

MINIMUM SANITATION PROTOCOLS FOR OFFSHORE GERANIUM CUTTING PRODUCTION

The purpose of this document is to establish *minimum* sanitation protocols to prevent the introduction of *Ralstonia solanacearum* race 3 biovar 2 into production greenhouses at off-shore facilities that produce geraniums intended for import into the United States.

The management pertains to geraniums propagated in “Increase”, and “Production Block” greenhouse facilities where the plant material originates from an “Elite Nucleus Block” (tissue culture). In order to prevent cross contamination of the “Increase” and “Production Block” facilities:

- There is to be a complete physical separation of Increase and Production Blocks which involves the use of dedicated structures.
- Personnel are not to enter the Increase Block if they have entered the Production Block on the same day.
- Equipment and protective clothing is dedicated for sole-use in Production and Increase Blocks when crop is present.

Minimum Standards for Greenhouse Structure and Material Requirements:

Facility Infrastructure: A Facility is a contiguous property that is used to produce a crop or crops by one owner. It is considered the regulated physical entity for purposes of these instructions. Individual structures that are located within a facility may be able to isolate problems. Resolution of these problems may involve actions against only certain greenhouses within a facility. This will be at the discretion of the APHIS and NPPO representatives.

All geranium plant material grown for the purpose of production of geranium cuttings destined for the US must be propagated and maintained in greenhouses. Greenhouses are covered (glass, polycarbonate, or polyethylene) to prevent splashing due to rain. Sides of greenhouse facilities are enclosed with screen. The outer perimeter of the greenhouse is to be surrounded by a buffer that may be composed of gravel or crushed rock, grass free of dicotyledonous plants/weeds, concrete or similar hard surfaces or weed-cloth and should be a minimum of 1m in width. The buffer should be sloped so that water drains away from the greenhouse and greenhouses are designed so that seasonal rain or flood waters do not enter greenhouses.

Greenhouse flooring varies among facilities. Hard surface floors such as concrete or crushed rock are essential. Regardless of flooring type, direct contact of production stock plants with the floor is prohibited. No native soil should be visible when crushed rock is used. Equipment surfaces in contact with stock production plants may not come in contact with the floor unless they have been surface disinfested prior to use. This includes the ends of hoses or watering wands which may be used to water production stock plants. Additional conditions pertaining to flooring are identified below.

Entry to the facility should be secure and should exclude any source of the pathogen from entering the facility. Trucks entering the facility must have tires cleaned and disinfected before entry into the production area or vehicles should be excluded from the production area.

Entry to the greenhouse must be through a door providing direct access to an area within each greenhouse that is equipped with wash station(s), foot bath(s), and protective clothing (aprons, lab coats, etc) to be used by all greenhouse entrants prior to entering plant production areas. Greenhouse design and the practices of hand and foot/shoe disinfection and the covering of clothing worn outside the greenhouse production area are implemented to significantly reduce the likelihood of introducing *R. solanacearum* race 3 biovar 2 into geranium facilities or production area. Sanitation practices are to be strictly enforced.

Wash stations: All exposed body parts (hands, arms, legs, etc) that may come in contact with plant material are to be washed with antibacterial soap prior to entering the production area of the greenhouse as described below.

Foot baths: The foot wear of all persons entering the greenhouse are to be brushed or rinsed free of soil and debris prior to entry of the facility; this will improve treatment efficacy and prolong the activity of the surface disinfectant. Each person entering the greenhouse facility is subsequently required to rinse shoes, boots or other foot wear in a reservoir of surface disinfectant (Table 1). The volume of disinfectant used in footbaths is to be adequate to ensure that soles and lower portions of footwear that may have come in contact with *R. solanacearum* race 3 biovar 2 are moistened with disinfectant and remain wet for the minimum amount of time required for the particular disinfectant to be effective. The disinfectant in these reservoirs is to be changed a minimum of twice daily, with debris in reservoirs being removed prior to replacement of the disinfectant into the footbath. The bottom surface of the reservoir shall be rough in texture to facilitate dislodging of any debris on the bottom of shoes/boots (this may be accomplished by placement of a mat inside the reservoir). Daily records are initialed by responsible personnel to demonstrate that foot baths etc. were properly maintained.

Protective clothing: Protective clothing (clean lab coats or aprons) is to be worn by all personnel upon entry of the greenhouse production area. The purpose of the protective clothing is to prevent contact of geraniums with employee clothing worn outside the increase and production areas. Protective clothing designated to come into contact with the geranium crop (washable lab coats and/or washable or disposable aprons worn over washable lab coats) is to be dedicated to each greenhouse and is to be put on after leaving the hand wash and foot bath station but prior to entering the plant production area. Protective clothing is to be removed prior to leaving each greenhouse but prior to entry into the hand wash/footbath area. If lab coats are worn between greenhouses within a production area, then they should be covered with or exchanged for an apron when workers enter the production area of each greenhouse. Protective clothing is to be stored so that clothing cannot contact the floor. Clothing is to be maintained free of debris, potting media, soil, or plant material. Protective clothing should be washed in detergent weekly or replaced in the case of disposable aprons.

Personal Hygiene: Sanitation of hands or forearms that come in contact with geraniums must be maintained throughout the period when workers are in the greenhouse facility during production. This may be accomplished by either dipping hands or forearms in disinfectant or by spraying hands and forearms with disinfectant. Workers may select to wear latex or equivalent gloves and these are also to be sprayed or dipped in surface disinfectant as described for hands and forearms. Workers must regularly surface disinfest their hands and forearms by dipping or spraying with disinfectant between groups of 10 plants or between definable production units of geraniums that are delimited by production practices (Table 2).

