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New Pest Response Guidelines

Ralstonia solanacearum race 3 biovar 2



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New Pest Response Guidelines: Ralstonia solanacearum race 3 biovar 2 was prepared by Joel Floyd, USDA-APHIS-PPQ-Emergency and Domestic Programs. The *Guidelines* was originally limited to geranium host plants, and was issued on February 27, 2003.

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Cover

Geranium—Wilting symptoms on geraniums caused by *Ralstonia solanacearum* race 3 biovar 2; courtesy of Wisconsin Department of Agriculture, Trade and Consumer Services

Potatoes—Symptoms of brown rot, *Ralstonia solanacearum* race 3 biovar 2, on potato tubers and plant; courtesy of Centro Internacional de la Papa (International Potato Center or CIP), Lima, Peru

Weed Host—Bitter nightshade, a weed host of *Ralstonia solanacearum* race 3 biovar 2; courtesy of Joel Floyd, USDA-APHIS-PPQ

Contents

Credits *page A-i*
Contributors i
Consultants ii
Cover ii
Contents *page ii-iii*

Chapter 1 *page 1-1*

Introduction *page 1-1*
Contents 1
Purpose 1
Status 1
Document Comprehensiveness 2
Commercial Suppliers or Products 2
Prevention of Infection 2
 Regulatory Officers 2
 Growers 2
Program Safety 3
Support for Program Decisionmaking 3

Chapter 2 *page 2-1*

Pest Information *page 2-1*
Contents 1
Introduction 1
Classification 1
Importance 2
 Race 1 3
 Race 3 3
History 3
 Geranium Hosts 3
 Current Status 4
 Solanaceous Hosts 4
 Current Status 5
Economic Impact 6
 Geranium Hosts 6
 Solanaceous Hosts 6
Hosts 6
Geographic Distribution 7

Chapter 3 *page 3-1*

Survey Procedures *page 3-1*
Contents 1
Introduction 1
Precautions 1
 Antimicrobial Soap, Lotion, or Disinfectant 1
 Disposable Gloves and Footwear 2
Detection Survey 2
 Procedure 2

Geranium Hosts	3
Solanaceous Hosts	4
General Survey	4
Targeted Survey	4
Delimiting Survey	6
Geranium Hosts	6
Solanaceous Hosts	6
List of Facilities	6
Working with Growers or Farm Managers	7
Traceback and Trace Forward Survey	7
Traceback Investigation	7
Analyzing Information	7
Positive Detection in Potatoes	8
Trace Forward Investigation	8
Water Sources and Irrigation Methods	8
Investigations at Processors	9
Monitoring Survey	9
Geranium Hosts	9
Solanaceous Hosts	9
Visual Inspection for Detection Survey	10
Greenhouse and Nursery Crops	10
Geraniums	10
Other Hosts in Greenhouses or Nurseries	11
Discarded Plants	11
Potato Cropping Systems	11
Potato Plants	11
Potato Tubers	12
Tomatoes and Other Solanaceous Crops	13
Visual Inspection for Delimiting Survey	14
Greenhouse and Nursery Crops	14
Potato Cropping Systems	14
Tuber Sampling	14
Delimiting Surveys in a General Growing Area	15
Sampling of Water Sources	15
Tomato and Other Solanaceous Hosts	15
Data Collection	15

Chapter 4 *page 4-1*

Identification	<i>page 4-1</i>
Contents	1
Introduction	1
Authorities	1
Plant Protection Act	2
Agricultural Bioterrorism Protection Act	2
Procedure for Sampling and Identification	3
Screening Nursery and Field Hosts	3
Labeling, Numbering, and Record Keeping	4
Storage and Shipping	4
PPQ Form 391	4
Saturday Delivery	5
Notification of State Officials of Submissions and Results	5
Sample Processing Time	5
Diagnostic Screening Laboratories	6

- Alternative Laboratory 6
- Required Permits 6
 - Intrastate Transfer 7
 - Interstate Transfer 8
- Diagnostic Tests to Determine Genus and Species 8
 - Validated Screening Test Kits 8
 - ELISA Test Kits 9
 - Water Testing 9
- Culturing 9
- Confirmatory Testing to Race and Biovar 9
- Positive Confirmations 10

Chapter 5 *page 5-1*

- Regulatory Procedures *page 5-1*
- Contents 1
- Introduction 1
- Instructions to Officers 1
 - Quarantine Actions Required 2
 - Geraniums 2
 - Potato Cropping Systems 2
 - Emergency Quarantine Action 2
 - Access to Private Property 3
 - Regulatory Records 3
- Overview of Regulatory Program After a United States Detection 3
 - Determining Origin of Infections 3
 - Geraniums 3
 - Potato Cropping Systems 4
 - Distribution of Lists of Positive and Suspect Properties 4
 - Geraniums 4
 - Potato Cropping Systems 5
- Further Investigations at Nurseries with Suspect Geraniums 6
- Placing Holds 7
 - Nurseries 7
 - Potato Storage Facilities and Grower Fields 7
- Record Keeping 8
 - Geraniums 8
 - Potato Cropping Systems 8
- Plants to Hold 9
 - Suspect Geraniums 9
 - Variety 9
 - Shipment 9
 - Potentially Infected Plants 9
 - Suspect Potato Cropping Systems 11
 - Grower Requirements Under Quarantine 11
- Issuing PPQ Form 523 Emergency Action Notification 11
- Geranium and Potato Volunteers and Cull Piles 12
- Harvesting Equipment, Storage Areas, and Conveyances 12
- Regulatory Requirements of Seed Producers 12
- Regulatory Requirements for Processors 12
- Regulated Articles 13
 - Cultivated Host Plants 13
 - Weed Host Plants 13
 - Pots and Media 14

Tools and Equipment 14
Removing Areas from Quarantine 14
Requirements for Potato Cropping Systems Under Quarantine 15
 Properties with Positive Testing or Associated Fields 15
 Fields Adjacent to Positive-Testing or Associated Fields, or Sharing Water 15
Storage Facilities 15
Volunteer Potatoes and Cull Piles 16
Harvesting Equipment, Storage Areas, and Conveyances 17
Regulatory Requirements of Seed Producers 17
Regulatory Requirements for Processors 17
Use of Chemicals 18

Chapter 6 *page 6-1*

Control *page 6-1*

Contents 1

Introduction 1

 Laws Pertaining to Pest Control 1

 Control Records 2

 Control Decisions and Oversight 2

Handling of Samples 2

Plant Disposal and Destruction Methods 3

 Burning or Incineration 3

 Steam Treatment Under Pressure 3

 Soil Fumigation 3

 Approved Landfill 3

Disinfection Procedures 4

 Tools and Equipment 5

 Exposed Irrigation Systems 5

 Holding Ponds 5

 Outdoor Soil or Holding Areas 5

Approved Disinfectants 6

Chapter 7 *page 7-1*

Environmental Compliance *page 7-1*

Contents 1

Introduction 1

Disclaimer 1

National Environmental Policy Act 2

Endangered Species Act 2

Federal Insecticide, Fungicide, and Rodenticide Act 3

Other Laws 3

Environmental Monitoring 3

Glossary *page 8-1*

References *page 1-1*

Appendix A *page A-1*

Resources *page A-1*

 Diagnostic Tools and Equipment 1

 Environmental Compliance 1

 Environmental Services 1

Appendix B *page B-1*

Forms and Questionnaires *page B-1*

Contents 1

PPQ Form 391 Specimens For Determination	2
Addendum to PPQ Form 391	4
Questionnaire for Nursery and Potato Cropping System Owners or Managers	5
Location	5
Questions	5
PPQ Form 523 Emergency Action Notification	7
Instructions	8
Procedure	8
Releasing Plants	8
Documenting Actions Taken	9
Disposal Memorandum	9
PPQ Form 526 Permit to Move Live Plant Plants or Noxious Weeds	10
	11

Appendix C *page C-1*

Diagnostic Validations and Isolation Methods *page C-1*

Contents	1
Introduction	1
Status of Commercial Diagnostic Kits	1
Non-plate, Rapid, Immunoassay Detection	2
Adgen Spot Check LF ^{TM2}	
Agdia ImmunoStrip ^{®2}	
CSL Pocket Diagnostic ^{TM3}	
Immunoplate Assays (ELISA)	3
Agdia PathoScreen ELISA	3
Comparison of Non-plate Immunoassay and Plate Immunoassay	5
Validation of Central Science Laboratory TaqMan [®] Assay	6
Validation of Sample and DNA Preparation Methods	6
Preparation of Media for Isolation	7
Selective Medium for <i>Ralstonia solanacearum</i>	7
Semi-selective Medium for <i>Ralstonia solanacearum</i>	7
Isolating Bacteria from Tissue Samples	9
Method A	9
Method B	9
Method C	9

Appendix D *page D-1*

Diagnostic Laboratories *page D-1*

Contents	1
Introduction	1
Determination of Race and Biovar	1
Determination of Genus and Species	2
State and Cooperating University Diagnostic Laboratories	3
Plant Diagnostic Networks and Private Laboratories	10

Appendix E *page E-1*

Pelargonium in the Nursery Trade *page E-1*

Appendix F *page F-1*

Comparison of Symptoms *page F-1*

Appendix G *page G-1*

Disinfectants *page G-1*

1

Ralstonia
solanacearum r 3 b 2

Chapter 1

Introduction

Contents

Purpose	page 1-1
Status	page 1-1
Document Comprehensiveness	page 1-2
Commercial Suppliers or Products	page 1-2
Prevention of Infection	page 1-2
Program Safety	page 1-3
Support for Program Decisionmaking	page 1-3

Purpose

Use *New Pest Response Guidelines: Ralstonia solanacearum race 3 biovar 2* as a guide when designing a program to detect, diagnose, contain, control, or eradicate this pathogen. Personnel within state departments of agriculture and others concerned with developing local survey or control programs should find the *Guidelines* useful.

United States Department of Agriculture–Animal and Plant Health Inspection Service–Plant Protection and Quarantine (USDA–APHIS–PPQ) developed the *Guidelines* through discussion, consultation, or agreement with staff at USDA–Agricultural Research Service (ARS), universities, industries, and state departments of agriculture.

The *Guidelines* will be updated as new information becomes available. Specific emergency programs should be based on information available at the time of the incident.

Status

Ralstonia solanacearum is a plant pathogenic bacterium that causes wilt diseases. Strains of *Ralstonia solanacearum* cause economic damage to diverse agricultural crops and are widespread (Hayward, 1991; Sequeira, 1994). *Ralstonia solanacearum* race 3 biovar 2 is of particular concern in the United States because of its affect on eggplant, geraniums, potatoes, and tomatoes. While race 1 is endemic to the southeastern United States where it can affect tomato crops, *Ralstonia solanacearum* race 3 biovar 2 is not known to occur in the United States and is considered of quarantine importance.

Document Comprehensiveness

This document is not intended to be complete and exhaustive, but provides a foundation based upon the literature available to assist further work. Some key articles were not available at the time of writing, and not all specialists and members of the research community were consulted for their advice.

Commercial Suppliers or Products

References to commercial suppliers or products should not be construed as an endorsement of the company or product by USDA.

Prevention of Infection

To minimize the effects of this disease and control actions that may be necessary, regulatory officers and growers should develop and implement effective sanitation procedures to ensure that the pathogen does not spread within their greenhouse or nursery facilities, associated support buildings, equipment or vehicles.

Regulatory Officers

Federal and state regulatory officers must conduct inspections and apply control measures to ensure that the disease or pathogen does not spread within or between greenhouses or nurseries, associated support buildings, equipment, vehicles, or fields, and does not escape into other production systems.

Important

Since inspectors could inadvertently spread *Ralstonia solanacearum* race 3 biovar 2 or other pathogens during the inspection process, federal and state regulatory officers conducting inspections—before entering and upon leaving each greenhouse and nursery location—must follow sanitation guidelines to prevent spreading contaminated plant material, soil, or water to other facilities. See [Precautions on page 3-1](#) for more information on this topic.

Growers

Geranium growers can minimize the impact of the disease, including the regulatory impact, by following these practices:

- ◆ Submit for testing plants that display wilt symptoms and do not recover after watering.
- ◆ Avoid sub-irrigation and shared watering systems.
- ◆ Use strict sanitary practices when handling or propagating plants.
- ◆ Do **not** place plants beneath the drip line of hanging geraniums.
- ◆ Maintain varieties of geraniums from foreign sources separate.

- ◆ Disinfect water systems and regularly treat irrigation water from any source.
- ◆ Keep greenhouses, areas around greenhouses, and irrigation water holding or overflow ponds free of weeds.

Domestic growers can implement the practices to minimize contamination of rooted cuttings for sale in the United States.

Growers can consult the document [Minimum Sanitation Protocols for Offshore Geranium Cutting Production](#), developed by USDA–Animal and Plant Health Inspection Service (APHIS) and used by off-shore geranium suppliers, for more information.

Program Safety

Safety of the public, and program personnel, is a priority consideration in preprogram planning and training, and throughout program operations. Safety officers and supervisors must enforce on-the-job safety procedures.

Support for Program Decisionmaking

USDA–APHIS–PPQ–Center for Plant Health Science and Technology (CPHST) provides technical support to emergency pest response program directors concerning risk assessments, survey methods, control strategies, and other aspects of pest response programs.

2

Ralstonia solanacearum r 3 b 2

Chapter 2

Pest Information

Contents

Introduction	page 2-1
Classification	page 2-1
Importance	page 2-2
History	page 2-3
Economic Impact	page 2-6
Hosts	page 2-6
Geographic Distribution	page 2-7

Introduction

Use *Chapter 2 Pest Information* to learn more about the classification, importance, history, host range, and distribution, of *Ralstonia solanacearum* race 3 biovar 2.

Classification

Use [Table 2-1](#) as an aid to the classification of *Ralstonia solanacearum* race 3 biovar 2.

TABLE 2-1 Classification of *Ralstonia solanacearum* race 3 biovar 2 (continued)

Domain	Bacteria
Phylum	Proteobacteria
Class	Betaproteobacteria
Order	Burkholderiales
Family	Ralstoniaceae
Strain	<i>Ralstonia solanacearum</i> (Smith 1896) Yabuuchi, <i>et al.</i> 1995 [race 3, biovar 2]
Synonyms	<i>Bacillus solanacearum</i> (Smith 1896), <i>Pseudomonas solanacearum</i> (Smith) Smith 1914, <i>Burkholderia solanacearum</i> (Yabuuchi, <i>et al.</i> 1992)
Approved common names (APS, 2003)	bacterial wilt (pepper, potato, tomato), southern wilt (geranium), brown rot (potato)

TABLE 2-1 Classification of *Ralstonia solanacearum* race 3 biovar 2 (continued)

Other common names	bacterial wilt of potato, bacterial wilt of solanaceous crops, brown rot of potato, brown rot of solanaceous crops, southern bacterial blight of tomato, southern bacterial wilt (CABI, 2003)
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Importance

Ralstonia solanacearum is a plant pathogenic bacterium that causes wilt diseases. Strains of *R. solanacearum* cause economic damage to diverse agricultural crops and are widespread (Hayward, 1991; Sequeira, 1994) (Table 2-2). Of particular concern in the United States is *R. solanacearum* race 3 biovar 2 because of its affect on eggplant, geraniums, potatoes, and tomatoes. While race 1 is endemic to the southeastern United States where it can affect tomato crops, *R. solanacearum* race 3 biovar 2 is not known to occur in the United States and is considered of quarantine importance.

TABLE 2-2 Host Range and Distribution of Strains of *Ralstonia solanacearum* [Adapted from Daughtrey (2003) and Denny and Hayward (2001)]

Race	Biovar	Hosts	Geographical Distribution
1	1, 3 or 4	Wide	Asia, Australia, Americas
2	1	<i>Musa</i> spp.	Caribbean Islands, Brazil, Philippines
3	2	Potato, other Solanaceae, geranium, others	Worldwide except United States and Canada
4	3 or 4	Ginger	Asia
5	5	Mulberry	China

The bacteria of all races of *Ralstonia solanacearum* can be transmitted through contaminated soil, irrigation water, equipment, or personnel. The bacteria may be spread by the following means:

- ◆ Shared water irrigation systems
- ◆ Transplanting and propagating infected plants
- ◆ Taking cuttings without disinfecting implements between plants
- ◆ Pinching buds of plants without sanitizing
- ◆ Transfer of contaminated soil (on equipment or shoes) to disease-free areas

The pathogen is **not** readily spread between plants by the following means:

- ◆ Splashing of water
- ◆ Leaf to leaf contact
- ◆ Aerial transfer

Infection occurs typically through the roots and wounding in root areas, a normal physiological process as rootlets grow. Bacteria are normally concentrated in the lower stem portions of the plant. Spread can be controlled in greenhouses by the strict application of sanitation practices.

The species is differentiated into five races according to host range (Buddenhagen, Sequeira, and Kellman, 1962), and into biovar classifications according to physiological tests (Hayward, 1964; Hayward, 1991).

Race 1

Strains of *Ralstonia solanacearum* race 1 biovars 1 and 3 have been found to occur on greenhouse ornamentals in the northern hemisphere (CABI, 2003). Race 1 also has a wider host range and is endemic to the southeastern United States.

Race 3

Ralstonia solanacearum race 3 is found worldwide **except** in the United States and Canada. Hosts of *R. solanacearum* race 3 biovar 2 are usually restricted to cultivated solanaceous species such as potato and tomato. Race 3 has been reported occasionally on eggplant (*Solanum melongena*), pepper (*Capsicum annum*) and solanaceous weeds (Martin & French, 1995). Some infected weed hosts remain symptomless. These weed hosts may enable race 3 biovar 2 to survive in a latent form within the host or in their root areas in the soil (Janse, *et al.* 2003).

History

Geranium Hosts

In the United States, a 1979 study of host tests with a strain of *Ralstonia solanacearum* isolated from *Pelargonium x hortorum* (= *P. zonale* hybrids or geranium) indicated the presence of race 3 because the isolate was not pathogenic on tobacco (Strider, *et al.* 1981). However, some reports of findings of *R. solanacearum* race 3 biovar 2 on *Pelargonium* spp. from Western Australia and Tanganyika are questionable (Pittman, 1933; Wallace, 1934).

In 1995, Kim and Olson (2003) received a strain of *Ralstonia solanacearum* race 3 biovar 2 from a Connecticut source. The strain was isolated from a geranium plant from Guatemala. They confirmed the presence of a similar strain on wilted geranium plants from greenhouses in Pennsylvania in 1999 and 2000 and from Delaware in 1999; the plants were imported from Guatemala, too.

In 1999, *Ralstonia solanacearum* race 3 biovar 2 was reported in New Jersey, New York, Ohio, Pennsylvania, South Dakota and Wisconsin on commercially grown geranium (*Pelargonium zonale*) imported from Guatemala (Hudelson, *et al.* 1999 and 2002; Nameth, 1999; SPRO, 2002). These findings resulted in a review of foreign site production facility practices that export geranium cuttings to the United States.

Also in 1999, the bacterium was detected in the United Kingdom on imported geranium cuttings produced in Kenya for the European market (Janse, *et al.* 2003). During September–December 2000, symptoms of bacterial wilt were observed in several geranium nurseries in Belgium and Germany (Janse, *et al.* 2003).

In February 2003, geranium plants imported from Kenya to the United States were implicated as the source of *Ralstonia solanacearum* race 3 biovar 2 that resulted in detections in 127 individual nurseries in 27 states. Imports from Kenya were halted and phytosanitary requirements implemented for all geranium imports from countries that have *R. solanacearum* race 3 biovar 2. In early 2004, another detection was made in the United States, this time traced backed to production greenhouses in Guatemala. This program, again conducted by federal and state regulatory officials, resulted in control actions in 453 nurseries in 41 states. Both actions resulted in the destruction of approximately two million geranium plants each.

In 2003 and 2004, detections in the United States have been limited to within greenhouses. The infections were contained and plants were destroyed. The disease was eradicated through industry practices or control programs.

Current Status

In late 2003, a certification program for geraniums imported to the United States was implemented. The program requires specific clean culture practices and regular testing. Geranium plants exported to the United States require phytosanitary certification and a statement that testing showed geraniums shipped to the United States are free of *Ralstonia solanacearum* race 3 biovar 2.

Solanaceous Hosts

In 1880, a strain of bacteria—now recognized as *Ralstonia solanacearum* race 1—infected tobacco in Granville County, North Carolina, causing symptoms of bacterial wilt¹. The disease became so severe that growers experienced losses of 25–100%, and the economies of entire communities were destroyed (Melton and Shew, 2000). At that time, the disease was named Granville wilt for the county in which it was first observed. Around the same time, an unidentified disease began to affect southern potato growers and tomato growers in Mississippi (Kelman, 1953).

1 Tobacco is not affected by race 3 (Janse, 1991).

When scientists first recognized bacterial wilts of solanaceous crops, they made no attempt to differentiate between races or strains. The host differential test to separate *Ralstonia solanacearum* races 1, 2, and 3, was developed in the 1960s; limited information regarding race designation was available before that time.

Since the 1950s, scientists have recognized that infections of *Ralstonia solanacearum* races 1 and 3 have hindered many solanaceous crops in both tropical and temperate regions. Race 1 has caused the greatest economic damage to potato, tobacco, and tomato crops in the southeastern United States. In Indonesia, Brazil, Colombia, and South Africa, races 1 and 3 caused the greatest economic damage (CABI, 2006). In the Philippines during the period 1966–1968, races 1 and 3 caused losses of 15%, 10%, and 2–5%, in tomato, pepper, and tobacco crops, respectively (Zehr, 1969).

In India, infection of tomatoes with *Ralstonia solanacearum* races 1 and 3 can result in the complete loss of a crop. In Israel, Volcani & Palti (1960) reported that potato losses were greater in the spring crop than the autumn crop due to high temperatures during tuber formation and maturity. Extensive losses of potato were reported in Greece during the period 1951–1953 (Zachos, 1957).

Current Status

Today, *Ralstonia solanacearum* race 1 is endemic in some areas of the southern United States, where management of the disease has reduced losses to 1–2% of production or \$10–15 million annually in tobacco, with similar losses in tomato and pepper.

Ralstonia solanacearum, the causal agent of potato brown rot, has been described as one of the world's most destructive plant bacterium. It has affected at least 3.7 million acres of potatoes in more than 80 countries, with estimated annual losses of more than \$950 million (DEFRA, 2003a). Losses due specifically to *R. solanacearum* race 3 biovar 2 cannot be quantified since reliable data are unavailable. Nevertheless, in the early 1990s this pathogen caused serious losses in the European potato industry.

Due to recent outbreaks, insurance claims in the Netherlands in 1998 and 1999—as a result of potato brown rot caused by *Ralstonia solanacearum* race 3 biovar 2—were estimated at U.S. \$2.7 million (Meiners, 2000). In addition, the U.S. floriculture industry incurred substantial losses when the bacterium was recently detected and confirmed in geranium cuttings imported from Kenya and Guatemala. For a limited number of nurseries, the cost of disposal and replacement of rooted geranium plugs due to race 3 biovar 2 was estimated to be around \$221,632, with total losses for 70 nurseries of \$1.4 million (L. Garret, USDA–APHIS–CPHST economist, personal communication). However, the total number of establishments that suffered losses due to *R. solanacearum* race 3 biovar 2 is unknown at this time, thus accurate losses due to this bacterium cannot be calculated.

