ACTION PLAN

for

Ralstonia solanacearum race 3, biovar 2

found in nursery facilities

Photo courtesy of the
Wisconsin Department of Agriculture, Trade and Consumer Protection

February 27, 2003

United States Department of Agriculture
Animal Plant Health Inspection Service
Plant Protection and Quarantine

This action plan provides guidelines and actions necessary for the eradication of *Ralstonia solanacearum* race 3 biovar 2 from US nursery facilities where it has been detected. This plan supplements information contained in Plant Protection and Quarantine (PPQ) Treatment, Emergency Programs, and Administrative Procedures Manuals.

It is to be used in conjunction with other agency regulations, guidelines, and manuals when conducting program activities. The information contained in this action plan are based on scientific information available at the time of writing in consultation with States and industry. The action plan will be updated as new information becomes available.

for Deputy Administrator/Plant Protection and Quarantine
Animal and Plant Health Inspection Service

2/27/03
Date

Please Note: There were two interim versions (2/19/03, 2/25/03) of this document distributed to field offices as drafts and initial guidance. This version 3 (2/27/03) is the first approved version available to growers and the public.

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For further information

Status of facilities, sample results, and regulatory guidance: consult the State Plant Health Director in your state
Media & public information: APHIS Legislative and Public Affairs, Meghan Thomas, phone 301-734-3266
Regional Contacts: PPQ Eastern Region, Lloyd Garcia, phone, 919-716-5709
PPQ Western Region, Clifford Smith, phone, 907-494-7568
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USDA, APHIS, PPQ Action Plan

Procedures for the handling of Ralstonia solanacearum race 3 biovar 2 potentially infected plant material at greenhouses receiving suspect geraniums from Kenya

Background information on *Ralstonia solanacearum* race 3 biovar 2 and the current situation

Races and biovars of *Ralstonia solanacearum* cause bacterial wilt diseases. The pathogen can be transmitted through soil, contaminated irrigation water, equipment, or personnel. It also spreads very easily by transplanting infected plants and propagative materials. Taking cuttings without disinfecting grafting knives between plants, pinching buds of plants, and other cultural practices may facilitate the spread of the pathogen within production facilities. However, the pathogen does not readily spread from plant to plant or by the splashing of water. Spread can be controlled in greenhouses by the application of sound sanitation practices. The bacterium is not spread aerially.

Ralstonia solanacearum is present in the United States, however *Ralstonia solanacearum* race 3 biovar 2, a newly detected, serious pathogen that could affect other important agricultural crops, it is not known to occur in the United States. In addition to a threat to other important crops, the wilting disease it causes in geraniums is lethal.

Wilting symptoms caused by *Ralstonia* species are similar to, and can be confused with, wilting symptoms caused by other pathogens on geraniums such as *Xanthomonas pelargonii* (the agent of bacterial blight). The primary symptom of *Ralstonia solanacearum* race 3 biovar 2, is wilting of leaves and/or abnormal yellowing leaves.

Growers must develop and implement effective sanitation procedures to ensure that the pathogen does not spread within their greenhouse facilities. Additionally, federal and state regulatory officers must conduct inspections and apply control measures to ensure that the disease does not spread within or between greenhouses or facility establishments and does not escape into other production systems. However, since inspectors could inadvertently spread *Ralstonia solanacearum* race 3 biovar 2 or other pathogens through the inspection process, federal and state regulatory officers conducting inspections, before entering and upon leaving each greenhouse, should disinfect shoes and hands and any equipment that comes in contact with plant material.

Recent confirmed detections of *Ralstonia solanacearum* race 3 biovar 2 reported in several states, have been traced to shipments that occurred during certain time periods from rooting stations in Michigan and New Hampshire. These facilities were apparently the recipients of infected geraniums from a facility in Kenya. The Michigan and New Hampshire rooting stations also handle geraniums of Guatemalan origin and might also have commingled and shipped geraniums from both the Kenya and Guatemala to certain US growers. At this time, only Kenyan plants from a facility there are suspected of carrying *Ralstonia solanacearum* race 3 biovar 2, but since commingling is likely to have occurred, all geraniums in these shipments are considered suspect. Shipments of geraniums from Kenya were subsequently halted on February 14, 2003.

Chronology of Destination Nurseries on Hold Lists:

Shipments from the Michigan rooting station during the period between December 2, 2002 and December 20, 2002 (weeks 49-51) shipped to 113 facilities that formed the basis of the priority list of destination nurseries sent out to the PPQ regions on February 14th (Western Region) and February 17th (Eastern Region). Additionally, a list of 19 facilities that received shipments during the same time period from the New Hampshire rooting station were sent to the PPQ Regions on February 19th as a priority list for those destination nurseries. These two lists were used as a priority for placing Emergency Action Notifications, with an expanded list of 1100 facilities (at that time) that followed as a second priority.

Since the lists of potentially problematic destination nurseries appears to be a changing situation, this document will not include further information on the specific chronology of the lists. Consult information provided by the PPQ headquarters and regions as this situation is updated. Consult appendix 3 for guidance on how shipping week numbers are defined.

All lists of destination nurseries are to be kept confidential within USDA or State cooperators.

Definitions:

Suspect geraniums – Geranium (*Pelargonium* sp.) plants, including cuttings from those plants, received directly or indirectly from any establishment identified as having plant material that may have been contaminated or infected with *Ralstonia solanacearum* race 3 biovar 2.