Tools: Greenhouse equipment used to harvest cuttings or for other functions involving contact with geraniums is to be surface disinfested prior to and between use on each stock production plant or stock production container. Alternatively, a set of knives can be permanently assigned to a specific bench and appropriately rotated and disinfested between plants on that specific bench. Carts and collection baskets are to be sprayed with a surface disinfectant on all surfaces that are likely to come into contact with geranium cuttings or equipment used in processing geranium cuttings. Equipment (knives, etc) used to harvest cuttings is to be soaked in surface disinfectant when not in use. The volume of disinfectant used to surface disinfest tools should be adequate to submerge entire blade or portions of tools that make contact with geranium plants. (Table 1).

Handling of cuttings and traceability: Upon harvest, cuttings are placed directly into new plastic bags or into plastic containers that can be surface disinfested before they are used again. Labels will accompany each bag of cuttings. The label system shall allow cuttings to be traced forward through the rooting stations (if cutting is sold as rooted cutting), or directly from the farm (if cutting is sold as un-rooted or callused) to the first wholesale grower customer. If an infestation is encountered all material that has been shipped to the United States and been traced back to the infested greenhouse will be subject to seizure and destruction in the United States.

Transfer of cuttings to grading facilities shall be done in a manner that ensures bags of cuttings or cuttings in plastic containers do not come in contact with any material that may harbor *R. solanacearum* race 3 biovar 2.

Greenhouse floors: Greenhouse floors should be maintained free of debris and weeds. Floors must drain properly to prevent long-standing puddles of water. Water in contact with flooring is never in contact with surfaces upon which plants are grown (splashing, watering, etc). Plant material (cuttings, trimmings, etc) that falls to the floor should not be retrieved by greenhouse workers who are in the process of handling production stock plants; it should be removed on a periodic basis (minimum weekly) and disposed of by other workers or by same workers after harvesting of cuttings is completed. Floors are to be sanitized at least once annually (Table 1).

Flooring throughout all greenhouses must be composed of concrete (or similar material) or crushed rock/gravel (which may be covered with weed-cloth) that is deep enough to cover all native soil. Any native soil coming through the rock will require immediate corrective action to reestablish a barrier. Floors in greenhouses are not permitted to be comprised of exposed soil/dirt; no exposed soil/dirt is allowed in greenhouses.

Production surfaces: The distance between the surface upon which the stock production plants are placed to the greenhouse floor and the types of surfaces upon which stock production plants are grown varies among greenhouse facilities. Stock production plants are to be elevated above the greenhouse floor to prevent contamination from splashing water and to ensure that there is no continuum between plant material and water, debris, or potting media on the floor. Surfaces upon which plants are grown should be disinfested (Table 1) prior to use and constructed such that water draining from pots does not make contact with other pots.

Potting Media: Geranium stock plants shall be grown in sterilized or fumigated APHIS approved media. New plastic bags or pots or recycled plastic pots that have been surface disinfested may be used as stock plant containers. Potting media and pots intended for use in the greenhouse shall not be stored on soil/dirt or turf surfaces.

Irrigation: The requirement for treatment of irrigation water is dependent upon its source and the water storage and delivery system in use. Water treatment is not required for irrigation water collected from properly sealed deep wells and where water is used directly or stored in tanks where there is no opportunity for contamination of the water supply by native soil or plant material/debris. Municipal water may also be used as a primary irrigation source without initial treatment. If water sources are derived from unsealed wells, rain-water collection systems, ponds, lakes, streams or any other type of open body of water or if water is recycled or recirculated, then treatment of water is required.

The water source is to be tested at least annually by APHIS-approved methods to ensure freedom from *R. solanacearum*.

Two independent water purification systems are to be available in each geranium production facility to safeguard the crop from infection by *R. solanacearum*. A back up system is required to ensure that the crop does not become infested with *R. solanacearum* due to failure of the primary water purification system. Water treatment systems effective against *Ralstonia solanacearum* race 3 biovar 2 are listed below.

Plants may not be irrigated using ebb and flow or flood irrigation systems as these methods may contribute to spread of *R. solanacearum* race 3 biovar 2. Irrigation systems should be constructed so that emitters are not in contact with potting media and/or are equipped with backflow devices to prevent contamination of the watering system. The last 1.2 meters of hose closest to the bench and hose-end implements used for hand watering geraniums should never come in contact with the floor of geranium production facilities, plant material or potting media. When such contact is made, the hose and watering implement must be treated with a surface disinfectant (Table 1). Hose ends used for watering shall be hung on a hook to prevent contact with the floor and with plant material on greenhouse benches. Never lay the hose ends on the floor or on greenhouse benches.

Records must be maintained of any breaches that occur in any part of the irrigation system, including the date, exact location, remedial measures taken, and tests performed to ensure that the irrigation system remains free of *R. solanacearum*.

Survey and Detection of *Ralstonia* in Greenhouse Facilities: All plant material used to propagate stock production geranium plants and the subsequent stock plants themselves must be free of *Ralstonia solanacearum* race 3 biovar 2 prior to introduction into the production stock greenhouse. Diagnostics and sampling protocols identified in the Testing and Sampling Plan are to be used for this disease indexing process. Records of disease testing are to be maintained and made available to APHIS upon request. In addition, plants are scouted regularly for signs of wilt, and tested for *R. solanacearum* as necessary. All plants that are to be discarded for any reason (except when all plants in a greenhouse are destroyed at the end of the production season) must be tested for *R. solanacearum* prior to destruction.

If a sample tests positive for *R. solanacearum*, the producer must notify the NPPO.

The NPPO issues a “stop ship order” on the greenhouse and notifies APHIS.

The NPPO and the producer will collect and submit samples to USDA APHIS for *R. solanacearum* race 3 biovar 2 determination.