Estimated losses to the potato, tomato, pepper, eggplant, and geranium industries are great. In solanaceous crops, economic losses due to *Ralstonia solanacearum* can be as low as 2%, or may reach 100% in certain regions of the world, such as Southeast Asia (CABI, 2006). Combined losses to potato, tomato, pepper and eggplant crops that are likely to be affected by *R. solanacearum* race 3 biovar 2 can reach \$70,191,390 annually². These estimates are based upon total value of the acres planted to these commodities in 2001 (ERS, 2003).

Economic Impact

Geranium Hosts

Like *Xanthomonas pelargonii*, infection by *Ralstonia solanacearum* race 3 biovar 2 can cause mortality in geraniums. However, growers can manage *Ralstonia solanacearum* by culling and following proper greenhouse sanitation practices. The economic impact of *Ralstonia solanacearum* race 3 biovar 2 on growers, importers, and distributors in the United States is due to the regulatory actions necessary to eradicate the disease once detected.

Solanaceous Hosts

Ralstonia solanacearum race 3 biovar 2 is a serious pathogen that causes brown rot or bacterial wilt of potato (Hayward, 1991; Janse, 1996). Bacterial wilt of potato has been estimated to affect 3.75 million acres in 80 countries. Annual global damage estimates exceed \$950 million (DEFRA, 2003a). It is adapted to cooler temperatures and would be particularly damaging to potato production regions of the United States.

While race 1 causes losses to tomato crops in Florida (Momol, *et al.* 2003), the affect of race 3 on tomatoes and other solanaceous crops in the United States is unknown. *Ralstonia solanacearum* race 3 biovar 2 causes mortality in geranium plants, but *Xanthomonas pelargonii* is a greater concern of growers. However, the regulatory affect can be more significant for growers with the disease in their facility.

Hosts

Excluding geranium (*Pelargonium* spp.), most hosts of *Ralstonia solanacearum* race 3 biovar 2 are in the family Solanaceae. The major crop host plant species include potato, tomato, peppers and eggplant. Other crop hosts have been implicated by laboratory testing. Weed hosts include many *Solanum* spp. See Janse, *et al.* (2003) for a complete review of hosts. [See Pelargonium in the Nursery Trade on page E-1](#) for more information on naming of geraniums.

² Excluding geranium, and based upon a minimal damage of 1.5%.

Geographic Distribution

Geographic distribution records listed below are of confirmed race 3 or of presumed race 3 based on pathogenicity to potato and tomato (CABI, 2003).

North America—Mexico (CABI, 2003).

EPPO Region—Belgium, France, Germany, Hungary, Netherlands, Lebanon, Slovakia and the United Kingdom (CABI, 2003).

Asia—Bangladesh, China (Fujian, Guangdong Guangxi, Hebei, Jiangsu, Taiwan, Zhejiang), India (Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal), Indonesia (Java), Iran, Japan (Kyushu), Nepal, Pakistan, Philippines, Sri Lanka (CABI, 2003), Republic of Korea (NIAST, 2002), Malaysia (Possier, *et al.*, 1999), and Thailand (Horita and Tsuchiya, 2001).

Africa—Burundi, Egypt, Ethiopia, Kenya, Libya, Réunion, Rwanda, South Africa, Zaire, Zambia, Zimbabwe (CABI, 2003).

South America—Argentina, Bolivia, Brazil (Goias, Parana, Pernambuco, Rio Grande do Sul, Santa Catarina, Sao Paulo), Chile, Colombia, Peru, Uruguay (CABI, 2003).

Central America and Caribbean—Cuba (French, 1998), Costa Rica, Dominican Republic, El Salvador (French, 1998), Guadeloupe, Haiti, Honduras, Nicaragua, Panama (CABI, 2003).

Oceania—Australia (New South Wales, South Australia, Victoria) Papua New Guinea (CABI, 2003).

3

Ralstonia solanacearum r 3 b 2

Chapter 3

Survey Procedures

Contents

Introduction	page 3-1
Precautions	page 3-1
Detection Survey	page 3-2
Data Collection	page 3-15
Delimiting Survey	page 3-6
Traceback and Trace Forward Survey	page 3-7
Monitoring Survey	page 3-9
Visual Inspection for Detection Survey	page 3-10
Visual Inspection for Delimiting Survey	page 3-14

Introduction

Use *Chapter 3 Survey Procedures* as a guide to conducting a survey for *Ralstonia solanacearum* race 3 biovar in geraniums, potatoes, and other solanaceous hosts.

Conduct a detection survey to determine if *Ralstonia solanacearum* race 3 biovar is present or absent in a defined greenhouse, nursery, or field area. After a new detection in the United States, or when a detection in a new area is confirmed, conduct a delimiting survey to define the geographic location of the disease. Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness.

Precautions

When visiting sites to conduct surveys or collect samples, regulatory officials must take strict measures to prevent contamination by plant pathogens between greenhouses, nurseries, and potato facilities during inspections. Acceptable methods of protection include the use of an effective antimicrobial soap, lotion, or disinfectant used according to the label instructions, or disposable gloves and booties changed between visits. [See Disinfection Procedures on page 6-4](#) for more information on disinfection of tools, irrigation systems, ponds, and soil.

Antimicrobial Soap, Lotion, or Disinfectant

Wash hands with an approved microbial soap. If **not** using a microbial soap, wash hands with regular soap and warm water to remove soil and debris. Afterward, use an alcohol-based antimicrobial lotion such as Purell® or an

equivalent product that contains 63% ethyl alcohol. Purell® has demonstrated efficacy against various resilient *Pseudomonas* spp. and so is effective against *Ralstonia solanacearum*.

If hands are free of soil or dirt, an alcohol-based antimicrobial lotion can be applied without washing. Unlike some antimicrobial soaps, an antimicrobial lotion is less likely to irritate the hands and thereby improve compliance with hand hygiene recommendations.

Disposable Gloves and Footwear

If soaps are **not** used, then disposable latex or nitrile gloves can be used as an alternative. Gloves must be changed between visits to greenhouses, nurseries, and potato facilities.

Footwear should be disinfected using a footbath or other appropriate method when moving between greenhouses or nurseries. If footbaths are **not** available use disposable booties available from hospital suppliers. Booties must be changed between visits to greenhouses, nurseries, and potato facilities.

Detection Survey

The purpose of a detection survey is to determine if a pest exists in an area. This can be extremely broad in scope—as when assessing the presence of the disease in a wide area—or it can be restricted to discovering if a specific pest is present in a certain area.

Based strictly on a negative result in a detection survey, it is **not** valid to claim that a pest does **not** exist in an area if results are negative. However, negative results are valuable for providing clues as to mode of dispersal, temporal occurrence, or industry practices, particularly when considered with results from similar areas or proximities.

Important

Detection surveys for geraniums infected by *Ralstonia solanacearum* race 3 biovar or other cultivated hosts in nurseries should be conducted by state inspectors in conjunction with federal PPQ inspectors. PPQ inspectors may inspect nurseries without a state inspector if they have permission of the nursery owner or manager and have advised the state of their visit.

Procedure

Follow this procedure when conducting a detection survey for *Ralstonia solanacearum* race 3 biovar 2:

1. Use visual inspection to examine cultivated host plants (geraniums, potatoes, and other solanaceous hosts) and solanaceous weeds for wilting symptoms. Be sure to include plants in the field that were started in greenhouses or nurseries in association with imported geraniums. [See Visual Inspection for Detection Survey on page 3-10](#) for more information on inspection procedures.

2. Collect samples of symptomatic plants. [See Procedure for Sampling and Identification on page 4-3](#) for instructions.
3. Submit samples for testing. [See Storage and Shipping on page 4-4](#) for instructions.
4. Use [Table 3-1](#) to determine if further action is required.

TABLE 3-1 Decision Table for Testing Plant Samples in a Detection Survey

If:	And:	Then:	And conduct further inspection of the following:
Samples test positive for the species <i>Ralstonia solanacearum</i> by ELISA or culture	Their origin is in the southern areas of the United States where race 1 occurs ¹	Conduct monitoring and further tests at a qualified, authorized diagnostic laboratory to determine race and biovar. See Monitoring Survey on page 3-9 and Identification on page 4-1 for more information.	<ul style="list-style-type: none"> ◆ Suspect geranium and potato shipments from infested greenhouses or fields, respectively, on hold. See Delimiting Survey on page 3-6 for more information. ◆ Any other hosts in nurseries suspected of having material at risk for <i>R. solanacearum</i> race 3 biovar 2. See Delimiting Survey on page 3-6 for more information.
Samples test negative for the species <i>Ralstonia solanacearum</i> by ELISA or culture	→	Release plants from regulatory control.	

1 While race 1 of *Ralstonia solanacearum* occurs in the southeastern United States on many of the same hosts as race 3, it is indistinguishable from race 3 without further diagnostic testing.

Suspect shipments of geranium, or potato and other solanaceous crops (including vegetable transplants), are those held because of their association with a positive detection from another source country, rooting station, nursery, potato or other solanaceous crop field or potato storage facility. Be sure to follow sanitation procedures during nursery visits. [See Precautions on page 3-1](#) for more information concerning sanitation.

Geranium Hosts

Use visual inspection to conduct a detection survey in greenhouse and nursery facilities. [See Visual Inspection for Detection Survey on page 3-10](#) for more information on this topic.

Random sampling of geranium (and potato) plants lacking symptoms is **not** recommended at this time because of a lack of sampling methodology and processing capability. Due to latency, infected plants may **not** show symptoms. There is currently no reliable test for asymptomatic plants, therefore symptomology is the most reliable indicator of possible infection.¹

Solanaceous Hosts

Use visual inspection to conduct a detection survey in solanaceous hosts. Detection surveys for *Ralstonia solanacearum* race 3 biovar 2 in potato growing areas can be general, or targeted to areas considered to be at greater risk. [See Visual Inspection for Detection Survey on page 3-10](#) for more information.

General Survey

Conduct a general survey at low-risk sites. Low-risk sites include fields with **little** or **no** history of solanaceous host production.

Targeted Survey

Detection surveys in potatoes, tomatoes, and other solanaceous hosts, should be targeted at particular crop production practices where the stage of production or geographic area may present some risk of infection and spread of the disease.

Proximity of Potatoes to Historically Positive Nurseries—Conduct surveys in potato fields if:

- ◆ Local greenhouses either imported geraniums that tested positive for the pathogen or received suspect varieties of geraniums associated with imported geraniums which tested positive for the pathogen.
- ◆ Potato fields are located downstream or adjacent to positive facilities that had holding or recirculating ponds for irrigation water.
- ◆ Potato fields may have been irrigated with surface water or other water sources downstream from potato processing plants where the effluent from processing is **not** treated.

Tomato and Other Solanaceous Hosts— Similar to potato field surveys, visual observations of wilt in tomato, pepper, and eggplant crops can be used in detection surveys. Conduct surveys of tomato and other solanaceous hosts if:

- ◆ Hosts were grown in the same production greenhouses as imported geraniums, since they may represent a high risk pathway for introducing infection into a field or home garden.
- ◆ Fields are located downstream or near nursery facilities that had imported geraniums which tested positive for *Ralstonia solanacearum* race 3 biovar 2, or had varieties of suspect geraniums that were associated with imported geraniums that tested positive for the pathogen.

Tomato Transplants—Tomato, eggplant, and pepper plants for commercial production are often grown as transplants in one area and shipped to another area for field planting. These avenues of

1 If available, testing of asymptomatic potato tubers might prove to be more reliable. Latent infections may be present for several years before field symptoms become visible. In the absence of symptoms, if a positive detection occurs in potato seed certification screening, normal traceback or trace forward investigations will indicate potential high-risk sites to survey and test. A potato sampling scheme over a large area may be the most efficacious method of sampling.

potential pathogen dissemination can be monitored for the presence of wilt. Send samples of symptomatic host plants to a diagnostic laboratory to detect *Ralstonia solanacearum* race 3 biovar 2.

Potato Seed Tubers—The most likely way for *Ralstonia solanacearum* race 3 biovar 2 to spread in potato growing areas is through infected tubers used for seed. Seed tubers are regularly sampled and screened as a part of potato seed certification programs. These programs, conducted at various institutions under the auspices of state university or state departments of agriculture, assure that commercial potato seed tubers are inspected for a variety of potato diseases before receiving certification.

Seed potato inspectors regularly look for bacterial ring rot (*Clavibacter michiganense* subsp. *sepedonicus*). Like *Ralstonia solanacearum* race 3 biovar 2, diagnostic laboratory tests are an integral part of confirming the presence of bacterial ring rot in symptomatic tubers. If diagnostic testing is performed to confirm the presence or absence of bacterial ring rot, tests should also be conducted to rule out the possibility that *R. solanacearum* race 3 biovar 2 is the causal agent.

Solanaceous Weed Hosts—*Ralstonia solanacearum* race 3 biovar 2 can survive in waterways and on weed hosts, including black nightshade (*Solanum nigrum* L.) and bitter nightshade (*S. dulcamara* L.) (Figure 3-1). For example, bitter nightshade growing in river water in Europe acted as a symptomless, secondary host in which the pathogen overwintered and multiplied to further contaminate irrigation water supplies (Janse, 1996). In conjunction with detection and monitoring surveys, collect potential weed hosts and send to the appropriate diagnostic laboratory, as with cultivated hosts of *R. solanacearum* race 3 biovar 2. In particular, weed hosts should be collected around holding ponds and run-off areas in or near fields with hosts that have tested positive.



FIGURE 3-1 Bitter Nightshade, a Weed Host of *Ralstonia solanacearum* race 3 biovar 2 [Image courtesy of Joel Floyd, USDA-APHIS-PPQ]

Delimiting Survey

After a new detection in the United States, or when a detection in a new area is confirmed, conduct a delimiting survey to determine the extent of the infestation or suspect areas in which to conduct further investigations.

Geranium Hosts

Commercial production of geraniums is limited to greenhouses, nurseries, and retail garden centers. Look for plants connected to circulating irrigation systems, water runoff, and collection ponds, to determine if the infection has spread to new areas on or off the grower's premises.

Traceback and trace forward investigations will help determine new facilities needing inspection. Develop lists of potentially infected facilities within and outside a state. These lists are **not** to be shared outside regulatory cooperators. If trace forwards to residences from positive nurseries can be identified, then these geraniums should also be inspected when possible.

Solanaceous Hosts

List of Facilities

A list of facilities associated with field grown potatoes from lots testing positive for *Ralstonia solanacearum* race 3 biovar 2 will be compiled. States will distribute the lists to field offices.

Important

Do not share lists of facilities with individuals outside our regulatory cooperators. Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited.

Working with Growers or Farm Managers

When notifying growers or farm managers on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of facilities that may have received *Ralstonia solanacearum* infected material.

It is important to understand that there are many different types of markets and facilities (i.e., seed lots, processing, or fresh market) that may have handled infested and potentially infested plant material and soil. Some growers operate on hundreds of acres with many different field sizes and seed lots, and store tubers in huge warehouses for long periods of time.

Procedure—Follow these steps when conducting an investigation:

1. Request that the grower or manager produce any documentation including invoices, shipping lists associated with the positive tested suspect tubers on the list of facilities.
2. Make copies of the records.
3. Locate any suspect material still on the premises.
4. Take photographs of the area where suspect plant material is located. Include photographs depicting conditions, including the proximity of neighboring plants, that may indicate broader contamination.
5. Make a record of the number of plants, type, and location including an accounting of suspect plants from that shipment not on the premises.
6. Inspect the area for the presence of cull piles and determine if any harvesting/processing equipment has been shared with other farms.

Traceback and Trace Forward Survey

Use traceback and trace forward investigations to determine priorities for delimiting survey activities after an initial U.S. detection. Traceback investigations attempt to determine the source of infection. Trace forward investigations attempt to define further potential dissemination through means of natural and artificial spread (commercial or private distribution of infected plant material). Once a positive detection is confirmed, investigations are conducted to determine the extent of the infestation or suspect areas in which to conduct further investigations.

Traceback Investigation

Analyzing Information

Traceback information gathered from seed lot tags and invoices can be used to determine the origin of the seed tubers. With timely submitted records from growers and tuber storage facilities, planning staffs on site can construct prioritized lists for further investigations. Information available from local water companies or farm organizations can be obtained to construct maps of water sources, irrigation channels, and connections in areas with suspect tubers and potentially infected tubers. Use this information to assess the need for further surveys of fields associated with irrigation water.

Positive Detection in Potatoes

Traceback investigations for positive potato detections should determine the source of infection by examination or analysis of the following:

- ◆ Certified seed documentation
- ◆ Potato storage facilities
- ◆ Irrigation practices and water sources
- ◆ Potato processing plants and water handling practices
- ◆ Proximity to greenhouse operations housing imported geraniums
- ◆ Any other potential movement of plant material, water, or machinery that could contribute to tracing the source of contamination

Trace Forward Investigation

Target seed or tubers for consumption associated with positive testing lots. Consider any distribution channels or irrigation water that might be pathways for further disseminating the pathogen, including but not limited to the following:

- ◆ Associated seed lots on a farm, in storage areas, or bins, that may have come in contact with positive testing lots
- ◆ Harvesting equipment or other vehicle movement history in fields and nearby fields planted with positive tested lots
- ◆ Irrigation or associated waterways running to other areas from fields with positive testing lots
- ◆ Weed hosts around contaminated waterways associated with positive testing fields

Water Sources and Irrigation Methods

For fields that test positive or are directly associated, gather information on irrigation water distribution systems documenting the water source for each area. This information can be obtained from local commercial water companies or irrigation district organizations. Maps of irrigation distribution systems should be available to overlay on agriculture field maps.

Water Sources—Water sources can include the following:

- ◆ Dry land farming without irrigation systems
- ◆ Ground water, which is pumped from wells
- ◆ Surface water, distributed through canals and irrigation ditches

Irrigation Methods—Irrigation methods can include the following:

- ◆ Flood
- ◆ Center pivot
- ◆ Solid-set with pipe and fixed risers for sprinklers
- ◆ Side-roll with pipes and sprinklers that can be rolled on large wheels

Investigations at Processors

The following information can be used to determine the extent of distribution of suspect tubers or contamination by contaminated water to various locations in the United States. Coordinate with your State contacts and regional office to gather this information. Additional resources may be needed to quickly gather this information and analyze it in order to hold suspect tuber shipments and potentially infected material.

Destination of Potatoes—Determine the destination of potatoes from known positive fields by documenting distribution channels that include washing facilities for table consumption potatoes and processors. Washing operations can often include flumes, or water within a processing plant used to carry potatoes from one area to the next. The water from flumes is often recirculated and held in ponds or tanks on the property.

Movement of Water—Document the movement of water within a facility and take tuber and water samples at facilities that handled suspect and potentially infested tubers.

Processing Environment—Determine the processing methods, temperatures tubers are subjected to, and the disposition of waste material from the processing plant. If waste material is a risk for further dissemination of the pathogen, it will be subject to regulatory actions.

Monitoring Survey

Conduct a monitoring survey if you have applied a control procedure and need to measure its effectiveness. [See Control on page 6-1](#) for more information on control options.

Geranium Hosts

Once a nursery has destroyed all suspect geraniums and disinfected growing areas, follow-up monitoring is necessary. Be sure to follow sanitation procedures during nursery visits in a monitoring survey.

Visual inspection, during the same or subsequent growing season, is appropriate in order to examine hosts for symptoms. If sampling protocols are available, sampling of water from irrigation systems or ponds may also be included with additional inspections of host crop areas in runoff areas adjacent to positive testing nurseries. [See Visual Inspection for Detection Survey on page 3-10](#) and [Visual Inspection for Delimiting Survey on page 3-14](#) for more information on inspection.

Solanaceous Hosts

Once *Ralstonia solanacearum* race 3 biovar 2 has been confirmed from a particular field, and infected and potentially infected plants have been destroyed, follow-up monitoring will be necessary. Use the following tools:

- ◆ Visual inspection in the field

- ◆ Collection of samples from cull piles, soil, potential weed hosts, and water, for several years and multiple times per season

See [Visual Inspection for Detection Survey on page 3-10](#) and [Visual Inspection for Delimiting Survey on page 3-14](#) for more information on inspection of host plants.

Visual Inspection for Detection Survey

Greenhouse and Nursery Crops

Use visual inspection as a tool when surveying for *Ralstonia solanacearum* race 3 biovar in greenhouse and nursery crops.

Conduct a visual inspection in nursery facilities by looking for plants with typical wilting symptoms. The absence of wilt symptoms, however, does **not** necessarily mean *Ralstonia solanacearum* race 3 biovar 3 is **not** present in the facility. Recent information suggests that some infected plants may **not** express symptoms, even when held for prolonged temperatures. This condition is known as latency.

Geraniums

Wilting symptoms in geraniums caused by *Ralstonia* spp. are similar to wilting symptoms caused by other bacterial pathogens such as *Xanthomonas campestris* pv. *pelargonii*, the agent of bacterial blight.

Therefore, when wilting symptoms are present, do **not** make a field diagnosis such as for *Xanthomonas campestris* pv. *pelargonii*. Always collect a sample for testing at the appropriate diagnostic laboratory. See [Comparison of Symptoms on page F-1](#) for photos of typical wilting symptoms in geraniums caused by both organisms.

***Ralstonia solanacearum* race 3 biovar 2**—The primary symptom in geraniums of infection by *Ralstonia solanacearum* race 3 biovar 2 is wilting of leaves and/or abnormal yellowing of lower leaves. *Xanthomonas campestris* pv. *pelargonii* causes the same symptoms, as well as leaf spots.

Plants infected by *Ralstonia* spp. also exhibit the following symptoms:

- ◆ Bacterial streaming when symptomatic plants are placed in water
- ◆ Vascular discoloration of the stem
- ◆ Roots changing to a brown color

Xanthomonas campestris* pv. *pelargonii—In geraniums, *Xanthomonas campestris* pv. *pelargonii* causes wilting of leaves and/or abnormal yellowing of lower leaves, as well as leaf spots. However, vascular discoloration is less pronounced or absent, and roots remain white.

Other Hosts in Greenhouses or Nurseries

Visual surveys of other hosts in greenhouses or nurseries should be conducted during geranium inspections. Symptomology in other infected hosts such as tomato, eggplant, and pepper may also include severe wilting. Weed hosts, however, generally do **not** display wilt symptoms.

Discarded Plants

In addition, inspect nursery waste areas for discarded plants. Ask the nursery owner or manager to identify cull piles. Examine them for the presence of recently discarded, wilting or dead geranium plants. Discarded host plants that are **not** completely dried up can still be prepared for sample submission. Document the source of the sample.

Potato Cropping Systems

Ralstonia solanacearum race 3 biovar 2 is readily carried in irrigation water. Therefore, its spread in a potato field can be rapid and evident by limited or widespread wilting of potato plants in a field. Symptoms may be widespread or concentrated in areas of a field where water accumulates.

Potato Plants

Conduct visual surveys of potato fields during periods when temperatures are high and symptoms of bacterial wilt will be most obvious if present.

Early—Symptoms of bacterial wilt in mature potato plants that result from infection by *Ralstonia solanacearum* race 3 biovar 2 may initially include the following:

- ◆ Wilting of leaves during the day with recovery at night (**Figure 3-2**)
- ◆ Some plants with only one or a few branches exhibiting wilt in the early stages



FIGURE 3-2 Left: Wilted Potato Plant with Symptoms of Brown Rot, Infected by *Ralstonia solanacearum* race 3 biovar 2. Right: Healthy Potato Plant [Image courtesy of Centro Internacional de la Papa, Lima, Peru]

Late—More advanced infection can result in the following symptoms:

- ◆ Entire plant wilts without recovery.
- ◆ Wilted leaves do **not** drop from the plant and can maintain their normal green color and size.
- ◆ Infected foliage is yellow and stunted.

