planted

the following fall. Prior to planting corms, they should be dipped in 170°F hot water and treated with a prophylactic (antifungal) agent.

Corm cultivation operation sites:

If *U. transversalis* is detected on above ground vegetation, a rigorous fungicide program needs to be implemented immediately and continued into the fall growing season; alternating systemic curative triazole and strobilurin fungicides with protectant fungicides. Fungicides should be applied and timed in accordance with the label instructions. The highest probability of eradication success would involve the immediate harvest of all corms (do not wait for flowers on young material) and the field should be disked. Failure to remove all green material and to harvest corms immediately will decrease the likelihood of eradication even with the use of fungicides and a reduction in the amount of green host material. A 120 day host free period for the entire nursery or farm should then be instituted. A scouting program for volunteer hosts must be initiated and all volunteer hosts removed. If volunteer plants are found, they must be removed immediately and the 120 day host free period restarted. Hot water treatment and fungicide dip will be applied to all corms harvested or to be planted.

Second tier survey

All host plants within a three mile radius of the initial find area, but outside the one mile radius, need to be surveyed visually. Outreach material should be delivered at time of initial survey. Special attention should be focused on areas where prevailing winds and weather conditions would have favored the dissemination of spores. All sanitation precautions for inspectors should be followed. If plants positive for GR are located, methods similar to those under the first tier survey should be employed and the survey area should be extended from the outermost find. If asymptomatic host material is present within the survey zone, a sentinel host material survey site should be established and re-inspected at least once per week. GPS coordinates need to be taken and a site map generated. A list of potential future sentinel sites within the defined area should be maintained. A laminated tag should be placed near the host material for quick reference (including contact information, brief pest information, and survey termination date).

Monitoring Surveys

Once a nursery has a positive confirmed infection of *U. transversalis* and all suspect gladiolus and potentially infected plants have been destroyed and affected areas disinfected, some kind of follow-up survey is necessary. Inspectional visits during the same and subsequent growing seasons are appropriate in order to examine hosts for symptoms. In areas where GR has been known to occur, two surveys should be conducted per year by trained personnel during periods of symptom expression.

Eradication

The following section summarizes some eradication techniques that have been utilized in states where GR has occurred. They are intended to be informative and useful in planning. In the event of disease detection, all host material must be immediately

destroyed (See Appendix 3). To discourage emergence of volunteer host plants, the infested area should be plowed on a bi-weekly basis or flooded. The area should be monitored for volunteer plants on a weekly basis for 120 days from completion of removal date. If volunteer plants are found, the plants must be removed immediately and the 120 day host free period should be restarted. Fields that were infested in the previous growing season should not be planted the following fall. Prior to planting corms, they should be dipped in 170°F water and treated with a prophylactic (antifungal) agent. A protectant fungicide spray program may also be instituted and at least bi-weekly inspections by nursery personnel and quarterly inspections by regulatory personnel should commence two weeks after planting or at green material emergence.

Corm cultivation operation sites: If *U. transversalis* is detected on above ground vegetation, a rigorous fungicide program needs to be implemented starting immediately and continuing into the fall growing season; alternate triazole and strobilurin fungicides with a protectant fungicide each time. Fungicides should be applied according to the label instructions. The highest probability of eradication success would involve the immediate harvest of all bulbs (do not wait for flowers on young material) and disking of the field. Failure to remove all green material and to harvest bulbs immediately will decrease the likelihood of eradication even with the use of fungicides and a reduction in the amount of green host material. A host free period for the entire nursery or farm for 120 days should then be instituted. A scouting program for volunteer hosts must be initiated and all volunteer hosts removed. If volunteer plants are found, they must be removed immediately and the 120 day host free period should restart. Hot water treatment and a fungicide dip should be applied to all corms harvested or to be planted.

<u>Residential sites:</u> All residential positive finds need to be removed immediately as well as any host material on the site.

<u>Host material within a 1 mile radius of a positive find:</u> For eradication with the highest probability of success, it is suggested that all green host material within this one mile radius be removed whether symptomatic or asymptomatic, including leaves, stems, and flowering parts.