Suspect plants - all other plants, regardless of host status, that may have become commingled during shipping from the rooting stations in question.

Commingled plants – Any plant, regardless of host status, that may have come in contact with suspect geraniums, shared a common watering system with suspect geraniums, or become contaminated through handling or unsanitary cultural practices such as pruning, de-budding and de-leafing. This would include all plants that were shipped in common containers (boxes, bags and racks), plants under ebb and flow, flood type irrigation, systems lacking backflow prevention, and plants located under hanging baskets of suspected geraniums. Included in this definition would be all pots, potting media, and equipment that were similarly contaminated.

Destination nursery - Any nursery, wholesale or retail, receiving suspect geraniums, directly or indirectly, from an establishment identified as having received plant material infected with *Ralstonia solanacearum* race 3 biovar 2.

Protocols:

Before visiting destination nurseries, regulatory officials are to take measures to prevent contamination by plant pathogens between greenhouse and nurseries during inspections, i.e., using effective antimicrobial soap or disinfectant used according to the label instructions. (An example is Gallex #GX-1027 is an antibacterial soap available from Galloway Chemical Division, P.O. Box 5301 Clearwater, FL 33758, 1-800-445-1143). Disposable latex or nitrile gloves and the kind of disposable booties available from hospital suppliers can be used as an alternative, and must be changed between greenhouse.

The following are instructions and guidance for actions for destination nurseries identified as recipients of suspect geraniums potentially infected with *Ralstonia solanacearum* race 3 biovar 2:

1) Contact Owner/Manager: Upon making contact with the destination nursery owner or greenhouse manager, inform them that our records indicate they have received geraniums suspected of being infected by *Ralstonia solanacearum* race 3 biovar 2. Check the destination nursery's records to assure shipments were received.

2) Notice of Holds to Facility: In cooperation with State authorities, issue an Emergency Action Notification ("EAN", PPQ form 523) to the facility that the suspect plant material is under quarantine until further notice. The authority is under 7 CFR 330, the following language should be included on the EAN:

"All geraniums shipped from the_____rooting facility between ____/___/_____ and ____/___/_____ are prohibited from movement pending further notification by USDA APHIS PPQ. Any other plant material received by those same shipments that may have been exposed directly, by commingling in shipping or since being received, by common irrigation systems, or by unsanitary nursery cultural practices are also subject to this hold. All host plants associated with the above exhibiting symptoms of wilt must be reported immediately to USDA APHIS PPQ and held until further notice. No other potential host material of Ralstonia solanacearum race 3 biovar 2 may leave greenhouses containing suspect plant material until further evaluations can be made"

   a) Pursuant to state law, state plant regulatory officials may issue their own hold orders. Language equivalent to the forgoing should be used.

   b) Consult your PPQ regional office for updates on specific rooting facility establishment names and shipping dates considered to be of risk for receiving potentially infected material.

   c) For guidance to nursery owners/managers on procedures to follow when plants with symptoms are found, please consult section 8, a).

   d) The issuance of an EAN prohibits interstate movement of held plant material. Movement to diagnostic laboratories within the state for testing is a function of state control and does not require a permit.

e) Supplemental, or amended EAN's with modified or additional wording will be required for subsequent actions after the initial holds, and if positive samples are confirmed. Not all actions can be specified in this document using future EAN language recommendations. These will involve temperature and time holding regimes, and orders to destroy or dispose of infected material, and disinfection orders. Consult with your State Plant Health Director (SPHD) and PPQ region, and use the guidance of the procedures below to issue additional EAN's for various situations.

3) Gather Information - Ask the facility owner/greenhouse manager to fill out the attached questionnaire, (Appendix 1) and consolidate records at the SPHD and/or PPQ regional offices.

4) Holding Plants: Once the suspect geraniums, suspect plants, and/or commingled plants as defined are located and held, determine the history of movement of the suspect plant material since the shipments in question were received, types of irrigation system used, safeguards against the backflow of water, and the extent of sanitary greenhouse practices in place.

Any other shipments of host material not suspected of coming from infected sources received by destination nurseries should be segregated from suspect plants.

a) Hold plant material that meets the following conditions:

1) Host plants showing symptoms of wilt;

2) Plants within 1 meter (39 inches) of any plant showing signs of wilt;

3) Suspect geraniums;

4) Suspect Plants;

5) Commingled plants such as:

i) Plants that may have come in contact with suspect geraniums during shipping, if in the same boxes, bags, or racks;

ii) Plants may be considered commingled by water contamination during shipping if watered on racks and dripping occurred on other plants, through common irrigation systems such as ebb and flow, flood, or those lacking backflow prevention and those plants located beneath hanging baskets of suspect geraniums or other suspect plant material.

iii) Plants that may have become contaminated through cultural practices such as pruning, de-leafing, de-budding, or pinching without proper sanitation. Proper sanitation includes procedures such as sanitizing hands or tools frequently, or using latex or nitrile gloves sprayed and sanitized of frequently to prevent contamination.

b) Plants that may have been held previously which fall outside the above parameters can be released. All other plants must be held and are subject to options, sampling, and testing procedures, disposal, and release and other conditions and measures listing in subsections 5) - 15) of this document.