- ◆ Leaves turn brown and dry.
- ◆ Infected stem cut in cross-section shows glistening beads of dark gray, slimy ooze.
- ◆ Symptomatic stem cut at ground level may show a whitish exudate at the cut surface.
- ◆ Cut stem placed in water produces visible bacterial streaming from the xylem.
- ◆ Continued development of the bacterial infection within the xylem system eventually results in death of the plant.

Bacterial Ring Rot—Bacterial ring rot, *Clavibacter michiganense* subsp. *sepedonicus*, occurs in potato growing areas of the United States and is another bacterial disease of potato that causes wilt symptoms.

The symptoms of bacterial ring rot differ from bacterial wilt by occurring typically on the upper most leaves or flower on the plant first and causing a slight rolling at the margins with some loss of color. As the infection progresses, leaf tissue between veins turns yellow. Eventually, entire leaves and stems become yellow, brittle, and die. Infection can be limited to one or two stems on a hill.

While the symptomology of these two bacterial diseases of potato may be different, the pathogens must be diagnosed with laboratory testing. Additionally, in areas of the southern United States where *Ralstonia solanacearum* race 1 exists, a sampling of wilted plants positive for *R. solanacearum* will require further testing to the race and biovar level at qualified, authorized diagnostic laboratories.

Other Diseases—Other diseases, as well as drought, can cause symptoms of wilt that are similar, so diagnostic tests must be performed on samples from symptomatic plants in order to confirm the presence of *Ralstonia solanacearum* race 3 biovar 2. [See Identification on page 4-1](#) for more information.

Potato Tubers

In tubers for seed or consumption, symptoms of *Ralstonia solanacearum* race 3 biovar 2 infection include the following:

- ◆ Grayish brown discoloration can be seen through the tuber periderm.
- ◆ Bacterial ooze accumulates around the tuber eyes or at the stolon end.
- ◆ Soil adheres to ooze.
- ◆ In more advanced levels of infection, squeezing tuber may cause more bacterial ooze to be exuded ([Figure 3-3](#)).
- ◆ Distinct grayish-brown vascular ring is visible when infected tubers are cut in cross-sections; can extend into the pith or cortex of the xylem tissue ([Figure 3-3](#)).
- ◆ Applying pressure to tubers cut in half forces grayish white droplets of bacterial slime ooze from the vascular ring.

- ◆ Infected tubers left in the soil continue to decay and secondary organisms invade to break them down.

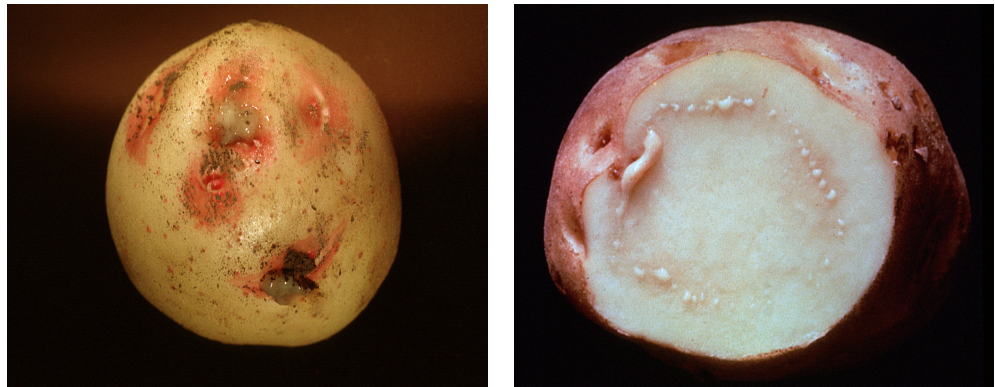


FIGURE 3-3 Symptoms of Brown Rot on Potato Tubers Exhibiting External Discoloration and Internal Infection with Ooze Exuding around the Vascular Ring of a Tuber cut in Cross-section [Image courtesy of Centro Internacional de la Papa, Lima, Peru]

Tomatoes and Other Solanaceous Crops

In a field, wilting and other symptoms may appear in several plants or all plants in a row in a furrow-irrigated crop. Wilting may also appear concentrated in plants in low areas of the field where water can accumulate.

The following symptoms can be found on tomato and other solanaceous plants infected with *Ralstonia solanacearum* race 3 biovar 2:

- ◆ Wilted leaves do **not** drop from the plant and can maintain their normal green color and size.
- ◆ Infected foliage can turn yellow and stunted.
- ◆ Severe wilt can cause leaves to turn brown and dry.
- ◆ Infected stem cut in cross-section can show glistening beads of dark gray, slimy ooze.
- ◆ Symptomatic stem cut at ground level may show a whitish exudate at the cut surface.
- ◆ Cut stem placed in water produces visible bacterial streaming from the xylem infected stem.
- ◆ Continued development of the bacterial infection within the xylem system eventually results in death of the plant.

Visual Inspection for Delimiting Survey

Greenhouse and Nursery Crops

Follow the same method as visual inspection for detection surveys. [See Greenhouse and Nursery Crops on page 3-10](#). Laboratory diagnosis according to prescribed procedures is necessary to determine the cause of wilting symptoms if bacterial infection is suspected.

Potato Cropping Systems

Inspect potato fields when temperatures are high, when symptoms of bacterial wilt are most obvious if present. Surveys should include the positive field as well as adjacent fields, those on a shared water supply, and fields that have had contact with potentially contaminated equipment.

The distribution of *Ralstonia solanacearum* in a potato field can be spotty. *Ralstonia solanacearum* is commonly found in areas of the field with poor drainage.

Delimiting surveys should include the following:

- ◆ Visual inspection of plants for symptoms, especially in wet areas of the field
- ◆ Visual inspection of weed hosts
- ◆ Sampling of soil and tubers near infected plants
- ◆ Inspection of plants near drainage canals or irrigation rigs
- ◆ Traceback and trace forward investigation of seed potatoes used in index field
- ◆ Compilation of field histories of the index and contact fields

Send plant and soil samples for testing to the appropriate diagnostic laboratory. [See Diagnostic Laboratories on page D-1](#) for a list of laboratories.

Tuber Sampling

Sampling is **not** required for fields or facilities with a direct link to positive testing seed lots or fields. The tubers in this category are automatically considered positive and are subject to control actions without testing.

However, tuber sampling for subsequent testing will be required for tubers that are potentially infested, including the following tubers:

- ◆ In adjacent fields,
- ◆ On a shared water source,
- ◆ Commingled in storage facilities, or
- ◆ Connected by a history of shared harvesting machinery.

Each tuber is a sampling unit. Repeat the sampling for each field, warehouse, or storage unit at a given location. Collect 300 samples in order to detect *Ralstonia solanacearum* at a 1% infestation level with 95% confidence. Collect samples throughout the entire building or storage area. If tubers are in bags, collect the 300 samples from different bags. Disinfect the samples before laboratory testing. [See Identification on page 4-1](#) for more information on laboratory testing. [See Potato Storage Facilities and Grower Fields on page 5-7](#) for more information on positive testing seed lots or fields.

Delimiting Surveys in a General Growing Area

Construct delimiting surveys in an area—based on known positive testing, associated positive testing, or potentially infested areas—from investigations of distribution channels and shared irrigation water. However, it may be necessary to do random samples in a general growing area to detect new infestations **not** discovered through investigations.

The delimiting survey in a general growing area can include random sampling of stored tubers and fields throughout a geographical area, with more intensive sampling near known infestations. As the distance away from the epicenter of known infestation increases, decrease the rate of random sampling. Based on the epidemiology and grower practices, an evaluation of risk and resources available will help determine the extent of these random sampling surveys.

Sampling of Water Sources

Test water sources near the positive fields, processing plants, or water sources used for irrigation, to delimit the spread of the bacterium.

Sample size is approximately 0.50 quarts; bottles should be sterilized. Collect water samples at a depth of 12 inches. Keep samples cool and in a dark location. Perform tests within 24 hours of collection. For best results, conduct sampling when water temperature exceeds 59 °F and populations of bacterium are highest in water. These conditions usually coincide with the period June through September.

Tomato and Other Solanaceous Hosts

[See Tomatoes and Other Solanaceous Crops on page 3-13](#) for a description of symptoms. In greenhouses or field grown crops, look for severe wilting. If a wilted plant does **not** recover after watering, collect a sample and have it tested. [See Procedure for Sampling and Identification on page 4-3](#) for more information.

Data Collection

Surveyors visiting sites to place holds or take samples should collect the following information:

- ◆ Date of collection or observations
- ◆ Collector's name
- ◆ Grower's field or greenhouse identification numbers

- ◆ GPS coordinates
- ◆ Variety of host plant grown
- ◆ Methods of irrigation
- ◆ History of farm machinery usage
- ◆ Observations of wilt and any other relevant information

In the absence of inspection officials, take the following actions immediately if wilting symptoms are noticed:

1. Mark the location.
2. Remove the plants from the bench or rack, or flag location in field.
3. Notify a state or PPQ inspector.
4. Place the whole plant inside two resealable plastic bags.
5. Label the sealed bags with the following information:
 - ❖ Date
 - ❖ Name of person responsible
 - ❖ Location of sample collection
6. Keep bagged plants cool or refrigerated until the inspector arrives. Do **not** freeze the sample.

4

Ralstonia
solanacearum r 3 b 2

Chapter 4

Identification

Contents

Introduction	page 4-1
Authorities	page 4-1
Procedure for Sampling and Identification	page 4-3
Diagnostic Screening Laboratories	page 4-6
Required Permits	page 4-6
Diagnostic Tests to Determine Genus and Species	page 4-8
Culturing	page 4-9
Confirmatory Testing to Race and Biovar	page 4-9
Positive Confirmations	page 4-10

Introduction

Use *Chapter 4 Identification* as a guide to identify *Ralstonia solanacearum* race 3 biovar 2. Accurate identification of this pathogen is pivotal to assessing its potential risk, developing a survey strategy, and determining the level and manner of control.

Authorities

The National Plant Germplasm and Biotechnology Laboratory (NPGBL), Beltsville, Maryland, must positively identify bacterial infections to the levels of race and biovar before initiation of any program quarantine activities. NPGBL also has the necessary registrations and containment approvals to handle select agents. State and cooperating university diagnostic laboratories are limited to determinations of bacterial infections to the level of genus and species—*Ralstonia solanacearum*. **See Diagnostic Laboratories on page D-1** for a list of laboratories and their addresses.

PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities:

- ◆ *Plant Protection Act* (7 CFR Part 330) (**See Plant Protection Act on page 4-2**)
- ◆ *Agricultural Bioterrorism Protection Act of 2002* (7 CFR Part 331) (**See Agricultural Bioterrorism Protection Act on page 4-2**)

Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring select agents are required to be registered. However, diagnostic laboratories that identify select agents are exempt from this requirement as long as they

complete APHIS–Centers for Disease Control and Prevention (CDC) Form 4 and destroy or transfer infected material to a laboratory registered with the APHIS Select Agent Program within the mandatory seven days. APHIS–CDC Form 4 is available at the Web site of the APHIS–Agricultural Select Agent Program.

Address **APHIS–Agricultural Select Agent Program**
Web site: http://www.aphis.usda.gov/programs/ag_selectagent/index.html
Telephone: 301-734-5960

Plant Protection Act

Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status. If any material is shipped interstate, the receiving laboratory must have a permit. For further guidance on permitting of plant pest material, consult PPQ Permit Services or visit the Web site of the PPQ–Permits Program.

Address **PPQ–Permits Program**
Web site: http://www.aphis.usda.gov/plant_health/permits/
Telephone: 301-734-8758

Agricultural Bioterrorism Protection Act

Federal regulation on *Agricultural Bioterrorism Protection Act of 2002* (7 CFR Part 331) specifies requirements for possession, use, and transfer of organisms listed as select agents and toxins. Once an unregistered diagnostic laboratory identifies a select agent, they must take the following steps immediately:

1. Notify staff at APHIS–Agriculture Select Agent Program.
2. Complete and submit APHIS–CDC Form 4 within 24 hours.
3. Destroy or transfer the agent to a registered entity within seven days.

In compliance with the *Act*, if a diagnostic laboratory held back part of a screened sample for voucher purposes and that sample (forwarded to NPGBL) came back as positive for a select agent, the diagnostic laboratory is required to notify the APHIS–Agricultural Select Agent Program immediately.

If the determination of the unregistered laboratory is to destroy the sample, this must take place within seven days of results notification and a PPQ officer must witness the destruction of the sample on or before the seven-day period expires. Clarification of this and other information related to adherence to the select agent regulations is available at the Web site of the APHIS–Agricultural Select Agent Program.

Address **APHIS–Agricultural Select Agent Program**
Web site: http://www.aphis.usda.gov/programs/ag_selectagent/index.html
Telephone: 301-734-5960

Procedure for Sampling and Identification

Some pre-identification and screening can be performed by field personnel assigned to the program. Only plants exhibiting wilting symptoms are to be sampled and submitted. See [Visual Inspection for Detection Survey on page 3-10](#) and [Comparison of Symptoms on page F-1](#) for more information.

Other bacterial diseases can also cause wilting symptoms, so screening by diagnostic laboratories is a necessary and required step. These must be state, cooperating university, National Plant Diagnosis Network laboratories, or recognized permitted private laboratories. See [Diagnostic Laboratories on page D-1](#) for more information.

Screening Nursery and Field Hosts

For sampling of plants showing wilting symptoms, the whole plant must be collected including roots, preferably without soil. 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.

1. Place the plant sample in a resealable bag and seal it.
2. Use a permanent marker to record the sample number on the bag. See [Labeling, Numbering, and Record Keeping](#) below for more information on sample numbers.
3. Place the bag inside a second resealable bag, along with a completed [Questionnaire for Nursery and Potato Cropping System Owners or Managers on page B-5](#). Fill in the form as completely as possible.
4. If plants in soil are submitted, place a separate plastic bag around the pot with a rubber band around the plant base to restrict soil spillage and contamination of the plant tissue.
5. If soil is left over, dispose of it properly.
6. Place double-bagged samples in a sturdy cardboard outer box with insulation to prevent movement within the box during shipping.

Entire samples of plants should be submitted, **not** sub-samples. Samples must include lower stems; exclude detached leaves or petioles. Samples that are dead or fermented upon arrival cannot be tested and will be rejected.

Labeling, Numbering, and Record Keeping

Generate sample numbers at the sampling site. Record the sample number on the following forms, and include a copy of each form with each sample:

- ◆ [PPQ Form 391 Specimens For Determination on page B-2](#) marked Urgent
- ◆ [Questionnaire for Nursery and Potato Cropping System Owners or Managers on page B-5](#)

For each sample, assign and record a unique identification number in the following format:

Example

XX-ABC-0001 where XX is your two-letter state code; ABC is a three letter, state assigned facility code; and 0001 is the sample number for that facility.

Use a unique sample number for each sample. Keep a log of assigned sample numbers.

If the diagnostic screening laboratory is a part of the National Plant Diagnostic Network (NPDN), samples may be submitted using a NPDN number format. If a bar-coded sample number system is available, be sure to include the bar-code in the sample on the PPQ Form 391. See **Table D-1 on page D-10** for a list of laboratories in the NPDN.

Inspectors must provide the sample date with all relevant collection information to their State Plant Regulatory Official or State Plant Health Director as soon as possible. This information should be communicated within a state and with the regional office program contact. If a sample tracking database is available at the time of the detection, enter collection information in the system as soon as possible.

Storage and Shipping

Samples must be shipped to approved diagnostic screening laboratories as soon as possible to prevent deterioration of plant tissue. [See Diagnostic Laboratories on page D-1](#) for a list of laboratories and their addresses.

If storage is necessary, place bagged samples in a cool location until ready for shipping. Do **not** place samples in a freezer. Samples may be held in a standard refrigerator at 39°F or at room temperature if less than or equal to 60°F.

Ship samples by overnight delivery. Ice packs are unnecessary.

PPQ Form 391

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.

Note the following additional information on the PPQ Form 391:

- ◆ Name of test kit used to make the *Ralstonia solanacearum* diagnosis
- ◆ Indication that the *Ralstonia solanacearum* test was strongly positive or weakly positive, etc. (If possible, a print-out of a digital image of the stick or plate should accompany the sample and paperwork.)
- ◆ Name of specific plant part that tested positive (Symptomatic or lower stem area should be the only tissue tested.)

Please call or fax NPGBL to notify that you are sending plant samples with suspected *Ralstonia solanacearum*. (If faxing, include all pertinent information and contact name and number).

Saturday Delivery

Only with specific permission from APHIS–PPQ headquarters should samples be sent on Fridays by FedEx®. Although it is possible to arrange Saturday delivery by overnight carriers to NPGBL, often samples are **not** delivered even with special arrangement with FedEx®. However, NPGBL will operate on Saturday if a large program is being conducted requiring additional hours of operation. If you have obtained permission from APHIS–PPQ headquarters to send samples for Saturday delivery, please send the FedEx® tracking number to NPGBL via email to Laurene Levy and Renee DeVries so they can notify their local Fedex® office to authorize Saturday delivery.

Address

Laurene Levy: Laurene.Levy@aphis.usda.gov
Renee DeVries: Renee.m.DeVries@aphis.usda.gov

Notification of State Officials of Submissions and Results

Notify the State Plant Health Director and State Plant Regulatory Officials 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.

Please do **not** call NPGBL to get sample results. The information will be reported to the regions and states from headquarters as soon as they are available.

Sample Processing Time

Growers and cooperators need to be aware that sample processing requires 48 hours to three weeks depending on the sample condition, number of samples that require processing, and the ease with which diagnostic tests can be performed. This is in addition to the time it takes to process and forward samples from the intermediate state and cooperating university diagnostic laboratories.

Diagnostic Screening Laboratories

To determine if an infection is due to *Ralstonia solanacearum*, samples of plants must be submitted through normal regulatory networks. These labs will perform diagnostic tests to the genus and species level. They will **not** determine the race and biovar. [See Determination of Genus and Species on page D-2](#) to find the appropriate laboratory.

Diagnostic screening laboratories receiving samples are to communicate the date of receipt with their State Plant Regulatory Official or State Plant Health Director. All relevant sample information and the diagnostic laboratory's species determination must be communicated as soon as possible within a state and with the PPQ regional office program contact.

If a sample tracking system is used, at a minimum enter the date received in the system, test results, the determination, date identification made, and who made the identification.

Alternative Laboratory

Laboratories within the National Plant Diagnostic Network can screen to genus and species for states that prefer **not** to or cannot screen their own samples. Any sample testing positive for *Ralstonia solanacearum* will be forwarded to National Plant Germplasm and Biotechnology Laboratory (NPGBL) in Beltsville, Maryland, for confirmatory testing. [See Plant Diagnostic Networks and Private Laboratories on page D-10](#) to identify the regional laboratory for your state.

States that cannot screen their own samples, or have not been able to make arrangements with another permitted laboratory out of state, can utilize Agdia, Inc. This private laboratory has the necessary permits and authorizations to screen to species—but **not** race or biovar. Samples testing positive for *Ralstonia solanacearum* will be forwarded to NPGBL for confirmatory testing.

Address	Agdia, Inc. 30380 County Road 6 Elkhart, IN 46514 T: (574) 264-2014, or 1-800-62-AGDIA Web site: http://www.agdia.com
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Other private laboratories will be identified as permits are approved.

Required Permits

If a state, university, National Plant Diagnostic Network (NPDN), or private laboratory performs a PCR assay and detects a presumptive positive outside the national survey process outlined here, they must abide by the requirements under the *Agricultural Bioterrorism Protection Act of 2002* (7 CFR 331). For more information, see [Agricultural Bioterrorism Protection Act on page 4-2](#).

Diagnostic screening laboratories receiving samples are to communicate the date of receipt with their State Plant Regulatory Official and/or State Plant Health Director. All relevant sample information, and the diagnostic laboratory's determinations, must be communicated as soon as possible within a state and with the PPQ regional office program contact.

Any laboratory that diagnoses samples to *Ralstonia solanacearum* race 3 biovar 2 is required, under the *Agricultural Bioterrorism Protection Act of 2002*, to immediately notify the APHIS–Select Agent Program regardless of whether a Plant Pest Permit is required.

Address **APHIS–Select Agent Program**
4700 River Road, Unit 2
Riverdale, MD 20737
Email: Agricultural.Select.Agent.Program@aphis.usda.gov
Telephone: 301-734-5960
Fax: 301-734-3652

The laboratory possessing the sample has seven days in which to destroy the sample. However, they must give PPQ the opportunity to witness the destruction. In addition, the laboratory director must complete APHIS Form 240. [See Agricultural Bioterrorism Protection Act on page 4-2](#) for more information.

For further guidance on permitting of plant pest material, consult the PPQ–Permits Web site.

Address **PPQ Permits**
Telephone: 301-734-7211, 6828 or 5055
Web site: http://www.aphis.usda.gov/permits/ppq_epermits.shtml

Intrastate Transfer

For inspectors shipping plant pest sample material to other labs to test for the presence of *Ralstonia solanacearum*—identification to species only, **not** biovar and race—no permit is required for shipment within the state. Persons shipping plant material to a diagnostic laboratory in their own state do **not** need to obtain a PPQ permit under either the *Plant Protection Act* (Regulations at 7 CFR Part 330) or PPQ Form 526.

Within state transfers of known *Ralstonia solanacearum* race 3 biovar 2 are regulated under the *Agricultural Bioterrorism Protection Act of 2002* (Regulations at 7 CFR Part 331). However, the submission of unknown samples or those only identified to *R. solanacearum*—**not** to race and biovar—are **not** regulated under this *Act*.

Interstate Transfer

States without their own department of agriculture or cooperating university laboratory for screening to genus and species may ship across state lines to use another state’s laboratory.

When shipping across state lines, the originating state must assure that the laboratory in the destination state has the necessary plant pest permit to receive interstate samples. Apply for the permit by completing [PPQ Form 523](#) [Emergency Action Notification on page B-7](#).

For the purpose of the *Agricultural Bioterrorism Protection Act of 2002* registrations are not necessary. As with intrastate submitted samples to diagnostic labs, interstate shipment of unknown potentially infected plants is **not** subject to regulations under this *Act*.

Diagnostic Tests to Determine Genus and Species

Serological tests are available to screen for this organism to the genus and species level at diagnostic laboratories. Special permits are **not** needed by these labs to do this test unless they are receiving samples from another state.

The three serological tests validated and recognized by the National Plant Germplasm and Biotechnology Laboratory are listed below. [See Diagnostic Validations and Isolation Methods on page C-1](#) for more information on these kits.

Validated Screening Test Kits

The National Plant Germplasm and Biotechnology Laboratory (NPGBL) in Beltsville, Maryland, evaluated the following three rapid serological tests for detection of *Ralstonia solanacearum*. None of the serological tests evaluated will determine the organism to race or biovar.