The producer is to supply APHIS with shipment data for shipping season from the affected greenhouse.

The greenhouse remains on hold until the race and biovar is determined by APHIS or its designee.

If the greenhouse tests positive for *R. solanacearum* race 3 biovar 2 the producer may not ship any product produced from the infested greenhouses.

The NPPO, APHIS, and the producer investigate the situation to determine the cause of the infestation.

If an infestation occurs then plants in the United States that are traced back to the infestation will be destroyed.

Training of Personnel: Personnel instruction is an important component of good management practices. Training shall be provided to cover all production practices required to prevent *R. solanacearum* race 3 biovar 2 from entering and becoming established. Training shall include not only practices performed in the greenhouse, but should provide a fundamental understanding of how *R. solanacearum* race 3 biovar 2 can spread from plants commonly encountered in workers' yards and environs and required sanitary practice to prevent infection. Management must ensure that staff members understand and follow the practices identified herein before entering the greenhouse. Access to geranium stock production facilities is limited to individuals certified to work in facilities, and a list of these personnel should be maintained.

Personnel will be provided training annually or more frequently as required. Facilities should maintain records of training and certifications.

Food is not to be allowed in or near the greenhouses.

Grading/packing facilities: Grading/packing facilities are subject to the same conditions as greenhouses with respect to the following elements of this plan as described above:

- Wash Stations
- Hand Washing
- Footbaths
- Protective Clothing
- Personal Hygiene
- Tools
- Handling of Cuttings
- Greenhouse Floors
- Production Surfaces
- Treatment of Water
- Training of Personnel

Table or counter surfaces upon which cuttings are handled must be periodically surface disinfested. Treatment of surfaces should be completed between bags or baskets of cuttings processed.

APHIS will amend these management practices as new knowledge and methods are developed / validated to detect and mitigate *Ralstonia solanacearum* race 3 biovar 2.

Treatments and Procedures

Wash stations:

Purpose: To prevent *Ralstonia solanacearum* race 3 biovar 2 from being introduced into greenhouses via contaminated skin (hands/forearms).

Composition: The wash station shall be located just inside the entry area of each greenhouse. It shall consist of a sink area that is supplied from a water supply that is from either a sealed well system or that has been treated for the purpose of *Ralstonia solanacearum* disinfestation. The water draining from the sink must not collect in the greenhouse; it is to drain immediately from the greenhouse facility.

Process: All exposed body parts (hands, arms, legs, etc) that come in contact with plant material are to be washed with antibacterial soap prior to each entry into the production area of the greenhouse. Proper handwashing protocols are as follows:

1. Wet: Wet hands first.
2. Soap: Apply antibacterial soap to palms.
3. Soap/Lather: Lather soap on hands, wrists and forearms
4. Wash: Work all surfaces thoroughly including wrists, forearms, palms, back of hands, fingers, and under fingernails – Rub hands together for a least 30 seconds.
5. Rinse: Thoroughly rinse with clean water. Be sure not to touch sides of sink.
6. Dry: Dry hands completely and use towel to turn off water (if foot pedal faucet is not available) to prevent hands from becoming reinfested.

Treatments: Antimicrobial soap and other appropriately labeled products (See Table 2).

Foot baths:

Purpose: To prevent *Ralstonia solanacearum* race 3 biovar 2 from being introduced into greenhouses via contaminated foot coverings.

Composition: A reservoir containing surface disinfectant effective against *Ralstonia solanacearum* is located between the entry of each greenhouse and the production area of each greenhouse. The bottom surface of the reservoir shall be rough in texture to facilitate dislodging of any debris on the bottom of shoes/boots (this may be accomplished by placement of a mat inside the reservoir). The depth of the footbaths is to be adequate to ensure that soles and lower portions of footwear that may have come in contact with *R. solanacearum* race 3 biovar 2 are wetted with disinfectant.

Process: The foot wear of all persons entering the greenhouse are to be brushed or rinsed free of soil and debris prior to entry of the facility; this will improve treatment efficacy and prolong the activity of the surface disinfectant. Each person entering the greenhouse facility is subsequently required to rinse shoes, boots or other foot wear in a reservoir of surface disinfectant. Foot coverings are to remain wet for a period adequate to allow for disinfectant to disinfect all contaminated footwear surfaces.

The disinfectant in these reservoirs is to be changed a minimum of twice daily, with debris in reservoirs being removed prior to replacement of the disinfectant into the footbath.

Personnel responsible for footbath maintenance shall read, understand, and execute formulation of footbath disinfectants; their accuracy should be verified by management. Daily records are initialed by responsible personnel to demonstrate that foot baths etc. were properly maintained.

Treatments: APHIS approved products containing active ingredients recognized as being effective against *Ralstonia solanacearum* or *Pseudomonas* spp. are identified at the end of this document. All treatments should be applied in strict accordance with the product label. Should a compound not be listed, please obtain approval from APHIS prior to application. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Protective Clothing:

Purpose: Protective clothing is used to prevent contact of geraniums with employee clothing worn outside the increase and production areas.

Composition: Protective clothing designated to come into contact with the geranium crop consists of washable lab coats and/or washable or disposable aprons. Protective clothing is to be dedicated to each greenhouse.

Process: Protective clothing (clean lab coats or aprons) is to be worn by all personnel upon entry of the greenhouse production area. Protective clothing designated to come into contact with the geranium crop (washable lab coats and/or washable or disposable aprons worn over washable lab coats) is to be dedicated to each greenhouse and is to be put on after leaving the hand wash and foot bath station but prior to entering the plant production area. Protective clothing is to be removed prior to leaving each greenhouse but prior to entry into the hand wash/footbath area. If lab coats are worn between greenhouses within a production area, then they should be covered with or exchanged for an apron when workers enter the production area of each greenhouse. Protective clothing is to be stored so that clothing cannot contact the floor. Clothing is to be maintained free of debris, potting media, soil, or plant material.