5) Options: For plants on hold after the above evaluations are made and before any testing, nursery owners or managers can elect to:

a) Rather than waiting for the appearance of symptoms and subsequent testing of those plants, growers may opt to destroy or dispose of plant material on hold under prescribed conditions. This is only an option available to the owners/managers who may prefer not to follow the procedures listed in 5 b) and c). This would apply to all suspect geraniums, other suspect plants, and commingled plants (as defined) including those plants within a 1 meter (39 inch) radius of any suspect plant. Prescribed disposal or destruction methods must be followed for that plant material and all benches, tables, floors, carts, and other tools and equipment that may have become contaminated must be disinfected according to prescribed procedures; or

b) Maintain the held plant material, including other plants determined to have been commingled in the same shipments as suspect geraniums, and any plant material within a 1 meter radius of nearest held material in place for a specified period of days and temperatures selected by the owner/manager as described in Appendix 4 to ascertain if wilting symptoms are expressed. Should this action be elected, the facility will be re-inspected at regular intervals by federal or state regulatory officials and at the end of the specified period. Destination nursery owners and managers must notify officials if any symptoms are found and follow procedures under 8 a) to maintain those samples until they arrive.

If wilting symptoms are expressed during the selected, specified time and temperature regime, and detections are verified as *Ralstonia solanacearum* race 3 biovar 2, then the time and temperature clock is reset since the last confirmed detection was observed to start another period for symptom expression based Appendix 4. Consult sections 6 c), d) and 7 c) for further guidance.

It is not necessary that this time and temperature regime be implemented immediately. Once begun, the days should be consecutive if at all possible, under the selected option. If heating system failures or weather conditions cause

temperatures to drop during the holding period, evaluations can be made to add days to the period to accommodate this.

Accurate records of average maximum and average minimum daily temperatures must be maintained by the owner/manager for future application of that information to meeting the conditions of the model described in Appendix 4.

> Various temperature reading devices can be used from simple thermometers, max-min thermometers, to a variety of inexpensive or more sophisticated temperature recording devices available on the market.

c) These temperature and time prescriptions for symptom expression (described in Appendix 4) can be applied retroactively, mainly in southern climates where warmer outdoor temperatures have occurred prior to holds. If this option is applied, request if records are available or maximum and minimum thermostat settings are recorded or known for each day for periods of time previous that the suspect geraniums were under prior to holds or notification. If weather data are also available, evaluations will be made of that information and information from the owner/manager with the assertion that no geraniums or host material showed wilting symptoms. (see: http://www.weatherpages.com/wxhistory.html)

6) Conditions for Release: held plants can be released from Emergency Action Notification conditions if the following can be demonstrated:

a) No suspect geraniums from specified suspect rooting and distribution facilities were received during the specified time periods, or;

b) if the option under 5 a) was elected by the owner/manager, and all suspect plant material and other plant material within a 1 meter radius of suspect material, including commingled plants as defined, were disposed of or destroyed according to approved procedures prior to symptom expression and testing, and associated areas and equipment was disinfected according to prescribed procedures and no additional wilting symptoms of plants not destroyed are noted subsquently; or

c) suspect geraniums and other host plants were held for a period of time as specified in Appendix 4 and no symptoms of wilt were or are observed under procedures described in 5 b) or 5 c), or;

d) All plants during times specified above showing signs of wilt similar to those caused by *Ralstonia solanacearum* have been tested and found negative for *Ralstonia solanacearum* race 3 biovar 2; **and**

e) all positive detections are handled in accordance with section 7; **and**

f) further traceback or traceforward investigations do not indicate a risk of dissemination of *Ralstonia solanacearum* race 3 biovar 2.

g) If plants are released, document actions on the PPQ form 523 (EAN) according to the provisions of section 16.

7) At facilities with plants testing positive for *Ralstonia solanacearum* race 3 biovar 2:

a) Place in double plastic bags and seal for disposal or destruction:

i) all geraniums testing positive for *R. solanacearum* race 3 biovar 2,
ii) all plants (host and non-host) within a 1 meter radius of positive plants,
iii) all plants considered to be commingled with plants testing positive by shipping, irrigation, or cultural practices.

b) All areas where those plants removed for disposal were situated, and other areas of risk because of exposure to infected plant material, will be disinfected according to prescribed disinfection procedures.

c) Facilities or greenhouses with positive tested material must have one full period under the time and temperature regimes described in Appendix 4 during which no positive detections were found before they are eligible for release.

8) Sampling procedures:

Federal and State officials must collect, process, and forward samples as soon as possible to screening laboratories as samples can begin to deteriorate rapidly, even under refrigeration. Upon observation of wilting geranium or other host plants in the greenhouse, or when plants or plant parts are still available from culled plants that the greenhouse manager indicated had shown wilting symptoms, then leaf , stem, and root samples will be taken, the greenhouse bench or other location from where plants taken and noted, and labeled according to instructions below:

a) Nursery owners or greenhouse managers in the absence of inspection officials that note the presence of plants with wilting symptoms should immediately notify state or federal inspectors, remove the plants from the bench or rack, note and mark the location where plants were taken, and seal them in double zip-lock bags, 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 (instruct them not to freeze the sample).

b) PPQ Officers or State officials must sample the whole plant 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.** If plants in soil are submitted, place a separate plastic bag around the pot with a rubber band around plant base to restrict soil spillage and contamination of the plant tissue. If soil is left over, dispose of properly.

c) Samples are placed in double zip-lock bags and placed in a cooled location or container until ready for shipping. Do not place samples in a freezer. Samples may be held in a standard refrigerator at 34°F (4°C) or at room temperature if less than or 60°F (15°C).