Communication and Notification:

Communication is imperative to the effective eradication and control of any new pest. This section highlights some communication channels that will ensure that the appropriate authorities are notified and that appropriate action is carried out.

Lines of Communication:

Before Confirmation of a suspect

- The inspector should immediately notify the State Plant Health Director (SPHD) and the State Regulatory Official (SPRO) of the State in which the suspected GR detection is located.
- State Laboratories will notify the SPRO and SPHD of results.

After Confirmation of a suspect

- PPQ Laboratories will notify the Regional Offices and the National Program Manager of GR results.
- The National Program Manager will notify Phytosanitary Issues Management (PIM) officials.
- The Regional offices will notify the SPHD and SPRO of all related states where facilities may be impacted by the trace back and trace forward investigations.
- *See appendix 7 for a list of contacts*

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Appendix 1: Management Practices for Growing GR-free Gladiolus

Introduction: These Management Practices may help prevent the introduction/establishment of gladiolus rust caused by *U. transversalis* and will increase the likelihood that it will be detected early, should an introduction occur. Any nursery may incorporate these practices into their Standard Operating Procedures (SOP).

Exclusion:

• Maintain production areas at less than optimum environmental growing conditions for the growth of GR and reduce other cultural practices conducive to optimal GR-growth conditions. Examples of adjustable environmental conditions include



temperature and humidity. Note: Be sure not to compromise the necessary growing conditions for gladiolus plants.

- Require specific sanitation protocol for personnel movement between greenhouses and growing areas. Enforce one-way movement of personnel from least restrictive to most restrictive areas of sanitation.
- Actively train personnel for identification of GR symptoms and signs and proper practices to guard against the spread of the disease.
- No other known hosts of GR should be grown on nursery grounds unless there is regular monitoring of those hosts.
- Confirm that host stock is propagated from materials originating on site or is received from shipping nurseries which are free from GR.
- All incoming host plants (buy-ins, transfers), regardless of origin, should be visually inspected for symptoms of GR by trained nursery personnel prior to being incorporated into the production area.
- Incoming host plants, propagative material, and corms should be stored away from the main production area.
- Incoming corms should be treated with hot water and a fungicide before planting.
- The mother stock plants should be located away from the main production area, *i.e.*, in a different structure with no connected air flow.

Prevention:

- Use an effective fungicide program for the control and prevention of GR on susceptible host plants.
- Offload incoming shipments to an area that can be cleaned of the leafy debris. Sweep debris from the receiving pad and the delivery truck; collect debris and bag for disposal.
- Avoid product returns of nursery stock from a receiver in a quarantined area. If unavoidable, contact your State Plant Regulatory Official (SPRO) - if in California, your County



Agricultural Commissioner - prior to accepting the nursery stock return. One-way movement of plant material through nurseries regardless of known GR-status may be

required. Otherwise, have a designated quarantine zone in the nursery that prevents returns from co-mingling with "clean" stock.

• If the infestations have been detected in a place of production, a hot water and fungicidal dip treatment of gladiolus corms before planting should be considered.

Monitoring:

- Nursery personnel should attend one or more GR workshops available through state agriculture departments and universities.
- All host buy-ins should be maintained separately from other hosts plants and periodically inspected for symptoms of the disease over the course of a growing season.
- Monitor host plants in surrounding area for symptoms and signs of *U. transversalis*.
- Identify sources of disease recognition fact sheets, and/or develop and distribute disease recognition fact sheets on host plants to educate all field nursery personnel.
- Record keeping: Maintain accurate shipping documentation identifying product, amount, date and origin or receiver for the purpose of identifying trace backs and trace forwards.
- If the disease is found in the area surrounding a nursery, immediately contact the department of agriculture in your state or your County Agriculture Commissioner.