Treatments: Protective clothing should be washed in detergent weekly or replaced in the case of disposable aprons.

Personal Hygiene:

Purpose: To prevent infection of geraniums by *Ralstonia solanacearum* race 3 biovar 2 and when introduced to limit spread in production greenhouses.

Composition: Plastic spray bottles or containers filled with adequate amount of surface disinfectants are to be used by each worker in the greenhouse.

Process: Sanitation of hands or forearms that come in contact with geraniums must be maintained throughout the period when workers are in the greenhouse facility during production. This may be accomplished by either dipping hands or forearms in disinfectant or by spraying hands and forearms with disinfectant. Surfaces are to remain wet for time required for disinfectant to kill *Ralstonia solanacearum* as defined by product label.

Workers may select to wear latex or equivalent gloves and these are also to be sprayed or dipped in surface disinfectant as described for hands and forearms. Workers are to dip or spray hands with disinfectant between groups of 10 plants or between definable production units of geraniums that are delimited by production practices.

There is to be no eating in the greenhouses.

Treatments: APHIS approved products containing active ingredients recognized as being effective against *Ralstonia solanacearum* or *Pseudomonas* spp. are identified at the end of this document. All treatments should be applied in strict accordance with the product label. Should a compound not be listed, please obtain approval from APHIS prior to application. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Tools Used to Propagate and Harvest:

Purpose: To prevent the infection/spread of *Ralstonia solanacearum* race 3 biovar 2 through production/harvesting of geraniums.

Composition: Tools include any piece of equipment used in the process of production and harvest of geraniums and their cuttings which may come in contact with geraniums or their cuttings.

Process: Greenhouse equipment used to harvest cuttings or for other functions involving contact with geraniums is to be surface disinfested prior to and between use on each stock production plant or stock production container. Alternatively, a set of knives can be permanently assigned to a specific bench and appropriately rotated and disinfested between plants on that specific bench. Carts and collection baskets are to be sprayed with a surface disinfectant on all surfaces that are likely to come into contact with geranium cuttings or equipment used in processing geranium cuttings. Equipment (knives, etc) used to harvest cuttings is to be soaked in surface disinfectant when not in use. The volume of disinfectant used to surface disinfest tools should be adequate to submerge entire blade or portions of tools that make contact with geranium plants.

Treatments: APHIS approved products containing active ingredients recognized as being effective against *Ralstonia solanacearum* or *Pseudomonas* spp. are identified at the end of this document. All treatments should be applied in strict accordance with the product label. Should a compound not be listed, please obtain approval from APHIS prior to application. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Handling of cuttings:

Purpose: To prevent cuttings and crop from becoming infested with *Ralstonia solanacearum* race 3 biovar 2 and to identify the source production greenhouse for geranium cuttings.

Composition: Cuttings are sections of geranium stems harvested for the purpose of export to the United States for future propagation. Plastic bags may not be recycled and must be stored to prevent contact with the floor or native soil. Surface disinfested plastic containers may be used for collection of harvested cuttings prior to the transfer of cuttings to plastic bags. Labels are plastic and information is marked in non-water soluble ink.

Process: Upon harvest, cuttings are placed directly into new plastic bags or into plastic containers that can be surface disinfested before they are used again. Labels will accompany each bag of cuttings. The label system shall allow cuttings to be traced forward thorough the rooting stations (if cutting is sold as rooted cutting), or directly from the farm (if cutting is sold as un-rooted or callused) to the first wholesale grower customer. If an infestation occurs then plants in the United States that are traced back to the infestation will be destroyed.

Transfer of cuttings to grading facilities shall be done in a manner to ensure that bags of cuttings or cuttings in plastic containers do not come in contact with any material that may harbor *Ralstonia solanacearum* race 3 biovar 2.

Treatments: Surface disinfectants should be used in strict accordance with the product label for cleaning plastic baskets used to collect cuttings. **APHIS approved** products containing active ingredients recognized as being effective against *Ralstonia solanacearum* or *Pseudomonas* spp. are identified at the end of this document. All treatments should be applied in strict accordance with the product label. Should a compound not be listed, please obtain approval from APHIS prior to application. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Greenhouse Floors:

Purpose: Greenhouse floors must be constructed and maintained in condition whereby they safeguard greenhouse-grown geraniums from infection by *Ralstonia solanacearum* race 3 biovar 2.

Composition: Flooring throughout all greenhouses must be composed of concrete (or similar material) or crushed rock/gravel (which may be covered with weed-cloth) sufficient to cover any exposed soil. Floors in greenhouses are not permitted to be comprised of exposed soil/dirt; no exposed soil/dirt is allowed in greenhouses.

Process: Greenhouse floors should be maintained free of debris and weeds. Floors must drain properly to prevent puddling. Water in contact with flooring is never in contact with surfaces upon which plants are grown (splashing, watering, etc). Plant material (cuttings, trimmings, etc) that falls to the floor should not be retrieved by greenhouse workers who are in the process of handling production stock plants; it should be removed on a periodic basis (minimum weekly) and disposed of by other workers or by same workers after

harvesting of cuttings is completed. Floors are to be sanitized at least once annually when greenhouses are cleared of stock production plants or before they are set up for the next growing season.

Treatment: APHIS approved products containing active ingredients recognized as being effective against *Ralstonia solanacearum* or *Pseudomonas* spp. are identified at the end of this document. All treatments should be applied in strict accordance with the product label. Should a compound not be listed, please obtain approval from APHIS prior to application. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Production Surfaces:

Purpose: To safeguard the geranium crop from infection by *Ralstonia solanacearum* race 3 biovar 2 via plant-to-plant spread or via the greenhouse floor.