d) On the inside bag holding the sample, write the sample number and on a card written with a Sharpie® permanent pen placed inside the second zip-lock sample bag (not in the bag containing the sample), legibly write the following:

(1) sample number (use numbering scheme described in 7e below)
(2) name of grower, nursery, or greenhouse facility
(3) location of facility, including phone number and e-mail address
(4) greenhouse number, section, bench number (as applicable)
(5) plant identification (common name, cultivar or variety where possible)
(6) number of plants, or percent of represented in sample.
(7) name and phone number of person taking the sample.

e) Complete a muticopy carbon PPQ form 391 (use form in Attachment 2 if carboned form not available) a copy to be included with each sample batch and then assign and record each sample a unique ID number of the following format:

XX-ABC-0001

(state two letter code—designate a three letter facility code--sample number)

f) When samples are forwarded to state laboratories for screening, assure that copies of PPQ 391's are kept with accurate sample numbers, name of inspector taking the sample, and all identifying greenhouse and bench information in order to locate potentially infected material in the vicinity of confirmed infected plants.

g) Samples must be sent by overnight delivery and ice packs are not needed or recommended. Packaging of all samples should be in a larger bag and made leak proof, then must be placed in a sturdy cardboard outer box.

h) Keep copies of all FedEx®, or other overnight forwarding courier's tracking airway bills. Record the name and phone number of the person responsible for the sample on the zip lock bag with other collection information and on the PPQ 391. Report all samples taken to the SPHD and fax this information to your Regional office.

i) Samples will be screened to the species level through the normal state regulatory networks (i.e., state departments of agriculture laboratories or their cooperating university laboratories). Persons shipping plant material for diagnostics as part of this program do not need to obtain a PPQ Permit under either the *Plant Protection Act* (Regulations at 7 CFR Part 330, the 526 Plant Pest Permit Form) or the *Agricultural Bioterrorism Protection Act of 2002* (Regulations at 7 CFR Part 331).

j) For those shipping plant pest material to other labs to test for the presence *R. solanacearum* (*i.e.*, identification to species only, not biovar and race), no permit is required for **intrastate** shipment, but for **interstate** shipments, the diagnostic laboratory needs a PPQ permit to receive shipments. In this case, it is the diagnostic laboratory in the other state that needs permits, not the person submitting the samples.

k) For further guidance on permitting plant pest material, contact PPQ Permit Services on (301) 734-7211, 6828 or 5055.

l) States that do not have their own state department of agriculture laboratory or cooperating university laboratory for screening to genus and species may use another state's laboratory as long at that laboratory has the necessary plant pest permit to receive interstate samples.

m) Screening Test Kit Availability and Validations:

The USDA-APHIS-PPQ-CPHST Laboratory in Beltsville, MD, has evaluated three rapid serological tests for detection of *Ralstonia solanacearum.* None of the serological tests evaluated will determine the organism to race/biovar.

Ordering and reimbursement information will be forthcoming from APHIS.

The three tests evaluated were:

i) **BID-*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√CHECK LF™**
ADGEN, LTD.
Nellie's Gate, AYR
Scotland, KA6 5AW
www.adgen.co.uk
Ph 44 1292 525275
Fx 44 1292 5255477

The tests were run as directed by the manufacturers instructions provided in the kits. We recommend that these kit instructions are followed while performing the tests. All kits indicated use on symptomatic plants, and for use on stem sections/segments, we followed those instructions. To conduct these tests we selected plant material that was chopped, mixed to distribute potentially infected material within the sample, and then the appropriate amounts were distributed to the test vials to conduct the test. The plant material was healthy and *Ralstonia solanacearum* – infected geranium.

All three tests detected the bacterium in infected plants with no reaction to healthy plants. There was a slight difference in sensitivity of the tests based on intensity of the band on the flow device. The consistently darkest and easiest test bands to read was the CSL Potato Brown Rot Pocket Diagnostic test kit. The Agdia BID-*Rs* ImmunoStrip test kit produced a lighter test band in a few instances, but did detect all infected samples. The ADGEN *Ralstonia solanacearum* SPOT√CHECK 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 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 test produced consistently medium dark to dark test bands read in about 10 minutes and were generally easy to score, and the ADGEN test produced medium dark to faint test bands instantly to 3 minutes, and in a few cases were hard to score.

All three will detect the pathogen, however, in our hands using samples with a varying range of quality we would recommend the CSL and Agdia tests for that range of samples and the ADGEN test for samples that are of good to fair quality and not of fair to poor quality.

n) As an alternative for states that cannot screen their own samples or have not been able to make arrangements with another permitted laboratory out of state, there is currently one private laboratory that has the necessary permits and authorizations to screen to species, (not race 3 biovar 2).

That laboratory is:

Agdia Inc.,
30380 County Road 6,
Elkhart, IN 46514
phone number: (574) 264-2014, or 1-800-62-AGDIA
www.agdia.com

Other private laboratories may be eligible as permits are approved and these establishment names will be provided.

o) If samples are sent out of state for screening, it is still the SPHD's responsibility of the sending state to assure that state laboratory possesses a PPQ plant pest permit to receive interstate samples and to track those samples once sent.

p) Once the plant material has been screened and is known to contain *Ralstonia solanacearum,* **forward the sample or culture as soon as possible by overnight carrier for confirmation to race and biovar**. There is only one diagnostic laboratory with the proper permits to test for the presence of *Ralstonia solanacearum* race 3, biovar 2. This is the USDA, APHIS, PPQ-CPHST laboratory in Beltsville, MD (*see address below*) whose director, Dr. Laurene Levy, has all necessary authorizations to receive samples submitted for diagnostics to the race and biovar level.