Appendix 2: Contact Information

National Program Leader;

Anwar Rizvi, Riverdale, Maryland, 301-734-4313, Anwar.S.Rizvi@aphis.usda.gov

Diagnostic Authority:

Mary Palm, Beltsville, MD, 301-504-5327, Mary.Palm@aphis.usda.gov

USDA/APHIS/PPQ - Western Region:

Phil Mason, Ft. Collins, CO, 970-930-5500, Phillip.A.Mason@aphis.usda.gov Roeland J. Elliston, Ft. Collins, CO, 970-494-7572, Roeland.J.Elliston@aphis.usda.gov

USDA/APHIS/PPQ - Eastern Region:

Anthony Man-Son-Hing, Raleigh, NC, 919-855-7331, Anthony.Man @aphis.usda.gov

States Plant Health Directors:

California - Helene Wright, 916-930-5500, Helene.R.Wright@aphis.usda.gov Florida - Paul Hornby, 352-331-3990, Paul.L.Hornby@aphis.usda.gov New York - Jonathan Staples, 518-438-3896, Jonathan.staples@aphis.usda.gov New Jersey - Ronald Blaskovich, 609-259-8650, Ronald.m.blaskovich@aphis.usda.gov Illinois- Stephen A. Knight, (847) 299-6939, Stephen.a.knight@aphis.usda.gov Minnesota - Kevin Connors, 612-725-1722, Kevin.j.connors@aphis.usda.gov Arizona - Jerry Levitt, 602-431-8930, Jerald.l.levitt@aphis.usda.gov Michigan - David McKay, 734-942-9005, David.r.mckay@aphis.usda.gov

States Department of Agriculture - States Plant Regulatory Officials:

California - Nick Condos, 916-653-1440, ncondos@cdfa.ca.gov
Florida - Richard Gaskalla 352-372-3505 x 106 gaskalr@doacs.state.fl.us
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Illinois Mark - Cinnamon 847-294-4343 mcinnamon@agr.state.il.us
Michigan - Kenneth Rauscher 517-373-1087 rauscherk@michigan.gov
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Arizona - G. John Caravetta 605-542-0996 jcaravetta@azda.gov

Industry Association:

Society of American Florists - Lin Schmale 703-838-5232 lschmale@safnow.org

Review Team:

Kathy Kosta, Courtney Albrecht, Anthony Jackson, Lin Schmale, Tim Schubert, Roland Elliston, Anthony Man-Son-Hing, Larry Brown, Anwar Rizvi, Pat Nolan.

Appendix 3: Disposal of infected material

To dispose of all infected host material in such a way that minimizes the risk of further spread.

Sanitation: When visiting an infested nursery to dispose of infected material, personnel are to take strict measures to prevent contamination by plant pathogens between nurseries during inspections. Regulatory personnel should wear disposable cover alls and decontaminate hands before and after touching host material. Shoes should also be decontaminated before entering and after leaving property. Acceptable methods of protection include the use of disposable gloves and booties changed between visits or an effective antimicrobial soap, lotion, or disinfectant. A 70% ethyl alcohol or 10% bleach (sodium hypochlorite) solutions can also be used as disinfestants.



Host Material: Infected host material may be encountered in a number of locations. The following list outlines the types of material that may be encountered at various locations.

- <u>Nursery Production:</u> (Whole potted plants, Whole plants in the ground, corms).
- **Retail Outlets:** (Whole potted plants, Corms)
- Corm Production: (Whole plants in the ground, Corms)
- <u>Cut Flowers Industry:</u> (Whole plants in the ground, Whole potted plants, Cut flowers stalks, Green Foliage)
- **Personal Property:** (Whole potted plants, Whole plants in the ground)
- <u>Public Property:</u> (Whole plants in the ground for landscape or along the highway)
- Natural Environments: (Whole plants in the ground that have naturalized)

Desired Disposal Method by Plant Part: Depending on the plant part, and the economic impact, several destruction methodologies are available below.

- Whole Potted Plants: All green plant material (leaves, stems, flowers) may be cut with a sharp knife near the root (without shaking and dispersing the spores) and should be double bagged and taken off site. All plant debris including leaves, stems, flowers, roots, and soil should be double bagged and taken off site. If destroying corms and pots is not acceptable, they may be immediately harvested or removed, and be dipped in 170oF hot water and treated with a prophylactic (antifungal) agent. Otherwise, they should be immediately removed, double bagged and taken off site.
- Whole Plants in the ground: All green plant material (leaves, stems, flowers) may be cut with a sharp knife near the root (without shaking and dispersing the spores) and should be double bagged and taken off site. All plant debris

including leaves, stems, flowers, roots, and soil should be double bagged and taken off site. If corms are to be saved, they should be immediately harvested, and be dipped in 170oF hot water and treated with a prophylactic (antifungal) agent. Otherwise, they should be immediately removed, double bagged and taken off site.