Composition: Production surfaces may be comprised of a variety of materials and designs but must ensure that they do not facilitate pot-to-pot spread and are of adequate distance from the floor to prevent infestation of geraniums.

Procedure: Stock production plants are to be elevated above the greenhouse floor to prevent contamination from splashing water and to ensure that there is no continuum between plant material and water, debris, or potting media on the floor. Surfaces upon which plants are grown should be disinfested prior to use and constructed so water draining from pots does not make contact with other pots.

Treatment: APHIS approved products containing active ingredients recognized as being effective against *Ralstonia solanacearum* or *Pseudomonas* spp. are identified at the end of this document. All treatments should be applied in strict accordance with the product label. Should a compound not be listed, please obtain approval from APHIS prior to application. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Potting Media:

Purpose: To safeguard geraniums from infection by *Ralstonia solanacearum* race 3 biovar 2 through the use of contaminated potting media.

Composition: Potting media does not contain native soil. All potting media must be steam pasteurized or fumigated with methyl bromide or Vapam and may be

reused after sterilization. Plants are potted in new plastic bags or surface disinfected pots. Plastic bags are not recycled.

Procedure: Geranium stock plants are to be grown in steam pasteurized media (2 hours at 80 °C starting when all 10 sensors reach 80 °C. Ten sensors must be placed 2" below surface. Sensors must be evenly spaced on left, right, and center. Two probes must be placed in the middle halfway from bottom and halfway from top. Sensors should be tested and calibrated before use) or fumigated media containing no native soil. Fumigation must be done with Methyl Bromide at the rate of 3 grams/liter for 72 hours at 70 °F or above. Vapam 3% must be applied at an equivalent of 50 ml per stock plant container volume for 72 hours. New plastic bags or pots or recycled plastic pots that have been surface disinfested may be used as stock plant containers. Potting media and pots intended for use in the greenhouse shall not be stored on soil/dirt or turf surfaces.

Treatment: Potting media must be treated with steam for 2 hours at 80 °C. Fumigation with Methyl Bromide for 72 hours or treatment with Vapam 3% for 72 hours is also acceptable. Pots to be recycled may be treated with surface disinfectants labeled for *Ralstonia*. Treatment must be in strict accordance with label.

Irrigation:

Purpose: Irrigation water is treated or source is protected from contamination by *Ralstonia solanacearum* race 3 biovar 2 which may infect geraniums in production greenhouses.

Composition: The requirement for treatment of irrigation water is dependent upon its source, the water storage and delivery system in use.

Procedure: Plants shall not be irrigated using ebb and flow or flood irrigation systems as these methods may contribute to spread of *R. solanacearum* race 3 biovar 2. Irrigation systems should be constructed so that emitters are not in contact with potting media and/or are equipped with backflow devices to prevent contamination of the watering system. The last 1.2 meters of hose closest to the bench and hose-end implements used for hand watering geraniums should never come in contact with the floor of geranium production facilities or with plant material or potting media. When such contact is made, the hose and watering implement must be treated with a surface disinfectant. Hose ends used for watering shall be hung on a hook to prevent contact with the floor and with plant material on greenhouse benches. Never lay the hose ends on the floor or on greenhouse benches.

Treatment: The requirement for treatment of irrigation water is dependent upon its source and the water storage and delivery system in use. Water treatment is not required for irrigation water collected from deep wells which are

properly sealed and where the water is used directly or stored in tanks with no opportunity for contamination of the water supply by native soil or plant material/debris. Municipal water may also be used as a primary irrigation source without initial treatment.

If the water source is from un-sealed wells, rain-water collection systems, ponds, lakes, streams or any other type of open body of water, or if water is recycled or re-circulated, then treatment of water is required.

The water source is to be tested at least annually by APHIS-approved methods to ensure freedom from *R. solanacearum*.

A minimum of two independent water purification systems are to be instituted for each geranium production facility to safeguard the crop from infection by *R. solanacearum*. An independent back up water purification system is required to ensure that the crop does not become infested with *R. solanacearum* due to failure of the primary water purification system.

Any water purification system must consist of:

Filtration of water through reed-bed systems and slow sand filters in combination with one of the following purification methods:

Ozonation (0.4 ppm residual ozone for a minimum of 4 minutes).

OR

Ultraviolet irradiation: 300J/m² of UV light at 254 nm with at least 50% light transmission.

OR

Peroxygen products: a minimum residual level of 4 mg per liter of peracetic acid for 2 minutes. This may be achieved by injection of irrigation water during pumping at 15-35 m³ per hour) with a commercial formulation of 50-100 ml/m³ of peracetic acid.

OR

Chlorine dioxide: Dosage of 0.1mg per liter of residual chlorine dioxide sustained for a two minute minimum reaction time will effectively kill *Ralstonia solanacearum*. This may be achieved by injecting

irrigation water with 5 mg per liter using a chlorine dioxide generator.

Disinfecting hose ends that have been in contact with greenhouse floors or other potentially hazardous surfaces may be treated with quaternary ammonium-based products which are recognized as being effective against *Ralstonia solanacearum*. Surface disinfectants should be used in strict accordance with the product label for sanitizing surfaces: in this case the watering hose.

Records must be maintained of any breaches that occur in any part of the irrigation system, including the date, exact location, and remedial measures taken, and tests performed to ensure that the irrigation system remains free of *R. solanacearum*.