Attention: DeVries/Levy
USDA, APHIS, PPQ-CPHST
BARC-East, Bldg. 580
Powder Mill Road
Beltsville, MD 20705 phone number: 301-504-8141

Diagnostic screening laboratories must write their determinations for each sample on the PPQ form 391 with the name and phone number of the responsible diagnostician, keep a copy, and follow the same sample packaging instructions as above. Samples should not be sent on Thursdays or Fridays because there is no Saturday delivery by overnight carriers to the Beltsville facility. Please call the above number to notify the CPHST Beltsville laboratory that you are sending samples of *Ralstonia solanacearum.* If infected stem plant material is not available because the plant was destroyed in the screening process, Dr. Levy will accept securely packaged cultures.

Notify the State Plant Health Director in the sample state of origin and fax the PPQ regional office of any sample forwarding information, completed documentation, including overnight freight tracking information. Once results are known, States will be notified by the PPQ regional office of the results. Some samples may take 2 days to process. Please do not call Laurene Levy to get sample results, this information will be reported to the regions and States from headquarters as soon as they are available.

9) Regulated Articles:

a) Host Plants: Plants, cuttings, or parts of the following hosts of *Ralstonia solanacearum* race 3 biovar 2 are regulated if tested positive, exposed by contact, or due to the conditions described in 4, a), 5):

Common Name (Cultivated Hosts)	Scientific Name
Geranium	**Pelargonium* spp.
Tomato	*Lycopersicon esculentum*
Peppers	*Capsicum spp.*
Eggplant	*Solanum melongena*
Potato	*Solanum tuberosum*
Bean	*Phaseolus vulgaris*
Bittergourd	*Momoridica charantia*
Beet	*Beta vulgaris*
Tobacco	*Nicotiana* spp.

*consult Appendix 5 for the *Pelargonium* species used most often in trade

Common Name (Weed Hosts)	Scientific Name
Black nightshade	*Solanum nigrum*
Climbing nightshade	*Solanum dulcamara*
Horsenettle	*Solanum carolinense*
Jimson weed	*Datura stramonium*
Purslane	*Portulaca oleracea*
Mustards	*Brassica* spp.
Lambsquarters	*Chenopodium album*
Bittergourd	*Momoridica charantia*

b) All plant material within one meter of positive tested hosts, including commingled, regardless of host status, will be considered regulated and if associated because grown under conditions described in 4 a).

c) Soil, planting or rooting media that has been in contact with positive tested plants and other plant material within a 1 meter radius, or under conditions described in 4 a).

d) Tools, implements, equipment, benches, greenhouses used in cultivation that may have contacted positive tested plant material through conditions described in 4 a).

e) Any other products, articles, or means of conveyance, of any character whatsoever, when it is determined by an inspector that they present a hazard of spread of *Ralstonia solanacearum* Race 3 Biovar 2 and the person in possession thereof has been so notified.

10) Quarantine Actions

a) Each property on which *Ralstonia solanacearum*, race 3, biovar 2 is found, or which is identified as a source or as a recipient of confirmed infected plants, will be subject of stipulations of issued Emergency Action Notifications (PPQ form 523) for the destruction of plant material and approved disinfection and handling procedures. Emergency Action Notifications, prohibiting interstate movement (and/or a comparable State notifications

prohibiting intrastate movement), are issued by PPQ field personnel to the property owners or managers of all establishments handling, moving, or processing articles capable of spreading *, Ralstonia solanacearum*, Race 3, Biovar 2.

b) An Emergency Action Notification may be issued pending positive identification and/or further instruction from the USDA, APHIS, PPQ Deputy Administrator.

c) All quarantine actions related to destruction are to be witnessed, supervised, and documented by a PPQ Officer whenever possible. If a PPQ Officer is not available, a State cooperating inspector can witness the disposal. Disinfections can be witnessed and documented by PPQ Officers or State cooperators.

d) If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific emergency action under the Plant Protection Act until emergency regulations can be published in the Federal Register.

e) The Plant Protection Act of 2000 provides for authority for emergency quarantine action. This provision is for interstate regulatory action only; intrastate regulatory action is provided under State authority. However, if the Secretary of Agriculture determines that an extraordinary emergency exists and that the measures taken by the State are inadequate, USDA can take intrastate regulatory action provided that the Governor of the State has been consulted and a notice has been published in the Federal Register. If intrastate action cannot or will not be taken by a State, the PPQ may find it necessary to quarantine an entire state.

f) A General Memorandum of Understanding between States and PPQ exists for each State and facilitates access to private property by PPQ Officers in conjunction with State inspectors to place facilities under notification and witness actions specified in the emergency action notification. Check with your SPHD for clarification.