- <u>Corms:</u> If destroying corms is not acceptable, they may be immediately harvested, and be dipped in 170oF hot water and treated with a prophylactic (antifungal) agent. Otherwise, they should be immediately removed, double bagged and taken off site.
- <u>Cut Flower Stalks:</u> All cut flower stalks should be removed, double bagged, and taken off site.
- <u>Green Foliage:</u> All green foliage should be removed, double bagged, and taken off site.

Off Site Disposal of Infested and Double Bagged Material: Host material, including leaf litter, must not be removed from the facility as trash. Material should be disposed of immediately within one day of removal. It may either be transported directly to an approved landfill and buried, or transported to a landfill after steam sterilization (212oF or 100oC for 30 minutes) and buried. The recommended burial depth is 2-4 inches below top soil.

- <u>Incineration (burning to ash):</u> Infected plants, all leaf debris may be disposed of by incineration at a facility or other location. Off nursery movement must be properly safeguarded. Burning may be through open burning or in an incinerator.
- <u>Burial:</u> Double bagged debris may be bagged, buried at an approved landfill and covered with 2-4 inches of soil.
- <u>Steam sterilization followed by burial:</u> Dry heat or steam commonly heated to internal temperatures of 212° F (100° C) for 30 minutes followed by burial in a landfill. The recommended burial depth is 2-4 inches below top soil.
- <u>Composting method:</u> As out lined in the California codified in California Integrated Waste Management Board regulations: Title 7, Division 7, Chapter 3.1 Article 7, Section 17868.3 (b) and (c) located at http://www.ciwmb.ca.gov/Regulations/Title14/ch31a5.htm#article7).
- Other methods deemed proper by the regulatory authorities may be used.

Extra Sanitary Protocols: All sanitation procedures should be followed at any step of the disposal procedure (see page 7). All vehicles used to transport infected material should be covered with a tarp during the transport. Prior to leaving the burial site, the vehicle, the tarp, and all equipment must be sanitized using an antifungal agent (70% ethyl alcohol or 10% bleach solution).



Appendix 4: Gladiolus Rust Incident Data Collection Form

Facility (Please provide facility map identifying all gladiolus and other host material areas).

Type of Inspection:		
Name of Inspector:	Phone:	
Routine plant inspection: Y or N Regulatory trace-forward/trace back a. Name of Facility:		
b. Location:		
	General Manager:	
d. Total acreage/area of Nursery &	STR &/or GPS:	
e. Number of Greenhouses/blocks in f. Number of plants:	n production:	
	retail; cut flower; research; plant dia	gnostics; tissue
h. List all other companies-facilities	s involved in production, sales, and d al nursery locations (i.e., trial fields;	
including tissue culture; breeding; r	diolus materials both domestic and i esearch; and propagative. (include li by facility; grown at off-shore facilit	icensed /
	from outside sources: potted gladiol; Corms; Seeds	
	for entry into nursery and for mover	ment between
greenhouses or other growing units.	·	

1. List chemical regiments used in gladiolus production in the facility. Give names of chemicals and intervals used.
II. Individual Gladiolus Growing Area or Greenhouse (The following information should be completed for each separate growing area or greenhouse type) A. Identification of Greenhouse/Field:
1. Type of growing area and name of unit (i.e., glasshouse; drop-side greenhouse; shade house; indexing, breeding, production field, research; tissue culture, etc.).
2. Who is the Growing area manager?
3. How many suspicious samples were collected by regulatory inspectors and submitted for identification to pathologist(s); (identify sites where samples were taken):
4. Was GR found anywhere on/in this area? In this Nursery? During this regulatory incident? Action taken?
5. Is entry to greenhouse/unit restricted to authorized personnel and/or monitored? Who is authorized to enter?
6. How is area secured?
B. Construction or configuration of growing area:
 Growing area (Circle one) Open Field (not under any type of structure) Enclosed Structure Construction (including shade houses)
2. Describe the airflow, what type of Air Intake and Exhaust system(s)?