Survey and Detection of *Ralstonia* in Greenhouse Facilities:

All plant material used to propagate stock production geranium plants and the subsequent stock plants themselves must be free of *Ralstonia solanacearum* prior to introduction into the production stock greenhouse. Diagnostics and sampling protocols are identified in the Testing and Sampling Plan. Records of disease testing are to be maintained and made available to APHIS upon request. In addition, plants are scouted regularly for signs of wilt, and tested for *R. solanacearum* as necessary. All plants that are to be discarded must be tested prior to destruction. Where positive results are attained, all plants in the greenhouse are to be destroyed, and the greenhouse decontaminated. The infested facility is to be quarantined, until such time that APHIS exporting authorities (NPPO) are satisfied that the infestation has been eliminated.

Training of Personnel: Instruction of personnel is an important component of good management practices. Training shall be provided to cover all production practices required to prevent *R. solanacearum* from entering and becoming established. Training shall include not only practices performed in the greenhouse, but should provide a fundamental understanding of how *R. solanacearum* can spread from plants commonly encountered in workers' yards and environs and required sanitary practice to prevent infection. Management must ensure that staff members understand and follow the practices identified herein before entering the greenhouse. Access to geranium stock production facilities is limited to individuals certified to work in facilities. A list of these personnel should be maintained. Personnel will be provided training annually; or more frequently as required. Facilities should maintain records of training and certifications.

Grading facilities:

Grading Facilities are subject to the same conditions as greenhouse with respect to the following elements of this plan as described above:

Wash Stations
Hand Washing
Footbaths
Protective Clothing
Personal Hygiene
Tools
Handling of Cuttings
Greenhouse Floors
Production Surfaces
Treatment of Water
Training of Personnel

Table or counter surfaces upon which cuttings are handled must be periodically surface disinfested. Treatment of surfaces should be completed between bags or baskets of cuttings processed.

APHIS will amend these management practices as new knowledge and methods are developed/validated to detect and mitigate *Ralstonia solanacearum* race 3 biovar 2.

Table 1. The following table provides a list of APHIS-approved surface disinfectants. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against *Pseudomonad* bacteria or *Ralstonia solanacearum*.

APHIS-approved Surface Disinfectants for <i>Ralstonia solanacearum</i>			
Trade Name	EPA Reg. No.	Active Ingredients	Use Sites
ANTHIUM DIOXIDE 5% AQUEOUS STABILIZED CHLORINE DIOXIDE	9150-2	Chlorine	Boot wash/shoe wash
BIO CLEAR HOME AIR & DUCT TREATMENT	9804-3-69883	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
BIO-FRESH CD	9804-3-65516	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
COIL & DUCT SPRAY	9804-3-46463	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
ECOTREAT	9804-3-7909	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
ENVIROCON	9804-3	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
TOTALINE COIL & DUCT SPRAY	9804-3-40536	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
VAPORENE 7420	9150-3-10778	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
SANIVEX	9150-3-74495	Chlorine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
Zero Tol	70299-1	Hydrogen dioxide	Greenhouse structures, benches, pots, watering systems, evaporative coolers, storage rooms, ventilation equipment, floors and other equipment
AFCO 4330	4959-16-833	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches

APHIS-approved Surface Disinfectants for <i>Ralstonia solanacearum</i>			
Trade Name	EPA Reg. No.	Active Ingredients	Use Sites
			and counter tops)
ALLSTAR IODO-4	4959-34-63131	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops), shoe wash/boot wash
BIOCIDE HOSPITAL LEVEL DISINFECTANT CLEANER/ DISINFECTANT	4959-16-51003	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
BIOENTRY IODINE DISINFECTANT	65020-4	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
CHEMLAND I-DYNE DISINFECTANT	4959-49	Iodine	Boot wash/shoe wash
CS-138 DISINFECTANT	4959-16-44637	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
CSAN 2339 IDOPHOR SANITIZER	4959-16-67829	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
DIONNE DIO DISINFECTANT I	4959-15-52437	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops), shoe/boot wash
IDO DISINFECTANT	4959-16-46983	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
IODO 175 CONCENTRATED IODINE	4959-34-4313	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops), shoe/boot wash
IODOPHOR SANITIZER	1677-22-541	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
KLENZADE MIKROKLENE DF	1677-58	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)

APHIS-approved Surface Disinfectants for <i>Ralstonia solanacearum</i>			
Trade Name	EPA Reg. No.	Active Ingredients	Use Sites
MIKROKLENE	1677-22	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
OAKITE TRISANITE	4959-16-1020	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
OASIS MIKROKLENE FOOD CONTACT SURFACE SANITIZER	1677-22	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
PEX O DINE 1.75	6836-81-1190	Iodine	Boot wash/shoe wash
SANI DINE	4959-15-64328	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)shoe/boot wash
SHIELD	4959-16-7116	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
TEXO FOOD THRASH	4959-16-1706	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
WESCODYNE	4959-16-1043	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
WEST AGRO ZZZ DISINFECTANT	4959-16	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
ZEP-I-DINE	4959-16-1270	Iodine	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
Lonza Formulation S-18	6836-77	Quat. ammonium	Farm, Poultry, Swine, and Mushroom Premise Sanitation Veterinary Practice/Animal Care/Animal Laboratory Disinfection
MAQUAT 128-MT	10324-112	Quat. ammonium	Outer clothing, field harvesting equipment, walls/floors of coolers, flower buckets, and greenhouse packing areas