11) Regulatory Measures:

a) There are no approved regulatory treatments (other than approved disposal or destruction methods) that will permit the movement or sale of infected plant material or exposed plants as defined in section 4, b) from locations with confirmed infections.

b) No plants exposed under conditions of section 4 including plant and parts belonging to any susceptible host species will be removed from any property on which*, Ralstonia solanacearum*, race 3, biovar 2 has been confirmed except for the purposes of disposal or destruction. This prohibition will remain in effect until the property is determined to be free of the disease, based on stipulations under sections 6 and 7.

c) Weed hosts in an infected greenhouse, or in contact with water or plant material from an infected greenhouse, should be removed and handled under the same conditions as other regulated cultivated host material.

d) No soil or media, or planting containers, flats, etc. in contact with suspect plant material or through potentially contaminating irrigation practices may be removed from any property on which *, Ralstonia solanacearum*, race 3, biovar 2 has been found, until testing of associated plant material with symptoms indicates it is free to move.

12) Destruction and Disposal Procedures:

a) For greenhouses or facilities with confirmed detections, or if the owner/manager elects to without waiting for symptoms and sampling under option 5 a), the eradication of *Ralstonia solanacearum*, race 3, biovar 2 requires the complete removal and destruction of infected plants (leaves, stems, flowers, roots), associated growing media, and associated growing containers of any plant material, regardless of host status, including material considered commingled as defined, and if within a 1 meter radius of positive tested plants.

b) The above listed regulated articles removed from the premises for the purpose of destruction or disposal must be contained in double, leak proof bags that are securely sealed.

c) All discarded soil or growing media from potentially infected lots must be removed and destroyed or disposed of by approved methods under section 13.

d) If such contaminated soil or media has been already been disposed of in a compost or other waste pile, cull piles, etc, it will be necessary to dispose of the entire pile because of contamination potential. Any culled host plants available, if they are living and still intact, from such piles, (or from any location), should be sent for testing. All such, plant material, media and waste will be double bagged and sealed before transport to disposal under prescribed methods.

e) If a facility has a potentially infecting recirculating irrigation system in contact with positive tested plants, then the irrigation system itself will be subject to disinfection procedures for that purpose.

13) Approved Disposal and Destruction Methods:

Incineration and steam sterilization are the preferred methods of disposal, however, these options may not be practical for large amounts of infected or potentially infected material that must be disposed of. In these cases, approved municipal landfills, as described below are an option. If landfills that have groundwater protection standards are not available in

the state, however, then incineration and steam sterilization under pressure are the only options:

a) Incineration

b) Steam sterilization under pressure - Follow the guidelines in the PPQ Treatment Manual, pages 3.4.1-3.4.2

c) An approved municipal landfill is one managed or owned by a city, town, or county that, by state regulation, meets conditions that would prevent leaching into groundwater of potential pollutants. This is normally the lining of the landfill bottom with clay or an impervious material, required now for most landfills, but not always in place, for protection against illegal or unintentional dumping of household or other hazardous waste, etc. The state counterparts should check with their appropriate agency to determine suitability of the municipal landfill for protection against leaching into groundwater.

> PLEASE NOTE: To be clear, this plant pathogen is not considered a hazardous waste, biomedical waste, epizootic, or any other such substance of concern for human health that the groundwater protection may be required for --*it is an agricultural pathogen only*—but there is a remote possibility that it could reach ground water for eventual take up from wells used for irrigation. The amount of infected material and concentration of the bacteria will be minimal relative to the volume of material, so the risk is low if the landfill has normal safeguards in place.

The regulatory official that witnesses the disposal of double bagged material from nurseries that use this option for disposal must assure the material is buried under two or more feet of soil. It is suggested that the landfill be notified in advance to arrange for a hole to be dug in the landfill in which the material can be disposed and then covered with soil.

14) Disinfection Procedures:

a) Disinfection of tools, equipment. Tools and equipment that may have come in contact with infected plants or contaminated soil will be cleaned of any soil or media and disinfected by approved disinfectants or by thoroughly washing with pressurized steam cleaning to raise the temperature to 212^{o}F and applied to the point of runoff. (see PPQ Treatment Manual, page 3.4.2).

b) Disinfection of Greenhouses. All potentially contaminated surfaces (benches, flats, walkways, footbaths, drainage areas under benches) and equipment within an infected greenhouse or area in contact with infected material will cleaned of any soil or media, and sprayed and soaked to the point of runoff with approved disinfectants or by pressurized steam cleaning to raise the temperature to 212^{o}F. (see PPQ Treatment Manual, page 3.4.2).

c) Exposed Irrigation Systems. A recirculating irrigation system, subirrigation system, one that has backflow occurrences from infected greenhouses must be drained and all parts, sumps, and pumps circulated with approved disinfectant solutions. This also includes sumps, pumps, and recirculating holding ponds. .

d) Holding Pond Decontamination Procedures- when holding ponds have become contaminated from irrigation systems in facilities with positive testing plants, guidance with come through consultations between headquarters, your regions and your state about possible environmental considerations.

e) Ground Decontamination Procedures- when plants have been held on the ground outside greenhouses in areas with positive tested plants, guidance with come from PPQ Headquarters and Regions in consultation with the State.

15) Approved Disinfectants:

For disinfection of all tools, benches, walkways, surfaces in contact with potentially infected plant material after removal of soil or media:

a) Several quaternary ammonia (20% ammonium chloride) products are approved for greenhouse use. Assure that the label specifies *Ralstonia* or *Pseudomonas* (a previous name for *Ralstonia*) and follow the rates listed on the label for disinfection of surfaces, tools, equipment, benches, walkways, gravel beds under benches, etc. (Some states such as California may have more stringent or specific disinfection compounds approved for their state).