a. Is humid air exhausted out of nursery block into environs or into other parts of the growing nursery?
b. Are individual sections of the nursery connected by the air ventilation system? Are houses ventilated; how & why? Are houses/areas maintained at a specified relative humidity? If so, what RH?
3. Are houses/area heated or cooled? What temperature(s) range & describe schedule?
4. If a cut flower grower; are houses subject to 12-hrs darkness during initiation of the flowering stage? If so, how is this accomplished and does it contribute to fungal growth conditions?
C. Sanitation Procedures: 1. Describe the sanitation procedures required of the employees having access to this area.
2. What chemicals, if any, are used for the decontamination procedures?
3. Have the environs and nursery landscape and/or neighboring properties contiguous with the Nursery been inspected for the presence of GR host material and GR infections? If yes, how far out?
4. How many times has the Nursery been inspected by State, County, and/or Federal inspectors during the past year? Are copies of the inspection reports available?

moved to other nurseries. Please include invoices for the time span one week prior to date GR infection is estimated to have begun and for all plants considered exposed. Also, include information for disposal areas of infected plant material if different than already described above.
III. Regulatory and Phytosanitary Information
A. Identify the Positive infection triggering regulatory investigation (may be infected at this nursery or an infection in an associated nursery).
B. Has there been a GR infection at this Nursery in the past? If so, when?
C. Is production area under inspection physically connected to another part(s) of the Nursery that handles gladiolus material in any capacity?
1. Is a retail outlet connected to the production nursery; either physically or under the same ownership? (i.e., contiguous spaces with retail shop and greenhouse connected; customers moving freely between areas, common air ducts, etc.)?
2. Do employees move freely between greenhouses, nursery, field blocks, retail outlet, etc? (for example, employees work in any/all areas of the nursery as needed in the course of a day as opposed to employees restricted from working in multiple areas of the nursery).
3. Describe preventative measures in place to stop fungal infections from spread between same entities listed in previous question. (This could be sanitation procedures already discussed, as well as additional physical barriers such as filters, etc. in place to prevent spores from spreading via air ducts).
4. Detail progression of plant material from start to finished product. (This may be a very

simple progression or can be very complicated depending upon the Nursery's set-up.

Example: The wholesale Nursery receives corms from broker; sticks corms in pots; grows to 4-in pot size and sells retail on site.)
5. List all sources of non-propagative gladiolus material that may have been on premises in the past year (such as cut flowers; retail potted materials; diagnostics; R&D materials, etc.).
6. Is any gladiolus material received under special permit(s)? - USDA Departmental Permit
- USDA Post entry Permit
- Other state or federal permits
7. List chemical regiments used in gladiolus production in the nursery. Give names of chemicals and intervals used.
8. Are spray records maintained and accessible to inspectors?
9. Are chemicals considered to be "curative" agents used in routine production as a "preventative" measure?
10. How are culls & plant debris disposed off site? At what intervals?
11. Is plant debris disposed immediately or left in greenhouse/field?
12. Does nursery/outlet utilize a "cull pile" and if so; where is the cull pile located and how long is it left before it is removed?
13. Does nursery "compost" its corms/culls?

•	te and/or intrastate and is it exported? To whom is as; potted plants; cut flowers; etc.)?
15. Are sales invoices available fo	or inspector(s) to view?
	icensed/patented varieties of gladiolus that are a be for production or for R&D-only)? If so, list the
17. What other companies are lice	nsed to sell your patented/licensed varieties?
18. Other information	
Name of the Inspector(s)	Date
Telephone - Office:E-mail:	Cell phone:
	ded by the above individual to the following: (list

Copies of this report will be provided by the above individual to the following: (list officials receiving copy from inspector):

Appendix 5: Emergency Action Notification (EAN) and Emergency Actions

Emergency Action Notification (EAN):

Federal Emergency Action Notification (EAN) will be issued to the infested nursery. States may issue hold notices. The EAN will remain in place until the nursery has satisfactorily completed the required sanitation measures. Prior to issuance of any notice, the inspector will evaluate the exposure level of all areas of the nursery and determine areas for inclusion/exclusion under the EAN. This evaluation will clarify the status of the areas at-risk to be regulated, and determine the required sanitation measures for plant, cut flowers, and other products. Many nurseries have numerous growing areas; some are divided by walls; some are under the same roof but divided by space; some are divided by long distance within the grounds of the nursery. These and other factors may be included in the evaluation of spread of the disease.