Address **Rs ImmunoStrip® Test**
Agdia, Inc.
30380 County Road 6
Elkhart, IN 46514
Web site: <http://www.agdia.com>
T: 800-622-4342
F: 219-264-2153

Address **Potato Brown Rot Pocket™ Diagnostic**
Central Science Laboratory (CSL)
Sand Hutton, York, YO41 1LZ
Web site: <http://www.csl.gov.uk>
T: 44 1904 462600
F: 44 1904 46211

Address ***Ralstonia solanacearum* SPOTCHECK LF™**
Adgen, Ltd.
Nellie's Gate, AYR
Scotland, KA6 5AW
Web site: <http://www.adgen.co.uk>
T: 44 1292 525275
F: 44 1292 525477

ELISA Test Kits

ELISA (Enzyme Linked Immunosorbant Assay) kits, tested and validated by NPGBL, Beltsville, Maryland, will also make diagnoses to the level of genus and species. [See Diagnostic Validations and Isolation Methods on page C-1](#) to learn more about the kits.

Water Testing

Methods of testing water for the presence of *Ralstonia solanacearum* race 3 biovar 2 have been developed by NPGBL, Beltsville, Maryland, but protocols have yet to be included in the *Guidelines*.

Culturing

Diagnostic laboratories can forward cultures after screening to genus and species has been performed. [See Diagnostic Validations and Isolation Methods on page C-1](#) for recommended methods of culturing. When sending cultures for race and biovar determination, the sender must assure that packaging is secure as with plant samples.

Confirmatory Testing to Race and Biovar

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 to NPGBL, Beltsville, Maryland, for confirmation to race and biovar.

NPGBL is the only laboratory with the proper permits to test for the presence of *Ralstonia solanacearum* race 3, biovar 2. Dr. Laurene Levy, NPGBL Director, has the necessary authorizations to receive samples submitted for diagnostics to the race and biovar level. Include the name of the kit used for the detection and specific descriptive information on the test results (i.e., intensity of the test band, OD of the ELISA well, or digital images of the test result).

Important

Entire samples of suspect *Ralstonia solanacearum* positives should be submitted to National Plant Germplasm and Biotechnology Laboratory (NPGBL)—not sub-samples. Samples must include lower stems. Samples should not consist of detached leaves or detached petioles. If samples are necrotic (brown) or fermented upon arrival at NPGBL, they will be rejected.

Address

**USDA-APHIS-PPQ-National Plant Germplasm and Biotechnology Laboratory
BARC-East, Bldg. 580
Attention: DeVries or Levy
Powder Mill Road
Beltsville, MD 20705
T: 301-504-7100
F: 301-504-8539**

Positive Confirmations

In compliance with the *Agricultural Bioterrorism Protection Act of 2002* (7 CFR Part 331) if a diagnostic laboratory held back part of a screened sample or culture for voucher purposes and the sample forwarded to the National Plant Germplasm and Biotechnology Laboratory (NPGBL), Beltsville, Maryland, came back as positive for *Ralstonia solanacearum* race 3 biovar 2, the diagnostic laboratory is required to notify PPQ-Permits that the sample exists.

Destruction must take place within seven days of notification that the sample is positive for *R. solanacearum* race 3 biovar 2 and a PPQ representative must have the opportunity to witness the destruction of the sample or culture within that time period. The responsible laboratory manager/plant pathologist must also complete the APHIS Form 2040. The form is available at the Permits Web site.

Address

PPQ-Permits

Telephone: 301-734-7211, 6828 or 5055

Web site: http://www.aphis.usda.gov/permits/ppq_epermits.shtml

NPGBL has the necessary permits and registrations to possess this select agent, and is also required by law to report the positive confirmation within 24 hours of detection.

5

Ralstonia
solanacearum r 3 b 2

Chapter 5

Regulatory Procedures

Contents

Introduction	page 5-1
Instructions to Officers	page 5-1
Overview of Regulatory Program After a United States Detection	page 5-3
Further Investigations at Nurseries with Suspect Geraniums	page 5-6
Placing Holds	page 5-7
Record Keeping	page 5-8
Plants to Hold	page 5-9
Issuing PPQ Form 523 Emergency Action Notification	page 5-11
Geranium and Potato Volunteers and Cull Piles	page 5-12
Harvesting Equipment, Storage Areas, and Conveyances	page 5-12
Regulatory Requirements of Seed Producers	page 5-12
Regulatory Requirements for Processors	page 5-12
Regulated Articles	page 5-13
Removing Areas from Quarantine	page 5-14
Requirements for Potato Cropping Systems Under Quarantine	page 5-15
Use of Chemicals	page 5-18

Introduction

Use *Chapter 5 Regulatory Procedures* as a guide to procedures that must be followed when surveying or controlling the plant pathogen *Ralstonia solanacearum* race 3 biovar 2.

Instructions to Officers

Agricultural officers must follow instructions for regulatory control measures, treatments or other procedures when authorizing the movement of regulated articles. A full understanding of the instructions and procedures is essential when explaining procedures to persons interested in moving articles affected by the quarantine and regulations. Only authorized treatments may be used in accordance with labeling restrictions. During all nursery visits, please assure that proper sanitation procedures are followed. [See Survey Procedures on page 3-1](#) and [Laws Pertaining to Pest Control on page 6-1](#) for more information.

Quarantine Actions Required

Geraniums

After investigations are performed—and additional consultations made if necessary—the unit of held suspect geraniums and potentially infected plants associated with them must be destroyed or disposed of. In addition, the area must be disinfected. [See Disinfection Procedures on page 6-4](#) for more information

Potato Cropping Systems

If a property has positive-testing or positive-associated fields, all plant material that can be reasonably removed from the field must be destroyed or disposed of. This includes cull piles and other plant debris.

Leave the field fallow for two years, and irrigate to promote volunteer sprouting. During the two years, the volunteer crop must be sampled, tested, and disked under during the growing season every four weeks to eliminate host material. Weed hosts in the field and along the edges must be sprayed with efficacious labeled herbicides to eliminate them from the area.

In the following two years, plant fields with non-host crops; irrigate to promote volunteer potato sprouting, disking any volunteer potatoes as sprouting occurs. Fields must be tested semi-annually for four years after an initial positive find. [See Survey Procedures on page 3-1](#) for more information.

Furthermore, no seed production can occur in the field for at least five years after detection of *Ralstonia solanacearum* race 3 biovar 2. Fields with susceptible hosts (potatoes or tomatoes) must be sampled for two seasons prior to any new seed production on the property.

Fields adjacent to positive testing or associated fields, or those on a shared water supply, may **not** grow host crops for two years, **nor** seed potatoes for two years, and must test and control any volunteer potato or weed hosts. Maintain strict sanitation of all vehicles entering and leaving the infested fields.

Storage facilities on properties with positive testing tubers must be held in quarantine until all potentially infested tubers are tested and either found to be negative or destroyed. The storage facilities must then be properly cleaned and disinfected with approved disinfectants in strict accordance with labeling. [See Disinfectants on page G-1](#) for more information.

Emergency Quarantine Action

If necessary, the Deputy Administrator will issue a letter directing PPQ field offices to initiate specific actions under the *Plant Protection Act of 2000* until emergency regulations can be published in the *Federal Register*.

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.

Access to Private Property

A General Memorandum of Understanding (GMU)—between states and PPQ—exists for each state. In certain circumstances, the GMU may facilitate access to private property, in the absence of landowner permission, by PPQ Officers in conjunction with state inspectors to place facilities under notification and witness actions specified in the PPQ Form 523 Emergency Action Notification (EAN). Check with your SPHD for clarification.

Regulatory Records

Maintain standardized regulatory records and database(s) in sufficient detail to carry out an effective, efficient, and responsible regulatory program.

Overview of Regulatory Program After a United States Detection

If *Ralstonia solanacearum* race 3 biovar 2 is detected on greenhouse geraniums or potatoes in the United States, follow this procedure:

1. Determine the origin of the infection.
2. Survey the extent of distribution of potentially infected geranium plants or potatoes.

Determining Origin of Infections

Geraniums

Important

There is no treatment for plants infected by *Ralstonia solanacearum* race 3 biovar 2. All greenhouse plants determined to be regulated and associated potting medium will be destroyed and areas sanitized before facilities can be released from quarantine. If new information indicates a more widespread infestation problem than originally assessed, or new information becomes available, these regulatory guidelines are subject to change.

Conduct a traceback investigation to determine the foreign source of infection and the extent of distribution of potentially infected plants in the United States from that source. Conduct a trace forward investigation to determine if potentially infected plant material had been distributed to other retail locations.

Investigations at United States geranium propagation facilities and their customers will be conducted. Results of investigations will allow implementation of a program of holding varieties or shipments that originated at a particular foreign facility during a shipping period. The irrigation methods, handling, and sanitation practices of each nursery will help assess if additional associated plant material is at risk of infection. Information

gathered in the investigation portion will be critical to determining which additional plants to hold and subsequent actions taken. Where guidance is needed, regional contacts will be consulted.

Potato Cropping Systems

Important

There are no treatments for plants or tubers infested by *Ralstonia solanacearum* race 3 biovar 2. Dispose of all positive testing and positive associated host plant material. If possible, test potentially infested material and assess the risk. All associated harvest, sorting, grading and storage equipment, machinery, structures, and areas involved in any phase of harvesting, sorting, cleaning, grading and storage, will be cleaned and disinfected before facilities can be released from quarantine. Fields where infested material was grown will be assessed for potential quarantine actions.

Once the extent of infestation is determined for a growing area, establish quarantine boundaries with growers, seed companies, storage facilities, processors, and distributors coming under restrictions determined by regulation. If new information indicates a more widespread infestation problem than originally assessed, or other information becomes available, these regulatory guidelines are subject to change.

Conduct a traceback investigation to determine the source of infected tubers and the extent of distribution of seed tubers in the United States from that source. Conduct a trace forward investigation to determine if potentially infected tubers have been distributed to growers or processing plants where processed material and water may be released to the environment. [See Traceback and Trace Forward Survey on page 3-7](#) for more information.

Information gathered in the investigation will be critical to determining which additional tuber lots and properties to hold, as well as which actions to take. Consult with regional contacts if guidance is needed.

Distribution of Lists of Positive and Suspect Properties

Geraniums

A list of nursery facilities will be compiled of geranium varieties and/or shipping windows that have been associated with positively tested, foreign origin geraniums. These lists will be distributed by state to the field offices, and are **not** to be shared with individuals outside our regulatory cooperators. Company names and locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited.

When notifying nurseries on the list, be sure to identify yourself as a USDA or state regulatory official conducting an investigation of nurseries that may have received *Ralstonia solanacearum* infected material. Speak to the nursery owner or manager.

It is important to understand that nursery facilities handling foreign geraniums are of several types. Companies ship cuttings and callused plants through PPQ–Plant Inspection stations. The cleared material is then either sent to rooting stations or to direct ship customers which are wholesalers.

Both types of facilities grow the plants for a period of time (usually 5 weeks) before being distributed to other entities. Rooting stations, under contract with the company, then ship orders to a variety of customer types including wholesalers and both large and small retail customers. Direct ship facilities can have their own network of retail stores or sell plants to other customers. Handling and cultural practices vary at all steps in the distribution.

During the investigation, request that the nursery owner or manager produce any tags, invoices, shipping lists associated with the positive tested suspect plants on the distributed list. Make copies of these records and locate the suspect plants still on the premises. Take photographs of the area where suspect geraniums are located and any greenhouse conditions including the proximity of neighboring plants that may indicate broader contamination. Make a record of the number of plants, type, and location including an accounting of suspect plants from that shipment **not** on the premises.

Traceback information gathered from plant tags and invoices can be used to determine the origin of the plants. With timely submitted records from plant distribution companies such as rooting stations or wholesale growers, PPQ–Emergency and Domestic Programs staff can generate lists of nurseries with implicated plant varieties. Considerable efforts have been made by the geranium industry to use bar-coding for plants and cuttings tracking that may have use in these investigations.

This information can be used to determine the extent of distribution of suspect geraniums at various locations in the United States. Coordinate with your state contacts and regional office to gather this information. Additional resources may be needed to quickly gather this information and analyze it in order to hold suspect geranium shipments and potentially infected plants.

Potato Cropping Systems

As a result of traceback or trace forward investigations, a list of facilities associated with field grown potatoes testing positive for *Ralstonia solanacearum* race 3 biovar 2 will be compiled. These lists will be distributed by state to the field offices, and are **not** to be shared with individuals outside our regulatory cooperators. Grower names and field locations on these lists are strictly confidential, and any distribution of lists beyond appropriate regulatory agency contacts is prohibited.

The lists will include three categories:

Positive Testing—Seed or ware potatoes, in fields, or as harvested, or in storage, at shipping points, or in processing facilities that are confirmed positive for *Ralstonia solanacearum* race 3 biovar 2.

Positive Associated—Seed or ware potatoes, in fields, or as harvested, or in storage, at shipping points, or in processing facilities that are directly linked to positive testing for *Ralstonia solanacearum* race 3 biovar 2. Direct links include but are **not** limited to the following types of potatoes:

- ◆ Planted in fields with same seed lots as positive tested lots,

- ◆ Commingled in the same storage bin or area with positive tested, and
- ◆ Irrigated from *R. solanacearum* race 3 biovar 2 confirmed contaminated water sources.

Potentially Infested—Seed or ware potatoes, in fields, or as harvested, or in storage, at shipping points, or in processing facilities that are indirectly linked to positive testing, for *Ralstonia solanacearum* race 3 biovar 2. Indirect links include, but are **not** limited to the following:

- ◆ Seed lots from the same company, or stored on the same property as positive testing lots
- ◆ Hosts in fields that share the same water supply as positive testing fields
- ◆ In fields adjacent to positive testing fields
- ◆ Fields that have a history of using harvesting equipment with positive testing fields

Further Investigations at Nurseries with Suspect Geraniums

Once a list of nurseries in the state is generated after the initial United States detection, it will be necessary to visit the locations to assess the extent of plant distribution and nursery irrigation and sanitation practices. Various greenhouse practices will prevent or facilitate the spread of bacteria of *Ralstonia solanacearum* race 3 biovar 2. Information must be gathered to determine what additional potentially infected plants or areas may be at risk for carrying the pathogen. For more information, see [Plants to Hold on page 5-9](#).

Examine the type of irrigation system and practices used at the nursery:

- ◆ Are plants under a sub-irrigation system where water floods the pots and penetrates from below?
- ◆ Do you notice hanging geraniums from suspect shipments?
- ◆ Were other plants (hosts or otherwise) under their drip line?
- ◆ Are cuttings of plants being made to propagate more plants?
- ◆ Do workers exhibit sanitary handling of plant material?

This kind of observational information should be documented by the inspector. In addition, interview the nursery owner/manager and ask if they have seen wilting geraniums or other hosts during the season in question. Ask if any wilting plants have been discarded and if so, where? Photographs of the facility and written observations can be useful in the investigation phase.

Ask the nursery owner/manager to complete and sign [Questionnaire for Nursery Owners Or Managers on page A-1](#).

The information gathered in the above questions and documentation will assist in determining if additional plants must be held. Consult with your regional contacts when there are questions concerning a particular facility practice that may affect the number of additional potentially infected plants held and the extent to which that practice is used.

Placing Holds

Nurseries

After a destination nursery is identified as having received plants from another source associated with positive testing geraniums, it is important to immediately place holds on all geranium varieties named on distributed lists and initially any other hosts plants in the facility. If the extent of potentially infected plants at that facility can be ascertained at that time, those **not** determined to be potentially contaminated can be immediately released.

A hold is interpreted as the prohibition of movement of those plants from the property until further evaluations can be made and control actions can be taken on suspect geraniums and other potentially infected plants.

After investigations are made, unless general contamination is suspected, the hold is **not** to be interpreted to:

- ◆ Prohibit the movement of all plants in a greenhouse;
- ◆ That plants **not** held cannot be cared for in a normal and sanitary manner; and
- ◆ Prohibit the relocation of suspect geranium shipments and potentially infected plants to a segregated area away from other plants; if the nursery owner/manager wishes to move held plants, assess the risk of such movement and keep records of where relocated plants are moved.

Potato Storage Facilities and Grower Fields

After a facility is identified as having received positive testing tubers (i.e., suspect tubers), it is important to immediately place holds on properties with suspect tubers and potentially infested tubers that are listed as the result of investigations or determined positive in testing. If holds were placed previously, and the extent of potentially infested tubers at that property can be ascertained after a thorough investigation, those lots or properties determined **not** to be at risk or potentially infected can be immediately released. This could result from negative testing results or new information discovered during investigations. A hold is interpreted as the prohibition of movement of plant material or tubers from the property until further evaluations can be made and control actions can be taken on suspect tubers and other potentially infected plant material.

After investigations are made, unless general contamination is suspected, the hold is **not** to be interpreted to:

- ◆ Prohibit the movement of all tubers in a facility or property; and
- ◆ Prohibit the relocation of suspect tuber shipments and potentially infected tubers to a segregated area away from other plants. If the facility owner/manager wishes to move held tubers to another area on the same property, assess the risk of such movement and keep records of where relocated tubers are and where they have been.

Record Keeping

Geraniums

Record keeping and documentation is important for any holds and subsequent actions taken. Rely on shipping records and information provided by the nursery owner/manager for how many plants remain, how plants have moved within the nursery, destination of plants sold, and cultural practices employed.

Keep a detailed accounting of the numbers and types of each plant variety held and/or destroyed in control actions. Consult a master list of varieties distributed with the lists of facilities. Draw maps of the greenhouse layout to located suspect geraniums, other potentially infected plants, and water runoff areas including recirculating pond locations. Take photographs of the facility layout, geranium placement, watering method, materials and methods used, plant labeling, and any other situations that may be useful for documentation and analysis.

Potato Cropping Systems

For positive potatoes, rely on shipping records and information provided by the grower, farm manager or facility manager for how many tubers remain, how plant material and tubers have moved within the facility, destination of tubers sold, origin of potato seed, and cultural and sanitation practices employed.

Keep a detailed accounting of the numbers and types of tuber or plant material held and/or destroyed in control actions. Consult a master list of varieties distributed with the lists of facilities. Draw maps of the facility layout to located suspect tubers, other potentially infected lots, cull piles, and water runoff areas. Take photographs of the facility layout, watering and sanitation methods, materials and methods used, labeling, and any other situation that may be useful for documentation and analysis.

Keep all written records filed with PPQ Form 523 Emergency Action Notification (EAN) copies, copies of sample submission forms, documentation of control activities, and related state issued documents if available. [See PPQ Form 523 Emergency Action Notification on page B-7.](#)

Plants to Hold

Complete [PPQ Form 523 Emergency Action Notification on page B-7](#) to place holds at destination nurseries for the following classes of plants:

Suspect Geraniums

Variety

Depending on the particular program directions, a particular variety or varieties of geranium plants may be considered suspect because they tested positive and traceback investigations to a particular foreign facility determined their overall infection risk. The variety level hold can be most appropriate at rooting stations or direct shipped growers that are large operations whose practice is to keep varieties separate.

Shipment

Depending on program directions and practices at a previous facility in the distribution, a level of suspect geraniums to hold may include the shipment of all host plants received from that facility.

Release all plants that are **not** considered potentially infected. The following categories are subject to hold.

Potentially Infected Plants

High Risk—Results of investigations at nursery facilities, and answers to the questionnaire, will determine the level of additional holds of potentially infected plants. After investigations are made, some of these conditions will require individual judgment and consultation with your regional office. The categories that define higher risk for potentially infecting plants include:

- ◆ Plants beneath the drip line of suspect geraniums—Because *Ralstonia solanacearum* bacteria are easily shed in water, plants directly below hanging geraniums, regardless of the plant species, are at high risk of soil contamination and must be included. This includes hosts and non-hosts. This is also true for plants stored under benches with suspect geraniums.
- ◆ Plants in the same pots with suspect geraniums—Infected geraniums in the same pots with other plants can easily infect them through water, soil, or root contact. This includes hosts and non-hosts.
- ◆ Plant propagation from suspect geraniums has occurred—Any plants propagated from suspect geraniums are at high risk for infection.
- ◆ Plants on a shared irrigation system with suspect geraniums—The most efficient method of spread for *R. solanacearum* is through water that came in contact with infected plants. It is therefore necessary to hold all

plants (hosts and non-hosts) that are on irrigation systems that allows for water flow from one plant to the next. These are various irrigation situations to look for:

- ❖ Sub-irrigation, ebb and flow, or flood irrigation: pots sit in a pan and are irrigated by flooding the pan in various ways.
- ❖ Backflow prevention: nearly all irrigation systems have check valves to as a safeguard to prevent contaminated water from backing up into the general water supply. If systems lack backflow prevention, there is a high risk of general contamination throughout the system from infected material.
- ◆ Plants in facilities where sanitary cultural practices are **not** in place— Using grafting knives for making cuttings or grooming plants without disinfection between varieties is a high risk factor in transmitting the pathogen to other host plant lots.
- ◆ Plants placed on the ground, on plastic sheeting or other material that allows puddling between plants— Because the pathogen spreads by water, there is a risk that puddling between plants will cause uptake of bacteria by health plants in the vicinity of infected. The extent to which this occurs needs to be assessed by observation and further evaluations or monitoring may be necessary.

Reduced Risk—The following greenhouse cultural practices are considered not as high of risk for spreading the pathogen to other plants. Evaluations can be made by the inspector as to the degree to which such practices may take place based on observations and responses to the questionnaire. It is recommended that the inspector consult with the supervisor, SPHD, SPRO or regional office if there is reason to believe the extent of these practices presents a greater risk and therefore requires additional destruction of plants. In some cases, after consultation, the degree to which these risk factors are practiced at the facility may determine the need for wider destruction of potentially infected plants, however, in most cases, these nurseries with plants in these reduced risk categories may be held and subject to follow-up inspections to inspect for wilt symptoms.

- ◆ Plants that were **not** segregated from suspect geraniums— Recent information shows that leaf-to-leaf contact between geraniums is not an effective method of pathogen spread, so there is little to no risk of plants in the same proximity of infected plants becoming contaminated by contact. An exception to this might be plants immediately adjacent to infected plants that were watered by hand (using a hose or a water-wand) where splash may transfer the pathogen to the soil in adjacent plants. Generally, drip irrigation and mist systems do **not** create a risk of splash to adjacent plants. When in doubt, consult your SPHD, SPRO, and regional contact.
- ◆ Plants that were pinched, defoliated, disbudded by hand— This method of grooming presents less risk of spread to non-infected hosts plants than by the use of cutting tools, but some degree of sanitation between

varieties is necessary. An exception to this is if little to no sanitary practices are in place for workers who perform plant grooming by hand and sanitation of hands should take place at least between varieties.

Suspect Potato Cropping Systems

Once initial detections are confirmed, regulated articles include the following:

- ◆ Host plant material including tubers and plants
- ◆ Soil
- ◆ Harvesting equipment including trucks, harvesters, trailers, machinery, tools, field boxes
- ◆ Worker footwear or implements
- ◆ Transport conveyances (if associated with, or suspected of being potentially infested)
- ◆ Storage facilities (if associated with, or suspected of being potentially infested)

Negative testing results may determine certain potential infested articles to be unregulated. Other hosts of *Ralstonia solanacearum* race 3 biovar 2 may occur in a potato area that is known to be from an infested area. The cultivated hosts are to be regulated in a manner similar to potato. [See Hosts on page 2-6](#) for more information.

Grower Requirements Under Quarantine

Properties with positive testing, or positive associated fields, will be required to destroy and/or dispose of all plant material that can be reasonably removed from the field. This includes cull piles and other plant debris.