   Two compounds that are registered for *Pseudomonas* are Physan® 20 (which is not approved for greenhouse where food crops are grown and not approved for use in California) and Green Shield® (approved for most uses with a special formulation, Green Shield®CA for use in California). Use rates as directed on the label.

b) ZeroTol® (27% hydrogen dioxide) is a compound that is not a quaternary ammonia, and has rates for disinfection including surfaces with soil or media contamination that cannot be cleaned. This product is also approved for use in disinfection of contaminated irrigation systems. Use rates as directed on the label.

16) Before release of a facility, PPQ Officers must document on the EAN:

a) that the prescribed time and temperature regime was held during which no positives for Ralstonia solanacearum race 3 biovar 2 were detected; or

b) if positives were detected, then all destruction, disposal, and disinfection actions taken with dates taken, location, and witnessed by whom. Also attach any documentation, receipts, etc. that document these actions.

17) Notify nursery owner/managers that their facilities may be subject to future monitoring by State or Federal officials for the presence of *Ralstonia solanacearum* race 3 biovar 2.

Appendix 1

In order to determine the possible extent of the current Ralstonia problem, it is necessary for facility owners or greenhouse managers to provide the following information, along with copies of relevant shipping documents. If GPS coordinates are not available, the address is sufficient.

Name of Nursery _____ Name of Owner _____
Address of Nursery _____GPS coordinates_____
(city)_____(state)_____ (zip code)_____
Phone number and contact name, title _____

1) This shipping season's dates and numbers of plants (geraniums and other species) received from the rooting stations in question. (Indicate the name of the rooting station).

2) The current location and numbers of all plants from these suspect shipments.

3) The history of movement of suspect plants within the facility.

4) The history of movement of suspect geraniums out of the facility.

5) The condition of plants (observations of wilting) since received. (numbers and variety showing symptoms).

6) Where within the facility were symptoms noticed? How many greenhouses, which benches?

7) The disposition of dead or culled plants and soil/potting media since received. (if disposed of, where?)

8) Approximate number of suspect plants, if any, sold to date in retail sales.

9) The destination of other suspect plants if removed from property (ie., sold to other businesses).

10) The location and number of plants of other hosts (tomato, eggplant, potato, tobacco, peppers) in facility inventory since receiving suspect shipments.

11) Irrigation system used (i.e., sub-irrigation, ebb and flow, drip, hand watering, etc.Is there backflow prevention in place?)

12) If flood, sub-irrigation, or ebb and flow, is practiced, identify the location of all plants sharing the same water source.

13) Describe the type of greenhouse benches used and floor composition.

14) What are the average minimum and maximum temperature maintained in greenhouses?

15) Is there a standard greenhouse sanitation protocol document available for your facility? Please provide a copy if so.

_____ _____

owner or manager signature *date*

Appendix 2 PPQ form 391 for sample submissions, use carboned form if available:

This report is authorized by law (7 U.S.C. 147a). While you are not required to respond your cooperation is needed to make an accurate record of plant pest conditions.

FORM APPROVED
OMB NO. 0579-0010

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTOIN SERVICE

SPECIMENS FOR DETERMINATION

Instructions: Type or print information requested. Press hard and print legibly when handwritten. Item 1 assign number for each collection beginning with year, followed by collector's initials and collector's number. Example (collector, John J. Dingle): 83-JJD-001. Pest Data Section - Complete items 14, 15 and 16 or 19 or 20 and 21 as applicable. Complete items 17 and 18 if a trap was used.

FOR USDA USE
LOT NO.
PRIORITY

1. COLLECTION NUMBER

2. DATE MO | DA | YR

3. SUBMITTING AGENCY ☐ State Cooperator ☐ PPQ ☐ Other .....

4. NAME OF SENDER

5. TYPE OF PROPERTY *(Farm, Feedmill, Nursery, etc.)*

6. ADDRESS OF SENDER

INTERCEPTION SITE

7. NAME AND ADDRESS OF PROPERTY OR OWNER

ZIP

COUNTRY/ COUNTY

8. REASON FOR IDENTIFICATION *("X" ALL Applicable Items)*

A. ☐ Biological Control (Target Pest Name)	E. ☐ Livestock, Domestic Animal Pest
B. ☐ Damaging Crops/Plants	H. ☐ Possible Immigrant (Explain in remarks)
C. ☐ Suspected Pest of Regulatory Concern (Explain in remarks)	J. ☐ Survey (Explain in remarks)
D. ☐ Stored Product Pest	L. ☐ Other (Explain in remarks)

9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".

10. HOST INFORMATION	11. QUANTITY OF HOST	
NAME OF HOST *(Scientific name when possible)*	NUMBER OF ACRES/PLANTS	PLANTS AFFECTED *(Insert figure & indicate number or percent)* ☐ Number ☐ Percent

12. PLANT DISTRIBUTION	13. PLANT PARTS AFFECTED			
☐ LIMITED	☐ Leaves, Upper Surface	☐ Trunk/Bark	☐ Bulbs, Tubers, Corms	☐ Seeds
☐ SCATTERED	☐ Leaves, Lower Surface	☐ Branches	☐ Buds	
☐ WIDESPREAD	☐ Petiole	☐ Growing Tips	☐ Flowers	
	☐ Stem	☐ Roots	☐ Fruits or Nuts	

14. PEST DISTRIBUTION
☐ FEW
☐ COMMON
☐ ABUNDANT
☐ EXTREME

15. ☐ INSECTS ☐ NEMATODES ☐ MOLLUSKS

NUMBER SUBMITTED	LARVAE	PUPAE	ADULTS	CAST SKINS	EGGS	NYMPHS	JUVS.	CYSTS
ALIVE								
DEAD								

16. SAMPLING METHOD | 17. TYPE OF TRAP AND LURE | 18. TRAP NUMBER

19. PLANT PATHOLOGY - PLANT SYMPTOMS *("X" one and describe symptoms)*
☐ ISOLATED ☐ GENERAL

20. WEED DENSITY ☐ FEW ☐ SPOTTY ☐ GENERAL

21. WEED GROWTH STAGE ☐ SEEDLING ☐ VEGETATIVE ☐ FLOWERING/FRUITING ☐ MATURE

22. REMARKS

23. TENTATIVE DETERMINATION

24. DETERMINATION AND NOTES *(Not for Field Use)*

FOR USDA USE
DATE RECEIVED
NO.
LABEL
SORTED
PREPARED
DATE ACCEPTED
RR

PPQ FORM 391 (JUL 86) *Previous editions are obsolete.*

Appendix 3:

Shipping Dates Related to Week Designations Used by Goldsmith Rooting Stations

week 45	11/4/02	11/8/02
week 46	11/11/02	11/15/02
week 47	11/18/02	11/22/02
week 48	11/25/02	11/29/02
week 49	12/2/02	12/6/02
week 50	12/9/02	12/13/02
week 51	12/16/02	12/20/02
week 52	12/23/02	12/27/02
Week 1	12/30/02	1/3/03
week 2	1/6/03	1/10/03
Week 3	1/13/03	1/17/03
Week 4	1/20/03	1/24/03
Week 5	1/27/03	1/31/03
Week 6	2/3/03	2/7/03
Week 7	**2/10/03**	**2/14/03**

Appendix 4

The development of wilt symptoms in geraniums infected by *Ralstonia solanacearum* race 3 biovar 2 (RSr3b2) is dependent upon temperature. Disease symptoms do not develop below 63°F, although RSr3b2 will remain alive in infected plants. As temperatures increase, fewer days are required to observe disease symptoms.

The following table will enable you to estimate when to inspect greenhouses or field sites for wilt of geranium caused by RSr3b2. To estimate inspection date, do the following:

1. Contact grower and request information on greenhouse temperatures from the date that suspect geranium were received until the present date. In general we have found that many growers do not keep accurate records. If records are not available, ask for thermostat settings. You will also need to know the date when plant material was placed in the greenhouse.

2. Average minimum temperatures are listed across the top row; average maximum temperatures are in the left hand column. Locate the closest max and min temperatures in the table for the time period under consideration. The cell where the appropriate column and row intersect contains an estimate of the number of days required to observe wilt symptoms. Determine the date for greenhouse inspection by adding the number of days from the table to the date when the suspect geraniums were placed in the greenhouse.

Number of Days to Symptom Development Using Average Daily Max Min Greenhouse Air Temperatures

	Minimum Temp (°F)									
Maximum Temp (°F)	**54**	**56**	**58**	**60**	**62**	**64**	**66**	**68**	**70**	**72**
65	**833**	**682**	**517**	**375**	**224**	**100**				
67	**242**	**205**	**169**	**132**	**94**	**60**	**43**			
69	**125**	**109**	**91**	**75**	**58**	**43**	**33**	**27**		
71	**80**	**70**	**61**	**52**	**42**	**33**	**27**	**23**	**20**	
73	**57**	**51**	**45**	**39**	**33**	**27**	**23**	**20**	**18**	**16**
75	**44**	**40**	**35**	**31**	**27**	**23**	**20**	**18**	**16**	**14**
77	**35**	**32**	**29**	**26**	**23**	**20**	**18**	**16**	**14**	**13**
79	**29**	**27**	**25**	**22**	**20**	**18**	**16**	**14**	**13**	**12**
81	**25**	**23**	**21**	**19**	**18**	**16**	**14**	**13**	**12**	**11**
83	**22**	**20**	**19**	**17**	**16**	**14**	**13**	**12**	**11**	**10**
85	**19**	**18**	**17**	**15**	**14**	**13**	**12**	**11**	**10**	**10**

Appendix 5

Common *Pelargonium* species in the nursery trade:

Pelargonium xhortorum: Zonal geranium or Florist's geranium. "Americana" geraniums fall in this group. This genus and species makes up 70-80% of the geraniums sold in the U.S. each year. Vegetatively propagated and most are tetraploids.

Pelargonium xhortorum: Seed geraniums. A smaller portion of the market. These are diploids and are reproduced via seed

Pelargonium peltatum: Ivy geranium. Approx 10-20% of the market is this species. Vegetatively propagated. Typically grown mainly in baskets (hanging above other crops). There are also seed propagated lines of this species.

Pelargonium domesticum: Regal or Martha Washington geraniums. This species is grown mainly as a flowering potted crop through florists and upper end retail garden centers. Vegetatively propagated. No more than 5-10% of the overall market is of this species. These are used as pot plants to display in the home and typically are planted out into the garden since they will not flower during the heat of the summer. The foliage and flowers are significantly different than the zonal geraniums.

Pelargonium spp.: Scented geraniums and other novelty types with unusual flowers, foliage or scented foliage. Very small market segment of unusual types. Vegetatively propagated. Broad diversity of genetics make up this group and difficult to type to species.