APHIS-approved Surface Disinfectants for <i>Ralstonia solanacearum</i>			
Trade Name	EPA Reg. No.	Active Ingredients	Use Sites
MAQUAT 615-HD	10324-72	Quat. ammonium	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
MAQUAT 615-LR	10324-109	Quat. ammonium	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, benches and counter tops)
MAQUAT 64 MN	10324-113	Quat. ammonium	Florist shops, wholesale florist, shippers, greenhouse packing areas, flower buckets, floors/walls of coolers, benches, and counter tops)
Physan 20	55364-5	Quat. ammonium	Greenhouses, hard surfaces, lawn and turf grass, seedlings, cut flowers, decorative fountains, pools, birdbaths, and plants
Consan Triple Action 20	58044-3	Quat. ammonium	Greenhouse hard surfaces, work area, benches, flower pots, buckets, flats, cutting tools, walkways, garden bird baths, and evaporative coolers
Formulation AE-90	47371-89	Quat. ammonium	Non-porous, inanimate surfaces such as floors, walls, metal surfaces, stainless steel surfaces, plastic surfaces, knobs, handles, and railings
Green Shield	499-368	Quat. ammonium	Work area, benches, pots, flats, flower buckets, cutting tools, greenhouse glass, walkways, evaporative coolers, decorative pools, fountains, and water displays
Triathlon	58044-3-59807	Quat. ammonium	Greenhouse hard surfaces, work area, benches, cutting tools, walkways, garden bird baths, and evaporative coolers

Table 2. a) Skin disinfectant active ingredients (CDC recommendations) in various formulations b) Specific products labeled for use on skin or clothing in the United States. Any product used for disinfection should be approved for use by the NPPO, labeled for use by the appropriate authority in the country where the facility is located, and must be efficacious against Pseudomonad bacteria or *Ralstonia solanacearum*.

Active Ingredients

ALCOHOLS (>60% ETHANOL)

CHLORHEXIDINE (0.5%-4% DEPENDING ON PREPARATION)

CHLOROXYLENOL (0.3%-3.75%)

IODINE AND IODOPHORS (7.5%-10% POVIDONE-IODINE)

QUATERNARY AMMONIUM COMPOUNDS (EFFICACY DATA LACKING)

TRICLOSAN (0.2%-2%)

Specific products useful for disinfection of skin and clothing (marked with an *) used

Product	Company	Phone number
*GX-1027 Antimicrobial Soap	Galloway Chemical	(800) 445-1143
Hibiclens; Hibistat	AstraZeneca	(800) 842-9920
*Canker Guard	Flo Tech. Inc.	(800) 335-6832
*Csan 154 QT Soap	Bell Chem Corporation	(407) 339-2355
EcoCare 250, EcoCare 260, EcoCare 350, *EcoCare 360	Ecolab	(651) 293-2848
*Triple Crown Super Healer; *Medi-Kwik AntiMicrobial & Fungicidal Skin Cleanser	Envirosafe, Inc.	(800) 227-9744
AgriCure; Pure & Clean Antibacterial Handwash with GermSAFE	International Laboratory Technology Corp.	(954) 893-1118
*QHS Quaternary Hand Sanitizer	Chemstar Products, Inc.	(813) 978-8648
FS Antimicrobial Hand Cleaner; FS E-2 Sanitizing Hand Soap; Acclaim Antibacterial Liquid Hand Soap	ZEP Manufacturing Co.	(800) 313-8439
*C-Soap	Genesis Technologies	(800) 825-5810

Ralstonia solanacearum in Geranium (*Pelargonium* spp.): Testing and Sampling Plan

Background:

In February 2003, the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) confirmed the presence of *Ralstonia solanacearum* race 3 biovar 2 in geraniums in four greenhouses in Illinois, Indiana, Michigan, and Wisconsin. This is the first time this pathogen has been detected in the United States since 1999. *R. solanacearum* race 3 biovar 2 is a bacterial pathogen that causes common wilt in geraniums and infects numerous Solanaceous plants (e.g. potatoes, tomatoes, eggplant, and peppers). It is also a major concern to the potato industry because the disease survives well in cold temperatures and renders potatoes unmarketable.

In 1999, *R. solanacearum* race 3 biovar 2 was confirmed in commercially grown geraniums in greenhouses, confined to the states of WI, NY, NJ, PA, and SD. The pathogen was traced back to infected geraniums that were shipped to US from greenhouses in Guatemala. Pathway analysis revealed the mode of entry of *R. solanacearum* race 3 biovar 2 into the US is through four states (FL, CA, NY, and TX), with the major ports of entry located at Miami, San Diego and LA, JFK, and El Paso and Houston.

In February 2003, *R. solanacearum* race 3 biovar 2 was identified in greenhouses in the US. Trace back investigations conducted by PPQ have determined that the origin of *R. solanacearum* race 3 biovar 2 was imported geraniums from Kenya. In December 2003, *R. solanacearum* race 3 biovar 2 was identified in greenhouses in the US. Trace back investigations conducted by PPQ have determined that the origin of *R. solanacearum* race 3 biovar 2 was imported geraniums from Guatemala. In the United States, *R. solanacearum* race 3 biovar 2 is a serious concern for the potato industry because of the cold tolerant nature of this plant pathogen, and the potential for its establishment and spread into major potato producing areas.

In an attempt to prevent the entry of this plant pathogen into US, APHIS PPQ will institute the following procedures for sampling and testing of host material in the country of origin before it can be eligible for entry into USA.

Sampling of geranium host material

Samples of geranium mother plants must be taken under authority of the National Plant Protection Organization (NPPO) or their designee. Samples should consist of crown tissue of **all suspect wilted plants** and should be taken throughout each greenhouse in the production facility. A sample of one plant for every 50,000 plants must be taken with a minimum sample of ten plants. Samples should be taken weekly and officials must sample crown tissue of the whole plant and including roots without the soil, if possible. Bare root plants are ideal. Since the pathogen is concentrated in the lower stem, the disease may not be detected from samples

if only leaf or partial stem samples are taken. All material that is wilted or showing other symptoms should be tested. **All plants that are to be discarded for any reason (except when all plants in a greenhouse are destroyed at the end of the production season) must be tested for *R. solanacearum* prior to destruction.** \

When a positive result is obtained, the test should be repeated to ensure accuracy. Be careful to follow the manufacturer's instructions exactly, since in some cases using too much or too little tissue can cause a false positive or false negative result.