The field will be left fallow for two years, and irrigated to promote volunteer sprouting. During these two years, in the growing season, the volunteer crop will be sampled, tested, and disked under every four weeks to eliminate host material. Weed hosts in the field and along the edges will be sprayed with efficacious labeled herbicides to eliminate them from the area.

Issuing PPQ Form 523 Emergency Action Notification

[PPQ Form 523 Emergency Action Notification on page B-7](#) (EAN) is issued to hold all host plants at facilities that have the suspect geranium or potato varieties and potentially infected plants or tubers connected to positive confirmations. Once an investigation determines there are plants that are **not** suspect geraniums or potentially infected potato tubers, they may be released and documented on the EAN.

The EAN may also be issued to hold potato or geranium plants when wilt is discovered pending positive identification. When a decision to destroy plants is made, or in the case of submitted samples once positive confirmation is

received, the same EAN for which the plants are on hold is used to document any actions taken such as destruction and disinfection. If varieties or shipments are to be held as separate units, it is advisable to issue separate EANs for each held unit of suspect geraniums or potatoes and potentially infected plants associated with that unit.

Electronic EANs can be used. [See PPQ Form 523 Emergency Action Notification on page B-7](#) for instructions. EANs are issued under the authority given in 7 CFR 330. States should issue their own hold orders parallel to the EAN to assure plants cannot move intrastate.

Address

<http://www.aphis.usda.gov/library/forms/>

Geranium and Potato Volunteers and Cull Piles

Fields with volunteer potatoes may come under regulations in a generally infected growing area may require sampling and testing if they are considered potentially infested.

Cull piles may be found next to fields or greenhouses, on other farm property areas, or near storage facilities or processors. These are potential reservoirs of the pathogen and are regulated until the risk level is determined by investigations and/or testing.

Harvesting Equipment, Storage Areas, and Conveyances

Potato harvesting equipment including trucks, harvesters, trailers, machinery, tools, field boxes, worker footwear or implements, transport conveyances, and storage facilities are subject to cleaning and disinfection if positive associated.

Regulatory Requirements of Seed Producers

Seed tuber producers must destroy and/or dispose of all positive tested and positive associated lots. They must test all lots in their inventory for the presence of *Ralstonia solanacearum* race 3 biovar 2. They must further clean and disinfect all equipment, storage areas, and conveyances used in their business enterprise. Regular testing for *R. solanacearum* race 3 biovar 2 must become a part of their seed certification program.

Regulatory Requirements for Processors

Companies that may have received potatoes from positive harvested fields are subject to holds, inspection, testing, and possible disinfection requirements. Processors can include facilities that wash potatoes for table consumption (ware potatoes), produce potato chips, fries, or starch. In all cases, the facilities

received positive testing or positive associated stock, or if tests are positive, the water and equipment used in processing and the waste products from processing will require regulatory action. Place holds on facilities, water sources, high risk plant material from processing until assessments are made to determine which parts of the facility will require disinfection, water treatment, and proper disposal of high risk waste material.

Processor receiving positive testing or positive associated potatoes must destroy or dispose of all of those potatoes in their inventory. All storage areas, processing equipment, and conveyances must be disinfected. All water sources or storage ponds recirculated for processing must be tested and decontaminated if found to be positive.

Regulated Articles

Cultivated Host Plants

Plants, cuttings, or parts of the following hosts of *Ralstonia solanacearum* race 3 biovar 2 are regulated if tested positive and considered potentially infected.

TABLE 5-1 Cultivated Hosts of *Ralstonia solanacearum* race 3 biovar 2

Geranium	<i>Pelargonium</i> spp. ¹
Tomato	<i>Lycopersicon esculentum</i>
Peppers	<i>Capsicum</i> spp.
Eggplant	<i>Solanum melongena</i>
Potato	<i>Solanum tuberosum</i>
Bean	<i>Phaseolus vulgaris</i>
Bittergourd	<i>Momordica charantia</i>
Beet	<i>Beta vulgaris</i>

¹ See *Pelargonium* in the Nursery Trade on page E-1

Weed Host Plants

Weed hosts of *Ralstonia solanacearum* race 3 biovar 2 are often symptomless but must be destroyed in greenhouses with positive detections including an area within one meter around the outside perimeter of the greenhouse. See [Control on page 6-1](#) for destruction/disposal and disinfection procedures.

TABLE 5-2 Weed Hosts of *Ralstonia solanacearum* race 3 biovar 2

Black nightshade	<i>Solanum nigrum</i>
Climbing nightshade	<i>Solanum dulcamara</i>
Horsenettle	<i>Solanum carolinense</i>

TABLE 5-2 Weed Hosts of *Ralstonia solanacearum* race 3 biovar 2

Jimson weed	<i>Datura stramonium</i>
Purslane	<i>Portulaca oleracea</i>
Mustards	<i>Brassica spp.</i>
Lambsquarters	<i>Chenopodium album</i>
Bittergourd	<i>Momordica charantia</i>

Pots and Media

Pots and soil, planting, or rooting media, that has been in contact with positive tested plant shipments and other potentially infected plant material are also regulated and subject to destruction, disposal, and disinfection procedures (See Control on page 6-1).

Tools and Equipment

Tools, implements, equipment, benches, and greenhouses, used in cultivation that may have contacted positive tested plant material are subject to disinfection. See Control on page 6-1 for more information.

Other regulated articles include 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.

Removing Areas from Quarantine

Plants held can be released from PPQ Form 523 Emergency Action Notification (EAN) conditions if any of the following can be demonstrated:

- ◆ No suspect geraniums from specified suspect rooting and distribution facilities were received at the destination nursery during the specified time periods.
- ◆ No other host plants from the facility have shown wilt symptoms, or if wilt was detected, they were subsequently sampled, tested, and found negative for *Ralstonia solanacearum* race 3 biovar 2.
- ◆ Suspect geraniums associated with positive confirmed detections of *R. solanacearum* race 3 biovar 2 were disposed of or destroyed along with associated potentially infected plants. This includes removal and destruction of weed hosts and the disinfection of nursery areas where destroyed plants were held including the tools and other equipment that may have come in contact with suspect geraniums and potentially contaminated plants. See Control on page 6-1 for more information.

Additionally, before release, further traceback or trace forward investigations should not indicate a risk of dissemination of *Ralstonia solanacearum* race 3 biovar 2 to other parts of the nursery property

Requirements for Potato Cropping Systems Under Quarantine

The information in this section has been summarized in [Table 5-3, "Requirements for Potato Cropping Systems Under Quarantine", on page 16.](#)

Properties with Positive Testing or Associated Fields

If a property has positive testing or positive associated fields, then destroy or dispose of all plant material that can be reasonably removed from the field. This includes cull piles and other plant debris. Leave the field fallow for two years, and irrigate to promote volunteer sprouting. During the two growing seasons, the volunteer crop must be sampled, tested, and disked under every four weeks to eliminate host material. Weed hosts in the field and along the edges will be sprayed with efficacious labeled herbicides to eliminate them from the area.

Fields in the following two years are to be planted with non-host crops and irrigated to promote volunteer sprouting and volunteer potato destruction. Fields must be tested semi-annually for four years after an initial positive find. [See Survey Procedures on page 3-1](#) for more information.

Furthermore, no seed production can occur in field for at least 5 years after detection of *Ralstonia solanacearum* race 3 biovar 2. Fields with susceptible hosts (potatoes or tomatoes) must be sampled for two seasons prior to any new seed production on the property.


Fields Adjacent to Positive-Testing or Associated Fields, or Sharing Water

Fields adjacent to positive testing or associated fields, or those on a shared water situation, may **not** grow host crops for two years, seed potatoes for two years, and must test and control any volunteer potato or weed hosts. Strict sanitation of all vehicle entering and leaving the infested fields will be maintained.

Storage Facilities

Storage facilities on properties with positive testing tubers must be held in quarantine until all potentially infested tubers are tested and either found to be negative or destroyed. The storage facilities must then be properly cleaned and disinfected with approved disinfectants in strict accordance with labeling. [See Disinfectants on page G-1](#) for more information.

TABLE 5-3 Requirements for Potato Cropping Systems Under Quarantine

If:	Then:	And in 1st and 2nd years:	And in 3rd and 4th years:	And for at least 5 years after detection:
<p>A property has positive testing or positive associated fields</p>	<p>Destroy or dispose of all plant material that can be reasonably removed from the field, including:</p> <ul style="list-style-type: none"> ◆ Cull piles ◆ Other plant debris <p>Storage facilities on the properties must be held in quarantine until all potentially infested tubers are tested and either found to be negative or destroyed. The storage facilities must then be properly cleaned and disinfected with approved disinfectants in strict accordance with labeling. See Disinfectants on page G-1 for more information.</p>	<p>Leave the field fallow and irrigate to promote volunteer sprouting. During the growing season of each year:</p> <ul style="list-style-type: none"> ◆ Volunteer crop must be sampled, tested, and disked under every four weeks to eliminate host material. ◆ Weed hosts in the field and along the edges must be sprayed with efficacious labeled herbicides to eliminate them from the area. 	<p>Plant with non-host crops and follow cultural practices from the 1st and 2nd years (i.e., irrigation and destruction of volunteer potatoes).</p>	<p>No seed production can occur in field after detection. Fields with susceptible hosts (potatoes or tomatoes) must be sampled for two seasons prior to any new seed production on the property.</p>
<p>Fields are adjacent to positive testing or associated fields, or are on a shared water situation</p>		<ul style="list-style-type: none"> ◆ Do not grow host crops or seed potatoes, ◆ Test and control any volunteer potato or weed hosts. ◆ Maintain strict sanitation of all vehicles entering and leaving the infested fields. 		

Volunteer Potatoes and Cull Piles

Fields with volunteer potatoes may come under regulations in a generally infected growing area may require sampling and testing if they are considered potentially infested.

Cull piles may be found next to fields, on other farm property areas, or near storage facilities or processors. These are potential reservoirs of the pathogen and are regulated until the risk level is determined by investigations and/or testing.

Harvesting Equipment, Storage Areas, and Conveyances

Potato harvesting equipment including trucks, harvesters, trailers, machinery, tools, field boxes, worker footwear or implements, transport conveyances, and storage facilities, are subject to cleaning and disinfection if positive associated.

Regulatory Requirements of Seed Producers

Seed tuber producers must destroy and/or dispose of all positive tested and positive associated lots. They must test all lots in their inventory for the presence of *Ralstonia solanacearum* race 3 biovar 2. They must further clean and disinfect all equipment, storage areas, and conveyances used in their business enterprise. Regular testing for *R. solanacearum* race 3 biovar 2 must become a part of their seed certification program.

Regulatory Requirements for Processors

Companies that may have received potatoes from positive harvested fields are subject to holds, inspection, testing, and possible disinfection requirements. Processors can include facilities that wash potatoes for table consumption (ware potatoes), or produce potato chips, fries, or starch.

In all cases, the facilities received positive testing or positive associated stock, or if tests are positive, the water and equipment used in processing and the waste products from processing will require regulatory action. Place holds on facilities, water sources, high risk plant material from processing until assessments are made to determine which parts of the facility will require disinfection, water treatment, and proper disposal of high risk waste material.

Processor receiving positive testing or positive associated potatoes must destroy or dispose of all of those potatoes in their inventory. All storage areas, processing equipment, and conveyances must be disinfected. All water sources or storage ponds recirculated for processing must be tested and decontaminated if found to be positive.

Before release of a facility, PPQ Officers must conduct a monitoring inspection and document on the EAN that no positives for *Ralstonia solanacearum* race 3 biovar 2 were detected or, if positives were detected, then all destruction, disposal, and disinfection actions taken with dates taken, location, and witnessed by whom.

Notify nursery owner/managers that their facilities may be subject to additional monitoring by state or federal officials for the presence of *Ralstonia solanacearum* race 3 biovar 2.

If investigations determine the quarantine restrictions on potato fields are adhered to over the prescribed time periods, actions are documented, and water sources are tested and free of infection, fields can be released from quarantine restrictions. Notify growers that their fields may be subject to additional monitoring by state or federal officials for the presence of *Ralstonia solanacearum* race 3 biovar 2.

Use of Chemicals

The PPQ *Treatment Manual* and this *Guidelines* identify the appropriately labeled chemicals, and describe the methods and rates of application. Concurrence by PPQ is necessary before using any other chemical or procedure for regulatory purposes.

6

Ralstonia
solanacearum r 3 b 2

Chapter 6

Control

Contents

Introduction	page 6-1
Handling of Samples	page 6-2
Plant Disposal and Destruction Methods	page 6-3
Disinfection Procedures	page 6-4
Approved Disinfectants	page 6-6

Introduction

Use *Chapter 6 Control* as a guide to the control of *Ralstonia solanacearum* race 3 biovar 2. USDA–APHIS–Plant Protection and Quarantine (PPQ) develops and makes control measures for *R. solanacearum* race 3 biovar 2 available to involved states. Environmental Protection Agency (EPA) approved and labeled treatments will be recommended when available.

Laws Pertaining to Pest Control

The [Federal Insecticide, Fungicide, and Rodenticide Act \(FIFRA\)](#) authorizes the [Environmental Protection Agency \(EPA\)](#) to regulate pesticides. All persons using and applying pesticides should understand the laws pertaining to pesticide use and application. The following are provisions of FIFRA that are most pertinent to emergency pest control programs:

- ◆ Restricted use pesticides must be applied by a certified applicator.
- ◆ Use of any pesticide inconsistent with the label is prohibited.
- ◆ Violations can result in heavy fines or imprisonment.

States can register pesticides on a limited basis for special local needs according to the following Sections:

- ◆ **Section 2(ee)**—Disinfections conducted against the target pest *Ralstonia solanacearum* must use a FIFRA Section 2ee until that name is specifically added to the label. This designation is necessary if the synonym *Pseudomonas* was used on the labels of recommended products. *Pseudomonas* is the genus name that was used previously, and is synonymous with *Ralstonia*.
- ◆ **Section 18**—EPA administrators can exempt federal or state agencies from FIFRA if it is determined that emergency conditions exist that require such exemptions.

- ◆ **Section 24**—A state can provide registration for additional uses of federally registered pesticides formulated for distribution and use within that state to meet special local needs in accordance with the purposes of this act.

For additional information concerning exemptions, see the [Emergency Programs Manual, Section 14](#). Contact [Environmental Services](#) staff to assure that any pesticide being considered as part of an eradication program conforms to pesticide use requirements. Obtain all required environmental documentation before beginning. [See Environmental Compliance on page A-1](#) for more information.

Control Records

Program personnel must maintain records and maps, noting the locations of all detections, the number and type of plants subjected to control actions, and the materials and formulations used in each treated area. Attach all documentation and receipts to the office copy of the PPQ Form 523 Emergency Action Notification. [See PPQ Form 523 Emergency Action Notification on page B-7](#) for more information.

Control Decisions and Oversight

Important

All quarantine actions related to destruction are to be witnessed, supervised, and documented by a PPQ officer whenever possible. Because *Ralstonia solanacearum* race 3 biovar 2 is listed as a select agent under the *Agriculture Bioterrorism Protection Act of 2000*, proper supervision and documentation of destruction of infected plant material is critical. If a PPQ officer is not available, a state cooperating inspector can witness and document the disposal. Disinfections can be witnessed and documented by PPQ officers or state cooperators.

Handling of Samples

Place all potentially infected plants in double plastic bags and seal for disposal or destruction, including the following:

- ◆ Shipments of suspect geraniums
- ◆ Associated potato plants or tubers that have tested positive for *Ralstonia solanacearum* race 3 biovar 2
- ◆ Pots, directly associated soil or potting media, and any other disposable item in contact with the plants or soil

Host weeds in positive tested facilities must also be removed and correctly disposed of or destroyed. [See Plant Disposal and Destruction Methods on page 6-3](#) for more information

Maintain an accurate count of each type (variety, etc.) of plant or tubers bagged for destruction and record this information.

If large inventories must be destroyed, the use of plastic bags may not be reasonable. Alternatives may include a dumpster with double layers of plastic lining that can be folded over the top and sealed to prevent debris from escaping during transport or storage. Contact your regional office or PPQ Headquarters for additional guidance.

All bench areas where plants were held, and other areas at risk for exposure to infected plant material, should be disinfected according to prescribed disinfection procedures. [See Disinfectants on page G-1](#) for more information.

Plant 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 waste. In these cases, use an approved landfill.

Burning or Incineration

All plant material and media must be incinerated or burned to the point of ash. If plastic pots are **not** accepted at the incinerator, remove them and properly disinfect or send to a landfill.

Steam Treatment Under Pressure

Use an autoclave, or follow the guidelines in the [PPQ Treatment Manual](#).

Soil Fumigation

Soil fumigation with an approved fumigant—such as methyl bromide or metam sodium—at the labeled rate will greatly reduce populations of the bacteria in infested fields. However, fields with host plant residue including tubers, roots, and stems, may still harbor adequate amounts of *Ralstonia solanacearum* for up to two years. Before fumigation, remove and destroy as much host plant material from the fields as possible.

Fumigation of the soil may **not** totally eliminate the pathogen due to the presence of bacterial reservoirs in buried plant host residue at the time of fumigation. Continued monitoring of the soil for the presence of *Ralstonia solanacearum* race 3 biovar 2 will be required.

Approved Landfill

An approved landfill is a state licensed municipal or private facility that is managed under state regulation to meet conditions that would prevent potential pollutants from leaching into groundwater.

See [Disposal Memorandum on page B-9](#) for a USDA Memorandum for Record concerning landfills that may be used to alleviate concerns of local officials, landfill operators, or the public.

Important

This plant pathogen is not considered a hazardous waste, biomedical waste, epizootic, or any other substance of concern for human health—it is an agricultural pathogen only. Proper disposal is required because there is a remote possibility that it could reach ground water for eventual uptake from wells used for irrigation of host crops. The concentration of the bacteria will be minimal relative to the volume of material disposed of, 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 or potato cropping systems must assure the material is buried under two or more feet of soil. Notify the landfill in advance to arrange for a hole to be dug in which the material can be disposed and covered with soil. If digging a hole is impractical for a landfill operation, another option is to cover the plant material with alternative daily cover or composted green waste normally used to cover refuse at landfills. These operations must be witnessed, supervised, and documented by a plant regulatory official.

The quantities of potato host material ordered for destruction may be impractical for the disposal options prescribed. Composting will likely heat the temperature of host material to sufficient temperatures necessary to kill the pathogen. The parameters of a composting disposal method must be proposed by a *Ralstonia* technical group. The efficacy of composting must be tested by CPHST prior to implementation as a program method. Care must be taken to control water run off from composting operations if implemented.

Disinfection Procedures

Use the following procedures to disinfect 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:

1. Clean away any soil or media.
2. Spray and soak to the point of runoff with approved disinfectants or by pressurized steam cleaning to raise the surface temperature to 212°F.

For more information, consult the following sources:

- ◆ [Approved Disinfectants on page 6-6](#)
- ◆ [Disinfectants on page G-1](#)
- ◆ [PPQ Treatment Manual](#)

Tools and Equipment

Use the following procedures to disinfect tools and equipment that may have come in contact with infected plants or contaminated soil:

1. Clean away any soil or media.
2. Apply approved disinfectants to the point of runoff, or thoroughly wash with pressurized steam to raise the temperature to 212°F.

See [PPQ Treatment Manual](#) for more information.

Exposed Irrigation Systems

Drain and clean the following systems:

- ◆ Recirculating irrigation system
- ◆ Subirrigation system
- ◆ Any system that does **not** prevent backflow of water from infected greenhouses

Clean all parts, sumps, and pumps, with approved disinfectant solutions. To be effective against *Ralstonia solanacearum* race 3 biovar 2, the systems must have ozonation with 0.4 ppm residual O₃ for 4 minutes with UV light of at least 300 j/m² at >50% transmission (PPQ, 2004).

Holding Ponds

When holding ponds have become contaminated from irrigation system runoff, contact your regional PPQ office and State about possible environmental considerations and treatment options.

Outdoor Soil or Holding Areas

When outdoor soil or holding areas have become contaminated during plant storage or runoff, contact your regional office and State about possible environmental consideration and treatment options.

Approved Disinfectants

Use the approved disinfectants in [Table 6-1](#) to disinfect all tools, benches, walkways, surfaces, and gravel beds under benches, in contact with potentially infected plant material after removal of soil or media. Be sure that the label specifies *Ralstonia* or *Pseudomonas*. Check with State labeling requirements. [See Disinfectants on page G-1](#) for more information

TABLE 6-1 Approved Disinfectants

Product	Notes
Quaternary Ammonia	Several quaternary ammonia (20% ammonium chloride) products are approved for greenhouse use.
Physan® 20	Physan 20 is not approved for greenhouse where food crops are grown. It is not approved for use in California
Green Shield®	Green Shield is approved for most uses. In California, use the special formulation Green Shield@CA.
Maquat® 615-HD or 615-LR	None
ZeroTol®	ZeroTol is 27% hydrogen dioxide. It 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.



Chapter 7

Environmental Compliance

Contents

Introduction	page 7-1
Disclaimer	page 7-1
National Environmental Policy Act	page 7-2
Endangered Species Act	page 7-2
Federal Insecticide, Fungicide, and Rodenticide Act	page 7-3
Other Laws	page 7-3
Environmental Monitoring	page 7-3

Introduction

Use *Chapter 7 Environmental Compliance* as a guide to environmental regulations pertinent to the plant pathogen *Ralstonia solanacearum* race 3 biovar 2 in geraniums, potatoes, and other solanaceous hosts.

A key element in designing a program or an emergency response is consultation with Environmental Services (ES), a unit of APHIS' Policy and Program Development staff. ES performs the following functions:

- ◆ Prepares environmental documentation such as environmental impact statements and environmental assessments to aid in making decisions
- ◆ Provides consultation on the topic of endangered species
- ◆ Coordinates pesticide registration and approvals for APHIS programs, ensuring that registrations and approvals meet the needs of programs and conform to pesticide use requirements

In addition, PPQ's Environmental Compliance team assists ES in the development of required documentation and implements any environmental monitoring that may be required of program activities. Refer to [Resources on page A-1](#) for additional information.

Disclaimer

All uses of pesticides must be registered or approved by appropriate Federal, State, and/or Tribal agencies before they can be applied. The information provided on pesticide labels may not reflect all of the actual information, including precautions and instructions for use, which you are required to follow in your specific state or locality. It is the responsibility of persons

intending to use a pesticide to abide by the label, including labeling that has been approved for the particular state or locality in which the chemical is to be used, and to comply with all federal, state, tribal, and local laws and regulations relating to the use of the pesticide. Staff within APHIS programs are responsible for their compliance with applicable environmental regulations, which often include measures above and beyond those listed on pesticide labels.

National Environmental Policy Act

The National Environmental Policy Act (NEPA) requires that federal agencies document the potential adverse effects of their actions. The process often requires public input. The exact nature of the documentation and public involvement is dictated by the potential for adverse effects and the significance of those effects.

It is likely that most pest control responses will include actions that need up to 30 days of public comment prior to initiation. Therefore, it is imperative to involve Environmental Services and Environmental Compliance early in the planning process. Doing so assures public involvement and a quick response.

Depending on the proposed program, NEPA requirements will be met with a categorical exclusion, environmental assessment, or environmental impact statement. Some programs can prepare their own NEPA documentation. Contact Environmental Services or Environmental Compliance if you are unsure which document should be prepared, or if you have little experience writing such documents. Refer to [Resources on page A-1](#) for contact information.

Endangered Species Act

The Endangered Species Act (ESA) requires that all federal actions, including emergency responses, do not harm federally protected threatened or endangered species. Before an action can begin, it must be determined if protected species are in the project area. If such species are present, measures must be put in place to protect them from potential adverse effects of the action. Such work requires coordination with the U.S. Fish and Wildlife Service and/or the National Marine Fisheries Service.

Several methods are available to ensure compliance with ESA, but the exact one chosen is dictated by the nature of the emergency, proposed response, and location. As soon as possible in the early stages of the response, contact staff at Environmental Services or Environmental Compliance, who can provide the necessary guidance and support in conducting the necessary analyses and developing the required documentation. Refer to [Resources on page A-1](#) for contact information.

Federal Insecticide, Fungicide, and Rodenticide Act

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires that chemicals used for control have approved labels and that all label requirements are followed. These requirements can include applicable uses, maximum application rates, handling instructions, and personal protective equipment. If no label is available for the emergency in question (i.e., the pest of concern is not listed as one for which the chemical may be used), it is possible to obtain a new label or a label exemption. If a label change is needed or no label can be located for your program needs, immediately contact Environmental Services, who can assist in label changes and emergency use exemptions. Refer to [Resources on page A-1](#) for contact information.

Other Laws

The National Environmental Policy Act, Endangered Species Act, and the Federal Insecticide, Fungicide, and Rodenticide Act, are of critical importance to all pest control programs, but other laws may apply depending on program locations and activities. These include the Migratory Bird Treaty Act, the Coastal Zone Management Act, and the Bald and Golden Eagle Protection Act. By including Environmental Services and Environmental Compliance early in program planning, guidance can be provided on meeting the requirements of these and other laws that may apply. Refer to [Resources on page A-1](#) for contact information.

Environmental Monitoring

Environmental monitoring of APHIS pest control activities may be required as part of compliance with the above laws, as requested by program managers, or as suggested to address concerns with controversial activities. This is especially true for less benign chemical controls and aerial application of chemicals.

Monitoring may be conducted with regards to worker exposure, quality assurance and control, off-site deposition, or program efficacy. Different tools and techniques are used depending on the monitoring goals, program chemicals, and control techniques. Environmental monitoring is coordinated by Environmental Compliance (EC). Staff from EC will work with the program manager to develop an environmental monitoring plan, conduct training to implement the plan, provide day-to-day guidance on monitoring, and provide an interpretive report of monitoring activities.

Glossary

approved landfill. State licensed municipal or private landfill that is managed under state regulation to meet conditions that would prevent leaching of potential pollutants into groundwater.

biovar. Group of bacterial strains that are distinguishable from other strains of the same species on the basis of their physiological characteristics.

chlorosis. Yellowing of normally green tissue due to chlorophyll destruction in infected plants.

decontamination. Application of an approved chemical or other treatment to contaminated implements, material, or buildings for killing or deactivating a pathogen.

detection survey. Survey conducted in an environmentally favorable area where *Ralstonia solanacearum* race 3 biovar 2 is not known to occur.

destination nursery. Any nursery receiving suspect geraniums or suspect plants from a rooting station or other nursery.

disposal. Method used to eliminate diseased plant material or material associated with diseased plant material, usually at an approved landfill.

ELISA. Acronym for Enzyme Linked Immunosorbent Assay, a serological laboratory technique used to determine the genus and species in a *Ralstonia* host sampling and testing program; excludes race and biovar.

Enzyme Linked Immunosorbent Assay. See *ELISA* above.

host. Plant which is invaded by a parasite or pathogen and from which it obtains its nutrients.

incineration. Burning of plants and associated soil or media that results in their complete destruction.

infection. Establishment of a parasite on or within a host plant.

monitoring survey. Survey conducted at a site where a disease was found and where an eradication program is being performed.

necrosis. Dead or discolored plant tissue.

pathogen. Micro-organism that can incite a disease.

PCR. Acronym for Polymerase Chain Reaction, a laboratory technique that amplifies DNA sequences in order to determine the race and biovar in a *Ralstonia* host sampling and testing program.

positive associated. Seed or ware potatoes, in fields, or as harvested, or in storage, at shipping points, or in processing facilities that are directly linked to positive testing for *Ralstonia solanacearum* race 3 biovar 2.

positive testing. Seed or ware potatoes, in fields, or as harvested, or in storage, at shipping points, or in processing facilities that are confirmed positive for *Ralstonia solanacearum* race 3 biovar 2.

potentially infested tubers. Seed or ware potatoes, in fields, or as harvested, or in storage, at shipping points, or in processing facilities that are indirectly linked to positive testing, for *Ralstonia solanacearum* race 3 biovar 2.

Polymerase Chain Reaction. See *PCR* above.

potentially infected plants. Within the nursery property, these include:

- ◆ All plants under the drip-line of hanging suspect geraniums
- ◆ Other plants that have been planted in the same pots with suspect geraniums
- ◆ All plants on a shared water irrigation system with suspect geraniums, including the following:
 - Ebb-and-flow
 - Flood
 - Sub-irrigation
 - Systems that lack of backflow prevention
- ◆ All host plants that may have been infected by a positive testing suspect geranium shipment through unsanitary greenhouse practices, including the following:
 - Failure to disinfect tools, hands, or equipment during grafting, pruning, de-budding
 - De-leafing between varieties
 - Plants on the ground with a non-porous surface such that puddling may occur under plants

potentially infected plants within nursery property. Includes all plants that are located under the drip-line of hanging suspect geraniums or on a shared irrigation system with a positive testing suspect geranium. Also includes plants that may have been infected by positive-testing suspect geraniums through unsanitary greenhouse practices.

positive testing geraniums. Geraniums in which *Ralstonia solanacearum* race 3 biovar 2 has been confirmed to be present by the USDA-PPQ-CPHST laboratory in Beltsville, MD.

shipment. All plants that were prepared for shipment and transported with suspect geraniums from a common supplier, i.e., listed on the invoice or packing list.

suspect hosts (geraniums or potatoes). Geraniums (*Pelargonium* spp.) that are associated with positive testing geraniums. These may have originated from a foreign country, a rooting station, or another nursery and be the same variety or in the same shipment depending on the circumstances suspect tubers. Also, potato tubers in fields or storage facilities that are associated with potato tubers that have tested positive for *Ralstonia solanacearum* race 3 biovar 2.

suspect geraniums. Geraniums (*Pelargonium* spp.) that are associated with positive testing geraniums. These may be a particular implicated variety or shipment that originated from a rooting station, direct ship facility, or another nursery.

suspect shipment. All host plants that were prepared for shipment and transported with suspect geraniums from a common supplier (listed on the invoice or packing list).

symptom. The external and internal reactions or alterations of a plant as the result of a disease.

traceback. To investigate the origin of infested plants through intermediate steps in commercial distribution channels to the origin.

trace forward. To investigate where infected plants may have been distributed from a source through steps in commercial distribution channels.

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Appendix A

Resources

Diagnostic Tools and Equipment

Central Science Laboratory
Sand Hutton
York
YO41 1LZ
United Kingdom
Telephone: +44 (0)1904 462000
<http://www.csl.gov.uk/>

Loewe Biochemica GmbH
Mühlweg 2a
D-82054 Sauerlach
Germany
Telephone: +49 8104 61620
<http://www.loewe-info.com/>

Neogen Europe Limited
(Acquired Adgen Ltd.)
Cunningham Building
Auchincruive

Ayr, KA6 5HW
Scotland, UK
<http://www.adgen.co.uk/>

Agdia Incorporated
30380 County Road 6
Elkhart, Indiana 46514 USA
Telephone: 574-264-2014
Toll free (USA): 800-622-4342
<http://www.agdia.com/>

BIOREBA AG
STA Laboratories – Colorado
1821 Vista View Drive
Longmont, CO 80504
Telephone: 303-651-6417
<http://www.bioreba.ch/>
<http://www.stalabs.com/>

Environmental Compliance

Environmental Monitoring, Categorical Exclusions—

USDA–APHIS–PPQ–Emergency and
Domestic Programs
Environmental Compliance
4700 River Road, Unit 150
Riverdale, MD 20737
Telephone: 301-734-8247

Environmental Services

FIFRA, ESA, Environmental Assessments—

USDA–APHIS–Policy and Program
Development
Environmental Services
4700 River Road, Unit 149
Riverdale, MD 20737
Telephone: 301-734-8565



Appendix B

Forms and Questionnaires

Contents

PPQ Form 391 Specimens For Determination	page B-2
Addendum to PPQ Form 391	page B-4
Questionnaire for Nursery and Potato Cropping System Owners or Managers	page B-5
PPQ Form 523 Emergency Action Notification	page B-7
Disposal Memorandum	page B-9
PPQ Form 526 Permit to Move Live Plant Plants or Noxious Weeds	page B-10

PPQ Form 391 Specimens For Determination

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. *See reverse for additional OMB information.* **FORM APPROVED OMB NO. 0579-0010**

FOR IIBIII USE

**U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION 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.

1. COLLECTION NUMBER		2. DATE MO DA YR		3. SUBMITTING AGENCY <input type="checkbox"/> State <input type="checkbox"/> PPQ <input type="checkbox"/> Other _____	
SENDER AND ORIGIN	4. NAME OF SENDER			INTERCEPTION SITE	
	6. ADDRESS OF SENDER				
	ZIP				
5. TYPE OF PROPERTY (<i>Farm, Feedmill, Nursery, etc.</i>)			7. NAME AND ADDRESS OF PROPERTY OR OWNER		
COUNTRY/ COUNTY					
8. REASON FOR IDENTIFICATION ("x" ALL Applicable Items)					
A. <input type="checkbox"/> Biological Control (Target Pest Name _____)		E. <input type="checkbox"/> Livestock, Domestic Animal Pest		F. <input type="checkbox"/> Possible Immigrant (<i>Explain in REMARKS</i>)	
B. <input type="checkbox"/> Damaging Crops/Plants		G. <input type="checkbox"/> Survey (<i>Explain in REMARKS</i>)		H. <input type="checkbox"/> Other (<i>Explain in REMARKS</i>)	
C. <input type="checkbox"/> Suspected Pest of Regulatory Concern (<i>Explain in REMARKS</i>)		D. <input type="checkbox"/> Stored Product Pest			
9. IF PROMPT OR URGENT IDENTIFICATION IS REQUESTED, PLEASE PROVIDE A BRIEF EXPLANATION UNDER "REMARKS".					
10. HOST INFORMATION					
12. PLANT DISTRIBUTION <input type="checkbox"/> LIMITED <input type="checkbox"/> SCATTERED <input type="checkbox"/> WIDESPREAD			11. QUANTITY OF HOST NUMBER OF ACRES/PLANTS		
			PLANTS AFFECTED (<i>Insert figure and indicate <input type="checkbox"/> Number <input type="checkbox"/> Percent</i>):		
13. PLANT PARTS AFFECTED					
		<input type="checkbox"/> Leaves, Upper Surface		<input type="checkbox"/> Trunk/Bark	
		<input type="checkbox"/> Leaves, Lower Surface		<input type="checkbox"/> Bulbs, Tubers, Corms	
		<input type="checkbox"/> Petiole		<input type="checkbox"/> Buds	
		<input type="checkbox"/> Stem		<input type="checkbox"/> Flowers	
		<input type="checkbox"/> Growing Tips		<input type="checkbox"/> Fruits or Nuts	
		<input type="checkbox"/> Roots			
14. PEST DISTRIBUTION					
15. <input type="checkbox"/> INSECTS <input type="checkbox"/> NEMATODES <input type="checkbox"/> MOLLUSKS		NUMBER SUBMITTED		LARVAE	
		ALIVE		PUPAE	
		DEAD		ADULTS	
		CAST SKINS		EGGS	
		NYMPHS		JUVS.	
		CYSTS			
16. SAMPLING METHOD					
17. TYPE OF TRAP AND LURE					
18. TRAP NUMBER					
19. PLANT PATHOLOGY - PLANT SYMPTOMS (" <i>X</i> " one and describe symptoms) <input type="checkbox"/> ISOLATED <input type="checkbox"/> GENERAL					
20. WEED DENSITY <input type="checkbox"/> FEW <input type="checkbox"/> SPOTTY <input type="checkbox"/> GENERAL					
21. WEED GROWTH STAGE <input type="checkbox"/> SEEDLING <input type="checkbox"/> VEGETATIVE <input type="checkbox"/> FLOWERING/FRUITING <input type="checkbox"/> MATURE					
22. REMARKS					
23. TENTATIVE DETERMINATION					
24. DETERMINATION AND NOTES (<i>Not for Field Use</i>)					
					FOR IIBIII USE
					DATE RECEIVED
					NO. LABEL SORTED PREPARED
					DATE ACCEPTED
					RR
SIGNATURE _____			DATE _____		

PPQ FORM 391 *Previous editions are obsolete.*
(AUG 02)

This is a 6-Part form. Copies must be disseminated as follows:

PART 1 - PPQ PART 2 - RETURN TO SUBMITTER AFTER IDENTIFICATION PART 3 - IIBIII OR FINAL IDENTIFIER
 PART 4 - INTERMEDIATE IDENTIFIER PART 5 - INTERMEDIATE IDENTIFIER PART 6 - RETAINED BY SUBMITTER

FIGURE B-4 Example of PPQ Form 391 [side 1]

OMB Information

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0010. The time required to complete this information collection is estimated to average .25 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

Instructions

Use PPQ Form 391, Specimens for Determination, for domestic collections (warehouse inspections, local and individual collecting, special survey programs, export certification).

BLOCK	INSTRUCTIONS
1	<p>1. Assign a number for each collection beginning the year, followed by the collector's initials and collector's number</p> <div style="border: 1px solid black; padding: 5px; margin: 5px 0;"> <p>EXAMPLE In 2001, Brian K. Long collected his first specimen for determination of the year. His first collection number is 01-BLK-001</p> </div> <p>2. Enter the collection number</p>
2	Enter date
3	Check block to indicate Agency submitting specimens for identification
4	Enter name of sender
5	Enter type of property specimen obtained from (farm, nursery, feedmill, etc.)
6	Enter address
7	Enter name and address of property owner
8A-8L	Check all appropriate blocks
9	Leave Blank
10	Enter scientific name of host, if possible
11	Enter quantity of host and plants affected
12	Check block to indicate distribution of plant
13	Check appropriate blocks to indicate plant parts affected
14	Check block to indicate pest distribution
15	<ul style="list-style-type: none"> • Check appropriate block to indicate type of specimen • Enter number specimens submitted under appropriate column
16	Enter sampling method
17	Enter type of trap and lure
18	Enter trap number
19	Enter X in block to indicate isolated or general plant symptoms
20	Enter X in appropriate block for weed density
21	Enter X in appropriate block for weed growth stage
22	Provide a brief explanation if Prompt or URGENT identification is requested
23	Enter a tentative determination if you made one
24	Leave blank

Distribution of PPQ Form 391

Distribute PPQ Form 391 as follows:

1. Send Original along with the sample to your Area Identifier.
2. Retain and file a copy for your records.

FIGURE B-5 Example of PPQ Form 391 [side 2]

Addendum to PPQ Form 391

On a separate piece of paper, record the following information. Place the paper in the outer double bag with the completed PPQ 391.

- 1.** Collection date.
- 2.** Submission date.
- 3.** Sample number [State code—Facility code—00000].
- 4.** Name and address of nursery.
- 5.** Contact name.
- 6.** Telephone number.
- 7.** E-mail address.
- 8.** Sample representing shipment invoice number.
- 9.** Week shipped.
- 10.** Specific number and type of plants in the shipment.
- 11.** Location of shipment in nursery (bench numbers, section numbers, etc.).
- 12.** Complete name of plant (genus, species, cultivar, color, any other).
- 13.** Name, agency and telephone number of collector.
- 14.** Lab name, telephone number and contact person.

Questionnaire for Nursery and Potato Cropping System Owners or Managers

Location

Please provide the following information:

1. Name of nursery.
2. Address, City, State, Zip Code.
3. Name of owner and telephone number.
4. Alternate contact person and telephone number.
5. GPS coordinates.
6. Type of facility:
 - ❖ Geranium rooting station
 - ❖ Geranium or potato direct ship wholesaler
 - ❖ Geranium, potato seed or potato table stock wholesaler
 - ❖ Retailer
 - ❖ Other

Questions

Please provide the information requested below. Your answers will help agricultural officials determine the extent of contamination in your nursery facility. Thank you for your cooperation.

1. Indicate the numbers of suspect geranium plants by variety and shipping date received from the foreign facility, rooting station, or wholesaler. Indicate the name of the shipper you received the plants from.
2. The current location and number of all suspect geranium varieties or shipments in question.
3. The history of movement of suspect geraniums within the facility.
4. The history of movement of suspect geraniums out of the facility. If shipped out, which customer did they go to? Indicate the quantity of each variety and the dates shipped.
5. The condition of plants since you received them. Are plants showing wilt? If so, indicate the number of affected plants, and the varieties.
6. If symptoms were observed this season, where within the facility were the wilting plants noticed and which variety?
7. If you had dead or wilted plants, what was the disposition of dead or culled plants and soil/potting media associated with those plants? If they were disposed of, where?

Appendix B

Questionnaire for Nursery and Potato Cropping System Owners or Managers

- 8.** What was the location and number of plants of other hosts (tomato, eggplant, potato, peppers) in your facility inventory since receiving suspect shipments?
- 9.** What type of irrigation system do you use? (i.e., sub-irrigation, ebb and flow, drip, hand watering, etc.) Is backflow prevention in place?
- 10.** If flood, sub-irrigation, or ebb and flow, is practiced, identify the location of all plants sharing the same water source with suspect geraniums.
- 11.** Do you filter or treat your irrigation water? Do you use water from an outdoor holding pond for irrigation recirculation or overflow?
- 12.** Describe the type of greenhouse benches used and floor composition.
- 13.** Do you ever store plants on the ground? If so, on what kind of surface?
- 14.** Do you keep you varieties or shipment segregated?
- 15.** Do you use a particular tagging or tracking system for geranium plants you distribute? If so, please describe the system used.
- 16.** Do you perform propagation of geraniums from the plants you receive from foreign sources?
- 17.** What kind of protection do your nursery workers use when handling plants? How often do they wash their hands or cutting implements?
- 18.** Do you use a disinfectant? If so, what is the name of the disinfectant?
- 19.** Is there a standard greenhouse sanitation protocol document available for your facility? Please provide a copy if so.

Signature of owner or alternate contact person

Date

PPQ Form 523 Emergency Action Notification

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0102. The time required to complete this information collection is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. **FORM APPROVED - OMB NO. 0579-0102**

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE PLANT PROTECTION AND QUARANTINE		SERIAL NO.	
EMERGENCY ACTION NOTIFICATION		1. PPQ LOCATION	2. DATE ISSUED
		3. NAME AND QUANTITY OF ARTICLE(S)	
6. SHPPER		4. LOCATION OF ARTICLES	
		5. DESTINATION OF ARTICLES	
9. OWNER/CONSIGNEE OF ARTICLES Name Address: UNITED STATES		7. NAME OF CARRIER	
		8. SHIPMENT ID NO.(S)	
PHONE NO. FAX NO. SS NO. TAX ID NO.		10. PORT OF LADING	11. DATE OF ARRIVAL
		12. ID OF PEST(S)	
		12a. PEST ID NO.	12b. DATE INTERCEPTED
		13. COUNTRY OF ORIGIN	14. GROWER NO.
		15. FOREIGN PHYTOSANITARY CERTIFICATE NO.	
		15a. PLACE ISSUED	15b. DATE

Under Section 412 and 414 of the Plant Protection Act (7 U.S.C. 7712 and 7714) and Section 2 of the Act of February 2, 1903 (21 U.S.C. 111) and Section 2 of the Act of July 2, 1962 (21 U.S.C. 194a) you are hereby notified, as owner or agent of the owner of said carrier and/or premises and/or articles, to apply remedial measures for an injurious agricultural pest as specified in item 12, in a manner satisfactory to and under supervision of an Agriculture Officer. Remedial measures shall be in accordance with the action indicated in item 16 and shall be completed within the specified time indicated in item 17.*

AFTER RECEIPT OF THIS NOTIFICATION, ARTICLES AND/OR CARRIERS HEREIN DESIGNATED MUST NOT BE MOVED EXCEPT AS DIRECTED BY AN AGRICULTURE OFFICER. THE LOCAL OFFICER MAY BE CONTACTED AT:

16. ACTION REQUIRED

TREATMENT
 RE-EXPORTATION
 DESTRUCTION
 OTHER

* Should the owner or owner's agent fail to comply with this order within the time specified below, USDA is authorized to recover from the owner or agent all costs, including the officer's time, incurred in order to properly dispose of or treat the specified articles.

17. AFTER RECEIPT OF THIS NOTIFICATION COMPLETE SPECIFIED ACTION WITHIN (Specify No. Hours or No. Days):	18. SIGNATURE OF OFFICER
ACKNOWLEDGEMENT OF RECEIPT OF EMERGENCY ACTION NOTIFICATION <i>I hereby acknowledge receipt of the foregoing notification.</i>	
SIGNATURE AND TITLE	DATE AND TIME
19. REVOCATION NOTIFICATION	
ACTION TAKEN	
SIGNATURE OF OFFICER	DATE

PPQ FORM 523 (JULY 2001) Previous editions are obsolete

FIGURE B-6 Example of PPQ Form 526

Instructions

Use PPQ Form 523 Emergency Action Notification to hold suspect geraniums and potentially infected plants at nurseries or other facilities. Only one form should be used to hold each lot of suspect geraniums and associated potentially infected plant material. The same form will be used to determine what is held or ordered destroyed, and what is released.

Forms can be completed by hand, or use the electronic version. Follow the instructions below.

Address <http://www.aphis.usda.gov/library/forms/>

Procedure

Block 1—Enter the name of location of the nearest PPQ office.

Block 3—Under “Name of Article” enter “*Pelargonium* spp.”, not “Geranium”.

Block 4—Enter the greenhouse numbers or other information indicating the location of the plants held.

Block 6—Enter the source nursery or foreign country shipper.

Blocks 7 and 8— Complete only if that information is known.

Block 12—To place plants within a nursery on “Hold”, enter for the “*Ralstonia solanacearum* race 3 biovar 2” as the pest. The authority under which actions are taken is 7 CFR 330 and the *Plant Protection Act*.

Block 15—Action Required text is as follows:

All geranium (*Pelargonium* spp.) _____ varieties received from _____ during the dates _____ are prohibited from movement from the nursery property pending further notification by USDA–APHIS–PPQ. Any other plant material received by those same shipments that may have been exposed directly, in shipping or since being received, by shared 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. The above listed plants and all potentially infested material after further investigations are conducted will be destroyed either by incineration, steam sterilization, or an approved landfill in accordance with USDA policies. Areas housing infected material shall be disinfected according to USDA protocols.

Releasing Plants


Block 16—After all hosts have been held at a nursery with suspect geraniums and investigations have determined which other plants are potentially infected because of shared water or unsanitary nursery practices, release all other plants and make a notation in Block 16.

Documenting Actions Taken

Block 19—After plants have been destroyed and the area sanitized, indicate the methods used (for example: incineration, steam sterilization, or disposal at approved landfill) and the name of the disinfectants used. Indicate which articles were disinfected.

Disposal Memorandum

Submit the Disposal Memorandum (Figure B-7) on USDA letterhead if approved landfill operators require documentation.



Memorandum For Record

March 28, 2003

Based on a review of available data, consultation with the U.S. Environmental Protection Agency (EPA) Office of Solid Waste Management and the APHIS Safety, Health, Environmental and Security Branch, we have determined that *Ralstonia solanacearum* race 3 biovar 2 does not present a risk to human health or the environment, with the exception of certain agricultural crops. We have based our conclusion on the following findings:

- ◆ *Ralstonia solanacearum* race 3 biovar 2 is not a human or animal pathogen
- ◆ EPA reports that *Ralstonia solanacearum* race 3 biovar 2 is not considered a hazardous or medical waste
- ◆ EPA reports that it can be disposed of in permitted solid waste
- ◆ It poses no threat to ground water
- ◆ There is a potential risk of infection for certain agricultural crops, especially potatoes but also tomatoes and peppers and other solanaceous plants
- ◆ It can be established in the environment through contamination of natural waterways, soil, and host plants

In order to prevent the release of *Ralstonia solanacearum* race 3 biovar 2 to the environment and to protect at-risk crops, APHIS requires the following mitigations in its *Ralstonia* eradication action plan:

1. Waste (whole plants, plant material, soil and equipment) shall be:
 - ◆ double-bagged in securely sealed, leak-proof plastic bags
 - ◆ disposed of in a state, local or tribally permitted solid waste landfill
 - ◆ covered to a depth of two feet with soil at landfill
2. A federal or state officials shall witness disposal at the landfill to ensure proper handling.

Copies of the action plan are available at the APHIS Web site at: <http://www.aphis.usda.gov/ppq/ep/ralstonia/index.html>

FIGURE B-7 Example of Disposal Memorandum

Appendix B

PPQ Form 526 Permit to Move Live Plant Plants or Noxious Weeds

PPQ Form 526 Permit to Move Live Plant Plants or Noxious Weeds

According to the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information is 0579-0054. The time required to complete this information collection is estimated to average 0.17 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

FORM APPROVED
OMB NO. 0579-0054

No permit can be issued to move live plant pests or noxious weeds until an application is received (7 CFR 330 (live plant pests) or 7 CFR 360 (noxious weeds)).

U.S. DEPARTMENT OF AGRICULTURE ANIMAL AND PLANT HEALTH INSPECTION SERVICE PLANT PROTECTION AND QUARANTINE PERMITS AND RISK ASSESSMENT, UNIT 133 RIVERDALE, MARYLAND 20737		SECTION A - TO BE COMPLETED BY THE APPLICANT					
APPLICATION FOR PERMIT TO MOVE LIVE PLANT PESTS OR NOXIOUS WEEDS		1. NAME, TITLE, AND ADDRESS (Include Zip Code)					
3. TYPE OF PEST TO BE MOVED * <input type="checkbox"/> Pathogens <input type="checkbox"/> Arthropods <input type="checkbox"/> Noxious Weeds <input type="checkbox"/> Other (Specify) _____ <small>This permit does not authorize the introduction, importation, interstate movement, or release into the environment of any genetically engineered organisms or products.</small>		2. TELEPHONE NO. ()					
A. SCIENTIFIC NAMES OF PESTS TO BE MOVED	B. CLASSIFICATION (Orders, Families, Races, or Strains)	C. LIFE STAGES, IF APPLICABLE	D. NO. OF SPECIMENS OR UNITS	E. SHIPPED FROM (Country or State)	F. ARE PESTS ESTABLISHED IN U.S.?	G. MAJOR HOST(S) OF THE PEST	
4.							
5.							
6.							
7. WHAT HOST MATERIAL OR SUBSTITUTES WILL ACCOMPANY WHICH PESTS (Indicate by line number)							
8. DESTINATION		9. PORT OF ARRIVAL		10. APPROXIMATE DATE OF ARRIVAL OR INTERSTATE MOVEMENT			
11. NO. OF SHIPMENTS	12. SUPPLIER		13. METHOD OF SHIPMENT <input type="checkbox"/> Air Mail <input type="checkbox"/> Air Freight <input type="checkbox"/> Baggage <input type="checkbox"/> Auto				
14. INTENDED USE (Be specific, attach outline of intended research)							
15. METHODS TO BE USED TO PREVENT PLANT PEST ESCAPE				16. METHOD OF FINAL DISPOSITION			
17. <small>Applicant must be a resident of the U.S.A. I/We agree to comply with the safeguards printed on the reverse of this form, and understand that a permit may be subject to other conditions specified in Section B and C.</small>					SIGNATURE OF APPLICANT (Must be person named in item 1)		
						18. DATE	
WARNING: Any alteration, forgery, or unauthorized use of this document is subject to civil penalties of up to \$250,000 (7 U.S.C. s7734(b)) or punishable by a fine of not more than \$10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. s1001).							
SECTION B - TO BE COMPLETED BY STATE OFFICIAL							
19. RECOMMENDATION <input type="checkbox"/> Concur (Approve) <input type="checkbox"/> Comments (Disapprove) <input type="checkbox"/> (Accept USDA Decision)		20. CONDITIONS RECOMMENDED					
21. SIGNATURE		22. TITLE		23. STATE	24. DATE		
SECTION C - TO BE COMPLETED BY FEDERAL OFFICIAL							
						25. PERMIT NO.	
PERMIT (Permit not valid unless signed by an authorized official of the Animal and Plant Health Inspection Service) Under authority of the Plant Protection Act of 2000, permission is hereby granted to the applicant named above to move the pests described, except as deleted, subject to the conditions stated on, or attached to this application. (See standard conditions on reverse side.)							
* For exotic plant pathogens, attach a completed PPQ Form 526-1.							
24. SIGNATURE OF PLANT PROTECTION AND QUARANTINE OFFICIAL		25. DATE	26. LABELS ISSUED	27. VALID UNTIL	28. PEST CATEGORY		
PPQ FORM 526 (OCT 2001) Previous editions are obsolete.							

FIGURE B-8 Example of PPQ Form 526 [side 1]

STANDARD SAFEGUARDS OF PERMIT

1. All pests must be shipped in sturdy, escape-proof containers.
2. Upon receipt of pests, all packing material media, substrate, soil and shipping containers shall be sterilized or destroyed immediately after removing pests.
3. Pests shall be kept only within the laboratory or designated area at the permittee's address.
4. No living pests kept under this permit shall be removed from confined area except by prior approval from State and Federal regulatory officials.
5. Without prior notice and during reasonable hours, authorized PPQ and State regulatory officials shall be allowed to inspect the conditions under which the pests are kept.
6. All pests kept under this permit shall be destroyed at the completion of the intended use, and not later than the expiration date, unless an extension is granted by this issuing office.
7. All necessary precautions must be taken to prevent escape of pests. In the event of an escape, notify this office.

FIGURE B-9 Example of PPQ Form 526 [side 2]



Appendix C

Diagnostic Validations and Isolation Methods

Contents

Introduction	page C-1
Non-plate, Rapid, Immunoassay Detection	page C-2
Immunoplate Assays (ELISA)	page C-3
Comparison of Non-plate Immunoassay and Plate Immunoassay	page C-5
Validation of Central Science Laboratory TaqMan® Assay	page C-6
Validation of Sample and DNA Preparation Methods	page C-6
Preparation of Media for Isolation	page C-7
Isolating Bacteria from Tissue Samples	page C-9

Introduction

Use *Appendix A* to learn more about laboratory tests used to detect the species *Ralstonia solanacearum*.

Validation studies were performed on various diagnostic kits for making determinations of *Ralstonia solanacearum* from geranium samples. Additional information on isolating the bacterium was also provided, as well as specific media recipes.

Information in this *Appendix* was provided by the USDA–APHIS–PPQ–CPHST–National Plant Germplasm and Biotechnology Laboratory (NPGBL), Beltsville, Maryland. [See Resources on page A-1](#) for information on contacting manufacturers of the diagnostic kits.

Important

All *Ralstonia solanacearum* diagnostics mentioned below determine species, not biovar or race. Buffers vary by manufacturer and often produce false positive reactions when used with another manufacturer's kit. Kit instructions must be followed while performing the tests. For best results, plant samples need to be symptomatic, and include the lower main stem area, or material near base of the first lateral shoot. Negative results do not necessarily indicate *Ralstonia solanacearum* is absent in the plant.

Status of Commercial Diagnostic Kits

Personnel at NPGBL contacted five companies to learn the status of their commercial *Ralstonia solanacearum* diagnostic kits: Adgen Ltd.¹, Agdia, Inc., Bioreba AG², Central Science Laboratory (CSL), and Loewe Biochemica

¹ Recently acquired by Neogen Europe Limited.

² STA Laboratories, Inc., in the United States.

GmbH. The companies reported **no** protocol or device changes, with the exception of Agdia, Inc. Agdia, Inc. had two new kits ready for evaluation: ImmunoStrip® and a rapid ELISA test. [See Resources on page A-1](#) for contact information for all manufacturers.

Non-plate, Rapid, Immunoassay Detection

Rapid detection devices such as strip or stick tests and lateral flow devices can be used to detect *Ralstonia solanacearum* in plant sap or from isolated bacteria, according to the manufacturers' recommendations. Testing bacterial cultures, however, would require plating on selective media, adding a few days to the testing procedure.

We tested the new kit, Agdia, Inc.'s, ImmunoStrip® alongside the CSL Pocket Diagnostic™ and Adgen's Spot Check LF™ lateral flow devices which were both unchanged from last year ([Table C-1](#)). The Agdia and CSL devices consistently detected *Ralstonia solanacearum* at 10⁷ cfu/ml and, in a few tests, at 10⁶ cfu/ml. The Adgen Spot Check LF™ performed very well in sensitivity testing, detecting as low as 10⁴ cfu/ml.

Adgen Spot Check LF™

The Adgen Spot Check LF™ for *Ralstonia solanacearum* produced lighter band intensities for the test and control bands when testing infected plant tissue, making the test more difficult to visually score compared to the Agdia and CSL kits. The Adgen kit instructs the user to sample leaf material, whereas CSL and Agdia instruct the user to sample from stems. In testing leaf and stem material, the Adgen Spot Check LF™ produced a false negative on infected stem material which tested positive with both the Agdia and CSL kits.

Agdia ImmunoStrip®

The Agdia device consistently detected *Ralstonia solanacearum* at 10⁷ cfu/ml and, in a few tests, at 10⁶ cfu/ml. When testing infected plant material, the new Agdia ImmunoStrip® was easier to visually score. The darker band color made low concentrations of *Ralstonia solanacearum* easier to see than either the CSL or Adgen devices. The Agdia ImmunoStrip® test requires 30 minutes to complete whereas both the CSL and Adgen lateral flow kits require about 3 minutes. This kit instructs the user to sample from stems.

CSL Pocket Diagnostic™

The CSL Pocket Diagnostic™ consistently detected *Ralstonia solanacearum* at 10⁷ cfu/ml and, in a few tests, at 10⁶ cfu/ml. This kit instructs the user to sample from stems.

TABLE C-1 Comparison of Non-plate Rapid Immunoassay Devices

	Company		
	Adgen Ltd.	Agdia, Inc.	CSL ¹
Part number	Spot Check LF™ Lateral Flow Device 14-850 (10 tests) 14-851 (100 tests)	ImmunoStrip® STX 33900 SK 33900 kit	Pocket Diagnostic™ for RS Lateral Flow Device
Incubation²	2-3	30	1-3
Comments	Macerate by shaking bottle containing ball bearings. Sample information can be written on cassette. Extra fee for brokerage.	Must keep strip upright and slightly immersed for 30 minutes. Tissue samples are ground in special bags. Includes information on testing bacteria.	Macerate by shaking bottle containing ball bearings. Sample information can be written on cassette.

1 Central Science Laboratory

2 Minutes

Immunoplate Assays (ELISA)

We compared Enzyme-Linked Immunosorbent Assay (ELISA) kits for *Ralstonia solanacearum* from four companies: Adgen Ltd., Agdia, Inc., Bioreba AG, and Loewe Biochemica GmbH. Of these, only the kit from Agdia, Inc. was modified in 2003 with new components. Total test time varies by manufacturer, ranging from 2 ½ hrs to 27+ hrs. In addition to testing plant samples, both Agdia, Inc., and Loewe include instruction on testing bacterial samples.

All four kits detected *Ralstonia solanacearum*; however, Agdia's kit performed the best by detecting more positives within a 30 minute period, within the 2 ½ hours for the entire test.

Agdia PathoScreen ELISA

Agdia's PathoScreen ELISA kit was very easy to use. The plate comes as pre-coated strips, saving time, labor, and avoiding waste. No dilutions were needed for the liquid components, whereas other kits required 1 to 2 dilutions per step, prior to dispensing. The Agdia ELISA required no incubator; the entire test is performed at room temperature. Turn-around time is very short, ≤ 2 ½ hrs total from start to final read, significantly less than the other kits.

Appendix C

Immunoplate Assays (ELISA)

Samples can be processed quickly and efficiently without a loss of assay sensitivity. The positive control reaction is strong, i.e.: OD = 2.30 at 650nm with 15 minutes incubation at room temperature.

Comparison of Non-plate Immunoassay and Plate Immunoassay

Both the rapid, non-plate immunodetection systems (strips, lateral flow devices) and plate immunodetection systems (ELISA) have similar reported sensitivity (Table C-2). For example, Agdia's ImmunoStrip® and ELISA tests both report a detection (low) limit of 10^5 cfu/ml; however, in our testing the low limit was 10^6 cfu/ml.

An advantage of using plate ELISA is the ability to process large numbers of samples in a short period of time. It is economical and allows for replicated testing. ELISA can be automated if desired.

In comparison, detection using rapid, non-plate, immunoassays is useful if you have fewer samples to test. It is direct and ready-to-use. It requires significantly less time (minutes versus hours or days). No buffer dilutions are required. Like ELISA, it does **not** require a spectrophotometer reader. It can be performed on-site.

TABLE C-2 Comparison of Plate Immunodetection Assays

	Company			
	Adgen Ltd.	Agdia, Inc.	Bioreba AG	Loewe Biochemica GmbH
Part number	Identikit 1131-09 (pre-coated)	PSP 33900 (pre-coated)	170575 (480) 170572 (960)	07056
Incubation¹	> 480 ²	≤ 150	≥ 1620	≥ 1560
Comments	Antibody pre-coated microplates with removable strips. Extra antibody binding step required for TAS. Several buffer dilutions required; extra fee for brokerage. Incubator required. Lacks instruction for testing bacterial samples.	Antibody pre-coated microplates with removable strips; no buffer dilutions; short incubations. All incubations at room temperature. Very rapid. Includes instruction for testing bacterial samples.	Uncoated; antibody coating step required; 10X and 5X buffers need diluting; overnight incubation step required in step 2. Incubator required. Lacks instruction for testing bacterial samples.	Uncoated; antibody coating step required; buffers must be made by user. Overnight incubation step required. Soaking is required in 3rd & 4th wash in each wash step. Incubator required. Includes instruction for testing bacterial samples.

1 Minutes

2 Triple Antibody Sandwich [TAS] protocol used with blue substrate

Validation of Central Science Laboratory TaqMan® Assay

PCR validation was performed using the PCR primer and probe sequences, and protocols provided by Dr. John Elphinstone, Central Science Laboratory, UK. The real-time test is based on the *Ralstonia solanacearum* TaqMan® assay (CSL SOP No. PLH7/32) for use in an ABI 9700 or 2400 thermal cycler. The test was converted with minor modifications to two other mobile, real-time PCR platforms: the Idaho Technologies R.A.P.I.D. field-hardened thermal cycler, and the Cepheid Smart Cycler. The CSL real-time PCR protocol for *Ralstonia solanacearum* detection was successfully transferred and validated in each of the two mobile PCR machines. (Protocol standard operating procedures [SOPs] can be provided separately for the sake of brevity.)

While both tests provide the same level of detection, the Cepheid Smart Cycler was easier to use overall. It required less specialized knowledge and incorporated OmniMix HS (Cepheid part number 900-0078), a ready-to-use, lyophilized bead for PCR that contains all reagents. After the bead is hydrated, primer, probe, and DNA are added together and the PCR reaction is ready to run on the cycler. The bead reduces preparation time and decreases the possibility of contamination of test samples, thereby reducing false positive results. Use of the CSL real-time PCR for *Ralstonia solanacearum* is recommended for PCR testing because of the ease of use, sensitivity, consistency, and reduced possibility of contamination. Furthermore use of the OmniMix beads make PCR tests easier for non-molecular clinicians.

The conventional *Ralstonia solanacearum* PCR test (Lee *et al.*, 2001) used in our lab last season remains pre-publication and is not available for wide distribution.

Validation of Sample and DNA Preparation Methods

In combination with the CSL Real-time PCR test, four extraction methods were evaluated:

- ◆ Modified hot alkaline DNA extraction method, as used for *Clavibacter michiganensis* subsp. *sepedonicus* (Lee *et al.*, 2001)
- ◆ Gene Releaser (BioVentures, Inc.)
- ◆ Qiagen DNeasy Plant Mini Kit (cat. no. 69104 and 69106)
- ◆ Qiagen QIAmp DNA Mini Kit (cat. no. 51304 and 51306) as in Poussier *et al.* (2002)

No PCR products were observed using the modified hot alkaline method. Products were observed using the Gene Releaser protocol, but manipulation of the tubes made the preparation difficult and possibly prone to

contamination in unskilled hands. The two Qiagen kits were the most consistent of the four methods tested, and either is recommended for use with the PCR tests evaluated.

Preparation of Media for Isolation

Several media have been described for isolation of *Ralstonia solanacearum*. Plant scientists prefer the following medias.

Selective Medium for *Ralstonia solanacearum*³

Step 1 Prepare 1% TZC stock solution.

How to Prepare 1% TZC Stock Solution (2, 3, 5 Triphenyl Tetrazolium Chloride¹)

1. Add 1 gram TZC to 100 ml dH₂O.
2. Filter solution.
3. Store @ 4C or -20C in dark bottle.

1 Also known as TZC, TTC, TPTZ, or Tetrazolium Red.

Step 2 Prepare basal medium.

How to Prepare Basal Medium

1. Per liter of dH₂O add each of the following:
 - ◆ 5g Glucose, sucrose or dextrose
 - ◆ 10g Peptone
 - ◆ 1g Casamino acids
 - ◆ 12-18g Agar
2. Adjust pH of to 7.0 with KOH if necessary.
3. Autoclave 20 min. @ 121 C.
4. Cool to 55 C.

Step 3 Add 5 ml of 1% TZC stock solution to basal medium.

Step 4 Pour ~20 ml per plate.

Step 5 Allow to set up overnight

Step 6 Incubate at 28 to 30C for 36-48 hours.

Step 7 Identify colonies.

Typical *Ralstonia solanacearum* colonies on this selective medium produce irregular-shaped, fluidal, mucoid, non-symmetrical colonies. Colonies are typically white with pink, light pink, or pale red centers.

Semi-selective Medium for *Ralstonia solanacearum*⁴

This medium was reported in Schaad *et al.* (2001) and French *et al.* (1995).

3 Also known as TZC medium, Kelman's medium, or TTC medium.

Step 1 Filter 10 ml Polymyxin B sulfate solution (8100 units/mg).

Step 2 Filter 5 ml 1% 2,3,5-Triphenyletrazolium Chloride.

Step 3 Prepare basal medium.

How to Prepare Basal Medium
1. Per liter of dH ₂ O add each of the following: <ul style="list-style-type: none">◆ 1g Casamino acids◆ 5 ml Glycerol or glucose◆ 10g Bacto Peptone◆ 15-17 g Bacto agar
2. Autoclave 20 min. @ 121 C.
3. Cool to 50 C.

Step 4 Add the following stock solutions dissolved in 70% ethanol:

- ◆ 0.5ml 1% Crystal violet
- ◆ 2.5ml 1% Bacitracin (73000units/g)
- ◆ 0.5ml 1% Chloramphenicol (water soluble)
- ◆ 0.5ml 0.1% Penicillin G sodium salt

Step 5 Optional: Add 2.5ml 1% Cyclohexamide to inhibit fungi.

Step 6 Add the following filtered solutions (dH₂O):

- ◆ 10ml Polymyxin B Sulfate Solution (8100units/mg)
- ◆ 5ml 1% 2,3,5-Triphenyltetrazolium Chloride

Step 7 Pour ~20ml/plate.

Step 8 Allow to dry overnight.

Step 9 Store at 4 C.

Step 10 Incubate at 35 C for 48 hours.

Step 11 Identify colonies.

Typical *Ralstonia solanacearum* colonies on this semi-selective medium produce creamy colonies. Red pigmented colonies are not *Ralstonia solanacearum*.

Isolating Bacteria from Tissue Samples

Several methods are available for isolating bacteria from plants. Three are described below. Isolation of *Ralstonia solanacearum* is best accomplished using symptomatic (i.e. wilting) plants. It is preferable to use vascular (xylem) tissue, especially from the lower stem area. Isolation from non-symptomatic plants is more difficult due to lower viable, bacterial populations.

4 Also known as mSMSA.

Method A

Method A was supplied by C. Allen (*pers. comm.*).

Step 1 Cut a small tissue section (~0.5 cm square), ideally from stem tissue. Surface sterilize a section of stem, if desired.

Step 2 Macerate in ≤ 1 ml sterile water.

Step 3 Allow to sit ≤ 15 minutes.

Step 4 Streak several plates directly, using loopfuls of the suspension. You may want to use more than one tissue sample and streak plates from each.

Method B

Method B was supplied by K. Rane (*pers. comm.*).

Squeeze out a drop of creamy ooze from a symptomatic petiole or stem and streak it out.

Method C

Method C was supplied by S. H. Kim (*pers. comm.*).

Step 1 Locate symptomatic leaves.

Step 2 Observe bacterial streaming from leaf, or oozing from a petiole or stem.

Step 3 Place a few petiole or stem segments (~3mm), or finely sliced leaf-blades, in a test tube containing 10ml, 0.1 M phosphate buffer (pH 7.0).

Step 4 Wait 15 minutes and streak plates.

Appendix C

Isolating Bacteria from Tissue Samples



Appendix D

Diagnostic Laboratories

Contents

- Introduction [page D-1](#)
Determination of Race and Biovar [page D-1](#)
Determination of Genus and Species [page D-2](#)

Introduction

Use *Appendix B* to identify diagnostic laboratories.

Important

Laboratories must notify their State Plant Regulatory Official (SPRO) (<http://www.aphis.usda.gov/npb/npbmemb.html#Membership%20Directory>) and State Plant Health Director (SPHD) (<http://www.ceris.purdue.edu/napis/names/sphdXstate.html>) when sending samples for race and biovar determination.

Important

States sending samples for *Ralstonia solanacearum* determination to other states must assure that the laboratory receiving samples has a Plant Pest Permit (PPQ Form 526) to receive those samples from out-of-state. Laboratories receiving samples from within their own states do not need permits. For more information, see: <http://www.aphis.usda.gov/ppq/permits/plantpest/index.html>.

Determination of Race and Biovar

Determination of race and biovar requires additional registrations for select agents under the *Agricultural Bioterrorism Protection Act of 2002*. Currently, the only laboratory with proper registrations to make these determinations for *Ralstonia solanacearum* race 3 biovar 2 is the USDA–APHIS–PPQ–National Plant Germplasm and Biotechnology Laboratory in Beltsville, MD. [See Identification on page 4-1](#) for information on packaging and documentation.

Address

USDA–APHIS–PPQ–National Plant Germplasm and Biotechnology
Laboratory
BARC-East, Bldg. 580
Powder Mill Road
Beltsville, MD 20705
T: 301-504-7100
F: 301-504-8539

Appendix D

Determination of Genus and Species

Determination of Genus and Species

The laboratories included in this section are limited to determination of genus and species.

State and Cooperating University Diagnostic Laboratories

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
Alabama	Dr. Jackie Mullen Extension Plant Pathologist Plant Diagnostic Lab Auburn University Auburn, AL 36849 T: 334-844-5508 jmullen@acesag.auburn.edu
Arizona	GO TO California .
Arkansas	Dr. Stephan Vann University of Arkansas Cooperative Extension Service, Plant Diagnostic Clinic 2001 Hwy 70 East Lonoke, AR 72086 T: 501-676-3124
California	Dr. Dan Opgenorth California Department of Food and Agriculture Plant Pest Diagnostics Laboratory 3294 Meadowview Road Sacramento, CA 95832-1448 T: 916-262-1100 F: 916-262-1190 dopgenor@cdfa.ca.gov
Colorado	Tamela Blunt Center for Crop Biosecurity Colorado State University Fort Collins, CO 80526 T: 970-491-6950
Connecticut	Dr. Sharon Douglas Connecticut Agricultural Experiment Station P. O. Box 1106 New Haven, CT 06504 T: 203-974-8499 sharon.douglas@po.state.ct.us
Delaware	Dr. Bob Mulrooney Department of Plant and Soil Sciences Room 152 Townsend Hall University of Delaware Newark, DE 19717 T: 302-831-4865 F: 302-831-0605 bobmul@udel.edu

Appendix D

Determination of Genus and Species

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
Florida	Dr. Tim Schubert FDACS-DPI P. O. Box 147100 Gainesville, FL 37614-7100 T: 352-372-3505 x 143 schubet@doacs.state.fl.us
Georgia	Dr. Jean Woodward University of Georgia Plant Pathology Department 2106 Miller Plant Science Bldg. Athens, GA 30602-7274 T: 706-542-9146 woodwar@uga.edu
Idaho	Liz Vavricka Plant Industry Lab Idaho State Department of Agriculture P. O. Box 790 Boise, ID 83701 T: 208-332-8640
Indiana	Agdia, Inc. Mike Tiffany 30380 County Road 6 Elkhart, IN 46514 T: 574-264-2014 info@agdia.com Dr. Karen Rane Plant Pest Diagnostic Lab Purdue University 915 W. State Street West Lafayette, IN 47907-2054 T: 765-494-5821 rane@purdue.edu
Iowa	Paula Flynn ISU Plant Disease Clinic 323 Bessey Hall Iowa State University Ames, Iowa 50011 T: 515-294-0581
Kansas	Dr. Ned Tisserat Plant Pathology Department Kansas State University Manhattan, Kansas T: 785-532-1387
Kentucky	Dr. John Hartman S-305 Ag Sci Bldg N University of Kentucky Lexington, KY 40546 T: 859-257-5779 jhartman@ca.uky.edu

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
Louisiana	<p>Dr. Clayton A. Hollier Department of Plant Pathology LSU AgCenter Baton Rouge, LA T: 225-578-2186</p> <p>Dr. Chris Clark Department of Plant Pathology LSU AgCenter T: 225-578-1381</p> <p>Dr. Gordon Holcomb Department of Plant Pathology LSU AgCenter T: 225-578-1386</p> <p>Craig Roussel or Tad Hardy Louisiana Dept. Agriculture and Forestry Baton Rouge, La. T: 225-952-8100</p>
Maine	<p>Bruce Watt University of Maine Pest Management Office 491 College Avenue Orono, ME 04473-1295 T: 207-581-3880 bwatt@umext.maine.edu</p>
Maryland	<p>Jennifer Dominiak Maryland Department of Agriculture 50 Harry S. Truman Pkwy. Room 345 Annapolis, Maryland 21401 T: 410-841-5920 dominijd@mda.state.md.us</p>
Massachusetts	<p>Dr. Robert L. Wick Department of Microbiology Morrill Science Center N203 University of Massachusetts Amherst, MA 01003 T: 413-545-1045 rwick@pltpath.umass.edu</p>
Michigan	<p>Dr. Richard Kaitany Geagley Laboratory Michigan Department of Agriculture 1615 S. Harrison Rd. East Lansing, MI 48823 T: 517-337-5091 kaitanyr@msu.edu</p>

Appendix D

Determination of Genus and Species

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
Minnesota	Gary Horvath Minnesota Department of Agriculture Laboratory Services Division 90 W. Plato Blvd. St. Paul, MN 55107-2094 T: 651-215-9063 F: 651-297-8787 gary.horvath@state.mn.us
Mississippi	Dr. Alan Henn, Associate Extension Professor Entomology and Plant Pathology Mail Stop 9655 Mississippi State, MS 39762 T: 662-325-4535 ahenn@ext.msstate.edu
Missouri	Dave Johnson, Plant Pathologist Missouri Department of Agriculture Plant Industries Division 115 Constitution Drive P.O. Box 630 Jefferson City, Missouri 65102 T: 573-751-8319 F: 573-526-7777
Nebraska	Jennifer Chaky Plant Pathology Department 448 Plant Science Building University of Nebraska, Lincoln Lincoln NE 68583 T: 402-472-8725
Nevada	Shouhua Wang Plant Pathologist Nevada Department of Agriculture Plant Division 350 Capitol Hill Avenue Reno, NV 89502 T: 775-688-1182x246 F: 775-688-1178 shwang@govmail.state.nv.us
New Hampshire	Cheryl Smith Extension Education, Adjunct Professor University of New Hampshire Spaulding Life Sciences Bldg., Room 242 Durham, NH 03824-3544 T: 603-862-3841 cheryl.smith@unh.edu

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
New Jersey	Dr. Glenn Freeman New Jersey Department of Agriculture Plant Laboratory Services P. O. Box 330 Trenton, NJ 08625-0330 T: 609-292-5484 glenn.freeman@ag.state.nj.us
New Mexico	NMDA Bureau Chief Entomology and Nursery Industries, MSC 3 BA Las Cruces, NM 88003-0005 T: 505-646-3207
New York	Margery Daughtrey Cornell University Long Island Horticultural Research & Extension Center 3059 Sound Ave. Riverhead, NY 11901 T: 631-727-3595 mld9@cornell.edu Karen Snover-Clift Cornell University Department of Plant Pathology 334 Plant Science Bldg. Ithaca, NY 14853 T: 607-255-7850 kls13@cornell.edu
North Carolina	Tom Creswell NCSU Plant Disease and Insect Clinic William Hall, Room 1104 100 Derieux Hall Campus Box 7211 Raleigh, NC 27695-7211 T: 919-515-3619 F: 919-515-7716 tom_creswell@ncsu.edu
North Dakota	GO TO Agdia, Inc.
Ohio	Nancy J. Taylor Extension Associate & Coordinator Plant Pest Diagnostic Clinic 110 Kottman Hall 2021 Coffey Road Columbus, Ohio 43210-1087 T: 614-292-5006 F: 614-292-7162 taylor.8@osu.edu

Appendix D

Determination of Genus and Species

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
Oklahoma	Dr. Brian Olson Oklahoma State University Entomology and Plant Pathology 119 NRC Stillwater, OK 74078-3033 T: 405-744-7126 olsonb@okstate.edu
Oregon	Acting Supervisor Plant Health Lab, Plant Health Section Oregon Dept. of Agriculture 635 Capitol St. NE Salem, OR 97301-2532 T: 503-986-4636 or 503-986-4661 F: 503-986-4786 jgriesba@oda.state.or.us
Pennsylvania	Dr. Seong Kim Pennsylvania Dept. of Agriculture Bureau of Plant Industry Plant Pathology Lab 2301 North Cameron Street Harrisburg, PA 17110-9408 T: 717-772.5221 F: 717-705.6518 skim@state.pa.us
Rhode Island	GO TO Agdia, Inc.
South Carolina	GO TO Agdia, Inc. Meg Williamson Clemson University Plant Problem Clinic 171 Old Cherry Road Clemson, SC 29634-0114 T: 864-656-3125 ppclnc@clemson.edu
South Dakota	South Dakota State University Brookings, SD 57006 T: 605-688-5157
Tennessee	GO TO Agdia, Inc.
Texas	Dr. Larry Barnes Texas Plant Disease Diagnostic Laboratory 1500 Research Parkway, Suite A-130 College Station, Texas 77845 T: 979-845-8032 barnes@tamu.edu

TABLE D-1 State and Cooperating University Diagnostic Laboratories

If in this state:	Then contact this laboratory:
Utah	Scott C. Ockey Plant Disease Diagnostician, Senior Research Associate Utah State University Old Main Hill Logan, UT 84322-5305 T: 435-797-2435 F: 435-797-1575 scotto@ext.usu.edu
Virginia	Washington Building, Room 703 1100 Bank Street Richmond, VA 23219 T: 804-786-3515 F: 804-371-7793 gokeefe@vdacs.state.va.us
Vermont	Scott Pfister Vermont State Plant Pathologist 103 S. Main Street Waterbury, VT 05671-0101 T: 802-828-3481 spfister@agr.state.vt.us
Washington	Jennifer Falacy Plant Pathology Project Coordinator Plant Pathology Laboratory 3939 Cleveland Avenue S.E. Olympia, WA 978501-4079 F: 360-586-5286 awagner@agr.wa.gov
West Virginia	Gary Gibson WVDA, Plant Industries Division 1900 Kanawha Blvd., East Charleston, WV 25305-0191 T: 304-558.2212 ggibson@ag.state.wv.us
Wisconsin	Annette Phibbs WDATCP, ARM, Plant Industry Division 4702 University Avenue Madison, WI 53705 T: 608-266-7132 F: 608-266-5855 anette.phibbs@datcp.state.wi.us
Wyoming	Mr. Gary Franc University of Wyoming Dept. of Plant Pathology P.O. Box 3354 Laramie, Wyoming 82071-3354 T: 307-766-2397

Plant Diagnostic Networks and Private Laboratories

Diagnostic screening can take place at a laboratory designated in the National Plant Diagnostic Network, or at Agdia, Inc. Currently, Agdia, Inc. is the only private laboratory able to perform this type of testing. Use [Table D-1 on page D-10](#) to identify the regional laboratory for your state.

TABLE D-1 : Regional Plant Diagnostic Networks (EPDN) and Private Laboratories

If in this state:	Then contact this laboratory:
Connecticut, Delaware, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, West Virginia	Northeast Plant Diagnostic Network (NEPDN) Cornell University Karen L. Snover-Clift Department of Plant Pathology 334 Plant Science Bldg. Ithaca, NY 14853
Colorado, Kansas, Montana, North Dakota, Oklahoma, South Dakota, northern Texas, Nebraska, Wyoming	Great Plains Diagnostic Network (GPDN) Kansas State University Ned Tisserat Department of Plant Pathology 4024 Throckmorton Hall Manhattan, KS 66506 T: (785) 532-1383 tissne@ksu.edu
Iowa, Michigan, Ohio, Indiana, Illinois, Minnesota, Missouri, Wisconsin	North Central Plant Diagnostic Network (NCPDN) Jan Bryne 114 CIPS Michigan State University East Lansing, MI 48824-1311 T: (517) 355-3504 byrnejm@msu.edu
Alabama, Louisiana, southern Texas, Arkansas, Mississippi, Virginia, Florida, North Carolina, Puerto Rico, Georgia, South Carolina, Kentucky, Tennessee	Southern Plant Diagnostic Network (SPDN) Richard Cullen Plant Disease Clinic UF Bldg 78 Mowry Road P.O. Box 110830 Gainesville, FL 32611-0830 T: (352) 392-1795 or 3631 (X 254)
American Samoa, Arizona, California, Hawaii, Idaho, Alaska, Nevada, New Mexico, Oregon, Guam, Utah, Washington	Western Plant Diagnostic Network (WPDN) Melodie Putnam 1089 Cordley Hall Corvallis, OR 97331-2903 T: (541) 737-3472 putnam@science.oregonstate.edu
Any state	Agdia Inc. 30380 County Road 6 Elkhart, IN 46514 T: (574) 264-2014 or (800) 62-AGDIA Web site: www.agdia.com



Appendix E

Pelargonium in the Nursery Trade

Geranium is a common name that can refer to plant species in the genus *Pelargonium* and the genus *Geranium*. The common nursery plant is one of several *Pelargonium* spp. listed below ([Table E-1](#)). The PPQ *Ralstonia* program does not regulate plants in the genus *Geranium*, which are primarily perennial landscape plants not known to be hosts of *Ralstonia solanacearum*.

TABLE E-1 Common *Pelargonium* in the Nursery Trade

Scientific Name	Common Name	Description
<i>Pelargonium x hortorum</i>	Zonal geranium or Florist's geranium	'Americana' geraniums are in this group. This genus and species makes up 70–80% of the geraniums sold in the United States each year. Vegetatively propagated and most are tetraploids.
	Seed geranium	A smaller portion of the market. These are diploids and are reproduced via seed.
<i>Pelargonium peltatum</i>	Ivy geranium	Approximately 10–20% of the market; vegetatively propagated. Typically grown in baskets (hanging above other crops). There are also seed propagated lines of this species.
<i>Pelargonium domesticum</i>	Regal or Martha Washington geranium	This species is grown mainly as a flowering potted crop through florists and upper end retail garden centers. Vegetatively propagated; less than 5–10% of the market. 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 from zonal geraniums.
Other <i>Pelargonium</i> 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.



Appendix F

Comparison of Symptoms



FIGURE F-1: Left: Early wilting caused by infection with *Ralstonia solanacearum* race 3 biovar 2 Right: Advanced wilting and abnormal leaf yellowing (chlorosis) caused by infection with *R. solanacearum* race 3 biovar 2 [Images courtesy of Wisconsin Department of Agriculture, Trade and Consumer Protection]



FIGURE F-2: Left: Abnormal yellowing caused by infection with *Ralstonia solanacearum* race 3 biovar 2 [Courtesy of the Plant Disease Diagnostics Clinic, University of Wisconsin-Madison, Cooperative Extension Service] Right: Close-up of wilting and necrosis caused by infection with *R. solanacearum* race 3 biovar 2 [Courtesy of Margery Daughtrey, Cornell University]



FIGURE F-3: Left: Wilting and mortality of geraniums caused by infection with *Ralstonia solanacearum* race 3 biovar 2. Right: Mortality (top) and early wilt (right) caused by infection with *R. solanacearum* race 3 biovar 2

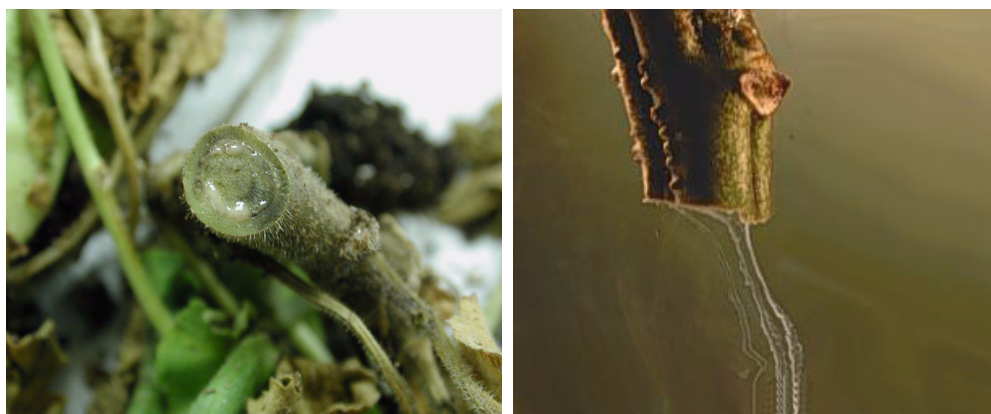


FIGURE F-4: Left: Cross-section of geranium stem with bacterial ooze caused by infection with *Ralstonia solanacearum* race 3 biovar 2. Right: Bacterial streaming from an infected stem immersed in water caused by infection with *R. solanacearum* race 3 biovar 2



FIGURE F-5: Left: Wilting caused by bacterial blight (*Xanthomonas pelargonii*) is indistinguishable from *Ralstonia solanacearum* wilting Right: Bacterial blight (*Xanthomonas pelargonii*) also causes characteristic spotting of leaf tissue [Images courtesy of M. Daughtrey, Cornell University]



FIGURE F-6: Left: Early wilting symptoms on pepper caused by infection with *Ralstonia solanacearum* Right: Advanced wilting and necrosis of potato caused by infection with *Ralstonia solanacearum* [Images courtesy of CABI, 2006]



FIGURE F-7: Left: Early tuber symptoms caused by *Ralstonia solanacearum* (Easily confused with ring rot caused by *Clavibacter michiganense* pv. *sepidonicus*) Right: Advanced tuber symptoms caused by *R. solanacearum* [Images courtesy CABI, 2006]

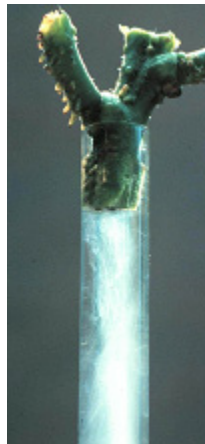


FIGURE F-8: Left: Bacterial streaming of infected stem immersed in water caused by infection with *Ralstonia solanacearum* (RS) [Image courtesy of UGA] Right: Bacterial ooze on tubers infected with *R. solanacearum* [Image courtesy of EMBRAPA]



FIGURE F-9: Left and Right: Advanced ring rot of tuber caused by *Clavibacter michiganense* pv. *sepidonicus* [Left image courtesy of CABI, 2006; right image courtesy of CIP]

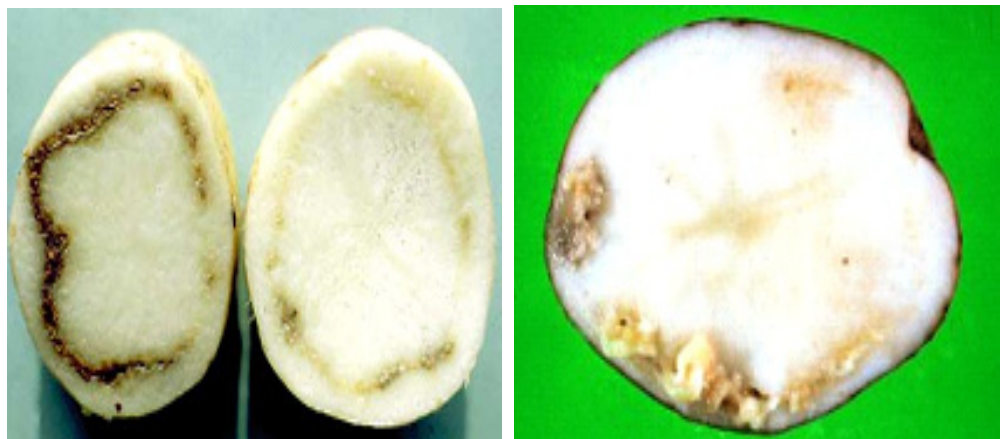


FIGURE F-10: Left and Right: Intermediate ring rot of tuber caused by *Clavibacter michiganense* pv *sepidonicus* [Images courtesy of Canadian Government of British Columbia and Manitoba]



Appendix G

Disinfectants

TABLE G-1 : Disinfectants Registered Against *Pseudomonas* spp. (*Ralstonia solanacearum* = *Pseudomonas solanacearum*)

Trade Name	EPA Reg. No.	Active Ingredients	Use Sites	Pertinent Bacteria
Green-Shield®	499-368	n-Alkyl dimethyl benzyl ammonium chloride; n-Alkyl dimethyl ethylbenzyl ammonium chloride; same as Physan 20	Work area, benches, pots, flats, flower buckets, cutting tools, greenhouse glass, walkways, evaporative coolers, decorative pools, fountains, and water displays	<i>Pseudomonas</i> spp.(unlabelled)
Physan 20	55364-5	Same as Green-Shield	Greenhouses, hard surfaces, lawn and turf grass, seedlings, cut flowers, decorative fountains, pools, birdbaths, and plants	<i>Pseudomonas</i> spp.
Zero Tol ^{ZT} ®	70299-1	Hydrogen dioxide	Greenhouse structures, benches, pots, watering systems, evaporative coolers, storage rooms, ventilation equipment, floors and other equipment	<i>Pseudomonas</i> spp.
Consan Triple Action 20	58044-3	n-Alkyl dimethyl benzyl ammonium chloride; n-Alkyl dimethyl ethylbenzyl ammonium chloride; same as Triathlon	Greenhouse hard surfaces, work areas, benches, flower pots, buckets, flats, cutting tools, walkways, garden bird baths, and evaporative coolers	
Triathlon™	58044-3-59807	Same as Consan Triple Action 20	Greenhouse hard surfaces, work areas, benches, cutting tools, walkways, garden bird baths, and evaporative coolers	
AE-90	47371-89	n-Alkyl dimethyl benzyl ammonium chloride; n-Alkyl dimethyl ethylbenzyl ammonium chloride	Non-porous, inanimate surfaces such as floors, walls, metal surfaces, stainless steel surfaces, plastic surfaces, knobs, handles, and railings	<i>Pseudomonas aeruginosa</i>

TABLE G-1 : Disinfectants Registered Against *Pseudomonas* spp. (*Ralstonia solanacearum* = *Pseudomonas solanacearum*)

Lonza S-18	6836-77	Octyl decyl dimethyl ammonium chloride; Dioctyl dimethyl ammonium chloride; didecyl dimethyl ammonium chloride; n-Alkyl dimethyl benzyl ammonium chloride	Farm, poultry, swine, and mushroom premise sanitation; veterinary practice, animal care, animal laboratory disinfection	<i>Pseudomonas aeruginosa</i> and <i>Pseudomonas cepacia</i>
MAQUAT 128-MT	1032 4-112	Octyl decyl dimethyl ammonium chloride; Dioctyl dimethyl ammonium chloride; didecyl dimethyl ammonium chloride; n-Alkyl dimethyl benzyl ammonium chloride	Outer clothing, field harvesting equipment, walls/floors of coolers, flower buckets, and greenhouse packing areas	<i>Pseudomonas aeruginosa</i>
MAQUAT 64 MN	1032 4-113	Octyl decyl dimethyl ammonium chloride; Dioctyl dimethyl ammonium chloride; didecyl dimethyl ammonium chloride; n-Alkyl dimethyl benzyl ammonium chloride	Florist shops, wholesale florists, shippers, greenhouse packing areas, flower buckets, floors/walls of coolers, design and packaging benches, and counter tops	<i>Pseudomonas aeruginosa</i>
MAQUAT 615-HD	1032 4-72	Octyl decyl dimethyl ammonium chloride; Dioctyl dimethyl ammonium chloride; didecyl dimethyl ammonium chloride; n-Alkyl dimethyl benzyl ammonium chloride; same as MAQUAT 615-LR	Greenhouses, hard non porous surfaces (flower buckets, floors, walls, coolers, design, packing benches and counter tops)	<i>Pseudomonas aeruginosa</i> , <i>Xanthomonas axonopodis</i> pv. Citri, and <i>vesicatoria</i>
MAQUAT 615-LR	1032 4-109	Same as MAQUAT 615-HD	Same as MAQUAT 615-HD	Same as MAQUAT 615-HD