Emergency Actions:

- 1. **The Emergency Action Notification (EAN)** will define treatments and required sanitation measures. Host plants within the nursery, for sale or under propagation will be inspected. Plants placed under EAN may not be removed from the nursery, from any holding area within the nursery, or moved within the nursery until they are found to be free of *U. transversalis*, or until the nursery is officially declared free-from *U. transversalis* and removed from quarantine status, or unless specifically approved by a State or Federal Regulatory Official.
- 2. **Suspected Samples** for official confirmation will be submitted to state identification authorities or United States Department of Agriculture.
- 3. **Trace Forward Investigation.** Initiate trace forward investigations. Identify shipments made prior to the discovery of *U. transversalis*. Notify your PPQ Regional Office of all interstate shipments made within the 15 days prior to the first positive detection of *U. transversalis* at the nursery. This includes all hosts plants in the nursery, not just those found infected.
- 4. **Trace Back Investigation.** Determine the origin of all infected host through trace backs. Trace back the plants to point of origin (propagator). Trace back nurseries should be inspected and result provide to the relevant regulatory officials. Determine all sources of other gladiolus that have been introduced into the facility within the past eight weeks. This may include propagative and non-propagative gladiolus material, including potted plants, cut flowers for floral arrangements, corms, and salesmen samples. Include such information in trace backs as determined necessary.
- 5. **Cull piles.** Record the location of any cull piles that may be contaminated with infected plant material. Check any cull piles for symptomatic plants, and plant material and sample if detected. Determine how the nursery disposes of culled plant material. (Refer to Destruction of Infested Plants, below and Appendix V for information.)

- 6. **Equipment.** Determine if equipment used at this nursery is shared with other facilities or field areas. Are equipment sanitation procedures in place at this nursery?
- 7. **Fungicides.** Determine if fungicides are used on the plants at the nursery. If fungicides were used, then record the date, material, amount and application rate.

Appendix 6: Emergency Action Notification Data Log for Infested Property

Date of EAN Action
1. Date infected plants and exposed radius of plants destruction complete:
2. Date & Results of Inspection One after destruction of infected plants:
a. Were more infected plants found? If so, identify the location in the facility & include map.
b. Date additional plants destroyed:
c. Note any additional regulatory actions taken:
3. Date & Results of Inspection 2 after original destruction of infected plants:
a. Were more infected plants found? If so, identify the location in the facility & include map.
b. Date additional plants destroyed:
c. Note any additional regulatory actions taken:
4. Date & Results of Inspection 3 after original destruction of infected plants:
a. Were more infected plants found? If so, identify the location in the nursery & include map.
5. Is further regulatory action initiated?
6. Host-Free period for 12-weeks; date beginning:

8. Date EAN rescinded:	
NOTES:	
Name of the Inspector(s)	
Date	
Telephone - Office:	Cell phone:
E-mail:	-

Copies of this report will be provided by the above individual to the following: (list officials receiving copy from inspector):

Appendix 7: A Quick Guide to Survey for Gladiolus Rust (GR) in Nurseries

Goal: To identify new infestations of GR Quickly. Sanitation: Always take strict measures to prevent contamination by plant pathogens between nurseries during inspections. Use disposable cover alls and decontaminate hands/ shoes before and after touching host material. Protect yourself with disposable gloves and booties changed between visits or an effective antimicrobial soap, lotion, or disinfectant.



Negative Results: Keep in mind that negative results do not guarantee that GR is absent from an area. However, they can support the notion that a pest is not present.

Who Should Conduct the Survey: Surveys should be conducted by state inspectors in conjunction with federal PPQ inspectors in conducting nursery inspections. PPQ inspectors may inspect nurseries without a state inspector if they have permission of the

nursery owner/manager and have advised the state of their visit.

Additionally, inspectors must have permission to survey on private property without a warrant.

When to Look: Each year, during regular nursery inspections. If this is not possible, another trip should be arranged.



Electron micrograph of

a rust sorus. Photo Credit: APS Net Glossary http://www.apsnet.org/education/illustratedglossary/PhotosS-V/uredinium.htm

How to Make Examinations: Inspectors should visually examine plants for signs and symptoms of infection. When warranted, samples are transported to a diagnostic laboratory for further examination by a trained and experienced plant pathologist. If necessary, plant samples will be incubated to encourage growth and sporulation of diseases, to aid in identification of the causal agent.

Which Plants to Examine: All Gladiolus and additional hosts should be examined at each nursery. This includes all plants in production, as well as culled and discarded plants. What Symptoms and Signs to look for: Symptoms and signs are similar regardless of which host is being examined, e.g. gladiolus or otherwise.

First, scan all host plants at a given location targeting plants with obvious yellowing (chlorosis) or death (necrosis). Areas with pronounced yellow spots should also be targeted areas. If the targeted plants do not look suspicious upon closer examination, you should still walk through all plants in the nursery scanning for symptoms. Even if no

plants express these "at-a-distance" symptoms, you should still scan all plants in the nursery.

Examine the leaves and stems of all plants for different stages of infection.

Latent Infection: Plants that are latently infected show no external symptoms. It is not possible to determine these plants using field survey techniques (sorry, that's the breaks). Plants may be infected and not show symptoms for a period of 8 to 20 days depending upon temperature and moisture conditions.

Early Infection: The first symptoms of GR are small yellowish spots.

Development of Pustules and Urediospores: The disease is easily recognized, being a "typical rust" with orange sori or pustules (small blister-like elevation of epidermis formed as spores emerge) on both sides of the leaves. The pustules tend to be elongated across the width of the leaves and contain many urediospores. The spores look like yellowish-orange powder to the naked eye. U. transversalis is named because the sori develop transversely across the width of the leaves, as compared to most rusts on monocots whose sori burst through longitudinally along the veins of the leaf. Development of Black Telia: As infection advances, another type of spore (teliospore) are produced as small black pustules surrounding uredia.

Advanced Infection: As damage increases, lesions turn dark brown to black.

If a Suspect is found: The whole plant should be bagged and sealed in double zip-lock bags and then labeled with the date, name of person responsible for the collection, and location where plant was taken. These plants need to be kept under refrigeration or in cool conditions until an identification can be made. You will need to determine the point of contact for a suspect identification in your state. This point of contact can provide more specific details for shipping and labeling that are specific to that diagnostic laboratory.

Timetable: The survey can piggyback on regular nursery inspections and should begin around mid summer. Inspections continue throughout periods of likely symptom expression.

Survey Records and Data: Survey data will include the following: date of nursery inspection, location of nursery, name and address of the owner, names and cultivars of the host plants, and pests or diseases detected. For samples from the non-nursery sources, similar information will be collected. All survey data will be submitted to the National Agricultural Plant Information System (NAPIS) database at the completion of the survey, including any new records of GR in a state or location. Distribution data may be mapped by NAPIS staff and made available on the CAPS web site.

For a more survey information, visit the Gladiolus Rust (Uromyces transversalis (Thum.)) Standardized Survey in Nurseries Producing Gladiolus Flowers and Corms companion document.

For more host information, visit the Pictorial Guide to Hosts of Gladiolus Rust Uromyces transversalis) companion document or the Lucid key to African cut flowers at the following URL.

http://www.lucidcentral.org/keys/v3/cutflowers/key/Cut%20Flower%20Exports%20of%20Africa/Media/Html/index.htm



Development of Black Telia around Pustules: U. transversalis fruiting bodes: uredinia (orange)



Erupting sorus on gladiolus leaf. Photo Credit: Christer Johansson