Serological Testing

Testing of samples may be done by a variety of approved lateral flow and strip serological test kits. Instructions for the operation of the tests should be followed as specified in the manufacturers instructions provided with each kit. Please read all materials associated with the kits and the kit components to verify proper storage of the reagents and flow devices. Check the manufacturers instructions for each new shipment since instructions may be modified to improve the performance at any given time.

i) *Rs* ImmunoStrip Test

Agdia, Inc
30380 County Road 6
Elkhart, IN 46514
www.agdia.com
Ph. 800-622-4342
FX 219-264-2153

ii) Potato Brown Rot Pocket™ Diagnostic

Central Science Laboratory (CSL)
Sand Hutton, York, YO41 1LZ
www.csl.gov.uk
Ph 44 1904 462600
FX 44 1904 46211

iii) *Ralstonia solanacearum* SPOT vCHECK LFT™

NEOGEN EUROPE, LTD.

Nellie's Gate, AYR
Scotland, KA6 5AW
www.neogeneurope.com

Ph 44 1292 525275
Fx 44 1292 5255477

Method of kit selection and observations

APHIS has tested the kits listed above as follows:

The tests were run as directed by the manufacturer's instructions provided in the kits. We recommend that these kit instructions are followed while performing the tests. All kits require use on symptomatic plants, and for use on stem sections and segments, we followed those instructions. To conduct these tests plant material was selected, chopped and mixed to distribute potentially infected material within the sample, and then the appropriate amounts

of plant materials were distributed to the test vials to conduct the test. The control plant material was healthy and *Ralstonia solanacearum* – infected geranium.

All three tests detected the bacterium in infected plants and did not react to healthy plants. There was a slight difference in sensitivity of the tests based on intensity of the band on the flow device. *It is important that the manufacturer's instructions be followed exactly. For example, an excess of plant material in a sample can cause a false positive reaction on some kits.*

We found the consistently darkest and easiest test bands to read was the CSL Potato Brown Rot Pocket Diagnostic test kit. The CSL test was consistently the fastest reacting (instant reaction to 3 minutes) and produced the darkest test bands that were the easiest to score

The Agdia *Rs* ImmunoStrip test kit, in early testing, produced a lighter test band, however modifications to the ACL strip have resulted in the production of darker bands on the flow device that make them easier to read. The Agdia test produced consistently medium dark to dark test bands and were generally easy to score.

The NEOGEN *Ralstonia solanacearum* SPOT vCHECK LF™ also detected infected positives, but in one test the band was so faint that it may have been missed. The test bands and control bands in the SPOTCHECK system were generally lighter than the other two tests, yet they were generally consistent with the CSL and Agdia systems in detecting a positive. The NEOGEN test produced medium dark to faint test bands instantly to 3 minutes, and in a few cases were hard to score.

In summary, all three tests will detect the pathogen, however, in our hands using samples with a varying range of quality, we recommend the CSL and Agdia tests for a wide range of samples. The NEOGEN test is useful for samples that are of good to fair quality and not of fair to poor quality.

Race 3 biovar 2 determination.

Weekly testing by the plant protection organization of the country of origin or their designee for *R. solanacearum* will be required before facilities can be certified to ship host material to the United States. Weekly testing will continue throughout the shipping season.

All plants that are to be discarded for any reason (except when all plants in a greenhouse are destroyed at the end of the production season) must be tested for *R. solanacearum* prior to destruction.

If a sample tests positive for *R. solanacearum* at the species level,

- NPPO notified
- NPPO notifies APHIS and immediate orders stop shipment for greenhouse.

- NPPO and producer should collect and submit samples to APHIS or other agreed upon authority for race and biovar determination.
- Producer will supply shipment data for the entire shipping season to APHIS from effected Greenhouse
- Greenhouse on hold until determination of race/ biovar is made.
- If positive the greenhouse cannot ship to the US
- An investigation should be conducted by the NPPO, APHIS and producer to determine the cause of the inspection.

If a positive find of *R. solanacearum*, race 3, biovar 2, is detected inside the U.S., the producer will provide, at APHIS request, records of customers who received potentially contaminated plants from the particular greenhouse implicated. If the traceability is adequate, APHIS will place a "stop-ship" order on the implicated greenhouse. However, if APHIS is not satisfied that the source of the problem is clearly traceable to one greenhouse, then an entire facility may, at APHIS discretion, be quarantined until such time that APHIS exporting authorities (NPPO) are satisfied that the infestation has been eliminated.

APHIS reserves the right to examine facilities that have tested positive for *Ralstonia solanacearum* prior to reestablishment of exports of host material to USA.

Diagnostics are subject to change as is the entire sampling protocol depending on new information as it becomes available. APHIS reserves the right to change and amend this document but will communicate changes prior to establishing new requirements.

SIGNATORY PAGE:

This work plan includes: Minimum Sanitation Protocols for Offshore Geranium Cutting Production, Testing and Sampling Plan, and a Signatory Page. The plan requires the participation of the host country National Plant Protection Organization (NPPO) inspection personnel to work in conjunction with APHIS when appropriate, and to provide the oversight needed to demonstrate that each production facility will carry out the procedures, sampling, and testing, described therein. Additionally the NPPO will provide the phytosanitary certification of inspection of all host material to accompany these articles. As required by §319.37-4, the additional declaration must state "These articles have been produced in accordance with the requirements in 7 CFR 319.37-5(r)(3)."

This work plan will remain in effect until further notice, or as agreed upon by both parties. Addendums may be added to this work plan as APHIS receives additional or updated information.

NPPO:
Date:

APHIS:
Date: