**Ralstonia solanacearum Race 4: Risk Assessment for Edible Ginger and Floricultural Ginger Industries in Hawaii**

Mathews L. Paret¹, Asoka S. de Silva¹, Richard A. Criley², and Anne M. Alvarez¹,³

---

**SUMMARY.** Fourteen species of ginger belonging to Zingiberaceae and Costaceae were evaluated for susceptibility to the bacterial wilt pathogen *Ralstonia solanacearum* (*Rs*) race 4 (ginger strains) by several methods of inoculation, including tests to simulate natural infection. Twelve of 14 species tested were highly susceptible to all strains of *Rs* race 4 upon stem inoculation, and susceptible plants wilted within 21 days. In contrast to previous reports that *Rs* strains from an invasive alien species, kahili ginger (*Hedychium gardnerarium*), are nonpathogenic on ornamental gingers, the kahili ginger strain wilted both ornamental and edible ginger (*Zingiber officinale*) species within 21 days. Pour inoculation to the base of 11 plant species to simulate natural infection confirmed the ability of *Rs* to invade all the tested species without root wounds. Shampoo ginger (*Zingiber zerumbet*) was the most susceptible (wilted in 26 days) whereas pink ginger (*Alpinia purpurata*) and red ginger (*A. purpurata*) were the least susceptible and wilted in 71 and 76 days respectively. Pathogen survival in potting medium was evaluated by enumerating viable cells in effluent water from drenched pots with and without infected edible ginger after stem or rhizome inoculation. *Ralstonia solanacearum* survived in plant-free potting medium for 120 days and for 150 to 180 days in potting medium with infected edible ginger. The ability of *Rs* race 4 to infect many ginger species without wounding and to survive for long periods indicates that high risks will be incurred if the kahili ginger strain is inadvertently introduced from the forest reserves into ginger production areas.

---

Bacterial wilt, caused by *Ralstonia solanacearum*, causes severe wilt in many crops and is widely distributed in tropical, subtropical, and temperate regions of the world (Hayward, 1991, 1994; Kelman, 1953). *Ralstonia solanacearum* is classified into five races based on the hosts affected, and five biovars based on the ability to use or oxidize several hexose alcohols and saccharides (Buddenhagen et al., 1962; Hayward, 1964). Race 1 strains (biovars 1, 3, and 4) are pathogenic to a broad range of hosts, including tomato (*Solanum lycopersicum*), tobacco (*Nicotiana tabacum*), and peanut (*Arachis hypogaea*); race 2 strains (biovars 1 and 3) infect banana (*Musa acuminata*), plantain (*Musa paradisiaca*), heliconia (*Heliconia spp.*), and other plants in the Musaceae family; race 3 strains (biovar 2) occur in cool upland areas in the tropics and cause severe wilt in potato (*Solanum tuberosum*), tomato, and geranium (*Geranium spp.*); race 4 strains (biovars 3 and 4) infect ginger; and race 5 strains infect mulberry (*Morus alba*). The strains in the race 3 group are a select agent under the U.S. Agricultural Bio-terrorism Protection Act of 2002 (U.S. Department of Agriculture, 2005). Pathogen diversity and the relationship among races, biovars, and phylotypes have been addressed recently (Alvarez, 2005; Fegan and Prior, 2005).

Edible ginger is a major vegetable and spice crop in Hawaii and in numerous locations in Australia, India, Indonesia, Jamaica, Japan, Malaysia, Nigeria, Sierra Leone, and the Philippines. Hawaii’s farmers harvested 4.3 million lb of edible ginger during the 2005–06 season and the total farm value was estimated at $3.0 million (Hudson, 2006b). Plants belonging to the Zingiberaceae and Costaceae families are prevalent in the forests of Hawaii and are grown for cut flowers as well as for the “ Lei” industry, which is important because of its aesthetic and symbolic value to traditional customs and the local tourism industry. Hawaii’s floriculture industry includes numerous economically important gingers from Zingiberaceae and Costaceae. The value of ginger cut flowers in Hawaii was $1.61 million in 2005–2006 (Hudson, 2006a), the predominant flowers being red ginger and pink ginger.

Bacterial wilt disease of edible ginger causes severe economic damage in many countries, including China, India, Indonesia, Japan, Malaysia, Mauritius, the Philippines, and the United States (Hawaii) (Alvarez et al., 2005; Kumar and Sarma, 2004). In Hawaii the disease was first reported on the island of Oahu in the early 1960s (Ishii and Aragaki, 1963; Quinon et al., 1964). Crop losses of edible ginger resulting from bacterial wilt exceeded 50% in 1998 and 1999 (Yu et al., 2003), and in 2005, the area harvested was reduced by 13% (Hudson, 2006b). In India, bacterial wilt is widespread on edible ginger and 100% yield losses have been reported (Dohroo, 1991; Mathew et al., 1979; Sarma et al., 1978; 1980).

---

This research was funded by the USDA Special Grants program for Tropical and Sub-tropical Agricultural Research (Award no. 2004-31335-15191) and USDA-ARS/Minor Crops Research (Award no. 59-5320-1-525).

We thank E.E. Trujillo, D.E. Gardner, M.T. Momol, and D. Cook for providing us with bacterial strains; and C.W. Morden and R.F. Baker of Lyon Arboretum, University of Hawaii at Manoa, for providing planting stocks.

¹Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, 3050 Male Way, Gilmore Hall 310, Honolulu, HI 96822

²Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, 3190 Male Way, St. John 102, Honolulu, HI 96822-2279.

This research was funded by the USDA Special Grants program for Tropical and Sub-tropical Agricultural Research (Award no. 2004-31335-15191) and USDA-ARS/Minor Crops Research (Award no. 59-5320-1-525).

We thank E.E. Trujillo, D.E. Gardner, M.T. Momol, and D. Cook for providing us with bacterial strains; and C.W. Morden and R.F. Baker of Lyon Arboretum, University of Hawaii at Manoa, for providing planting stocks.

²Corresponding author. E-mail: alvarez@hawaii.edu.
Sharma and Rana, 1999). Bacterial wilt has been reported for other members of the Zingiberaceae family, including Alpinia [Alpinia spp. (Hayward, 1994)], turmeric [Curcuma longa (Velluppyalai, 1986)], galanga [Kaempferia galanga (Hc, 1986)], siam tulip (Curcuma alismatifolia), and mioga [Zingiber mioga (Tsuchiya et al., 2004, 2005)]. The pathogen was isolated from yellow ginger (Hedychium flavescens), white ginger (Hedychium coronarium), and kahili ginger plants growing at a single location on the island of Oahu in Hawaii (Aragaki and Quinon, 1965).

A ginger strain of Rs originally isolated from edible ginger has been used in a biocontrol program to reduce populations of kahili ginger, an invasive Zingiberaceae species in the tropical forests of Hawaii. The ginger strain was reportedly specific for kahili ginger and nonpathogenic to important ornamental gingers and common plant species in Hawaii (Anderson and Gardner, 1999). Based on this premise, the pathogen was introduced into Hawaiian forests, including Hawaii Volcanoes National Park on the island of Hawaii (Anderson and Gardner, 1996, 1999). However, other studies indicated ornamental ginger species were susceptible to the ginger strain (Paret et al., 2006). Thus, further studies were needed to assess the host range using a representative set of the Rs strains isolated from edible ginger and kahili ginger.

Survival of Rs races 1, 2, and 3 in water, soil–sand mixtures, and roots of symptomless solanaceous hosts has been documented (Elphinstone et al., 1998; Granada and Sequeira, 1983; Hayward, 1991), but there is a lack of information on survival of race 4 ginger strains in soil or potting media that are commonly used in Hawaii to produce rhizomes in bag culture (Hepperly et al., 2004). Because the industry has increased the use of potting media for production of ornamental gingers (Criley et al., 2005; Kuchny et al., 2005a, b), survival of Rs race 4 populations in the potting medium may be a factor in disease spread.

The objective of the current study was to assess the pathogenicity of Rs race 4 strains on plants belonging to the Zingiberaceae and Costaceae families, and to determine the ability of the pathogen to survive in a potting medium commonly used for potting culture in Hawaii.

Materials and methods

Pathogenicity and host range studies. Ralstonia solanacearum strains used for the experiments (A4515, A5192, and A3450) were selected from a larger collection of 55 well-characterized ginger and tomato strains to represent the genetic diversity observed in the local population (Yu et al., 2003). For host range studies we selected two edible ginger strains (A4515 and A5192; isolated from farms on the island of Hawaii), one kahili ginger strain (A4679; isolated from the island of Hawaii), and three tomato strains (A5370, A3450, and A5345; isolated respectively from Hawaii, Trinidad, and Florida). A nonpathogenic strain of Enterobacter cloacae (Ecl) A5149, an endophyte of the ginger rhizome (Nishijima et al., 2004), isolated from the island of Hawaii was used as a negative control. Bacterial strains were streaked on a modified tetrazolium chloride (TZC) medium (Norman and Alvarez, 1989) and incubated for 48 to 72 h. One to two colonies of Rs were transferred to 3.0 mL distilled water (dH₂O), vortexed, and adjusted spectrophotometrically to Å₈₀₀nm = 0.1, which corresponds to 10⁸ cfu/mL. Seeds of important ornamental gingers such as red ginger, pink ginger, white ginger, red ginger lily (Hedychium coccineum), siam tulip, white turmeric (Curcuma zedoaria), Globba (Globba spp.), yellow ginger, shampoo ginger, kahili ginger, beeche ginger (Zingiber spectabile), torch ginger (Etherea elatior), and edible ginger belonging to the Zingiberaceae family; and spiral ginger (Costus barbatus), belonging to the Costaceae family, were collected from the Waimanalo Farm and Lyon Arboretum (University of Hawaii at Manoa, Manoa, HI) and planted in plastic pots (2 L) filled with potting medium (Sunshine Mix 4 Aggregate Plus; Sun Gro Horticulture Canada Ltd, BC, Canada). Two- to three-month-old plants were inoculated into the stem using a syringe to deliver 1.0 mL of the inoculum for each strain tested. Wilt symptoms including flagging and yellowing of leaves were recorded for 5 to 21 d after inoculation (DAI).
a 1-mL subsample was taken to initiate a 10-fold dilution series (10\(^{-1}\) to 10\(^{-7}\)); 100 \(\mu\)L of each dilution was plated onto modified SMSA medium (Engelbrecht, 1994) to assess the viable \(R_s\) population. The same method was used to assess the bacterial population in the potting medium. A 1-g soil sample was vortexed for 10 s in 10 mL dH\(_2\)O, and allowed to settle. One milliliter of the supernatant was diluted to enumerate colony counts by the previously mentioned methods. The effluent water and potting medium samples from this experiment were analyzed until 240 DAI.

Three methods of inoculation—pouring inoculum into plant-free potting medium, stem inoculation, and rhizome inoculation—were compared in another experiment to determine the effect of the bacterial inoculation method (hence, plant part colonized by the pathogen) and subsequent release of \(R_s\) into the potting medium and effluent water. For the first inoculation method, 100 \(\mu\)L of each dilution was placed onto modified SMSA medium (Engelbrecht, 1994) to assess the viable \(R_s\) population. The same method was used to assess the bacterial population in the potting medium. A 1-g soil sample was vortexed for 10 s in 10 mL dH\(_2\)O, and allowed to settle. One milliliter of the supernatant was diluted to enumerate colony counts by the previously mentioned methods. The effluent water and potting medium samples from this experiment were analyzed until 240 DAI.

### Table 1. Pathogenicity of *Ralstonia solanacearum* (\(R_s\)) on various species in Zingiberaceae and Costaceae.

<table>
<thead>
<tr>
<th>Bacterium Strain no.*</th>
<th>Host of origin</th>
<th>Origin</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs race 4</td>
<td>A4515</td>
<td>Edible</td>
<td>HI</td>
<td>4/4</td>
<td>2/4</td>
<td>3/3</td>
<td>3/3</td>
<td>1/1</td>
<td>0/1</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>1/1</td>
<td>0/1</td>
<td>3/3</td>
</tr>
<tr>
<td>A5192</td>
<td>Edible</td>
<td>HI</td>
<td>3/4</td>
<td>2/4</td>
<td>5/5</td>
<td>3/3</td>
<td>2/2</td>
<td>1/1</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>2/3</td>
<td>1/1</td>
<td>0/1</td>
<td>3/3</td>
</tr>
<tr>
<td>Rs race 1</td>
<td>A5370</td>
<td>Tomato</td>
<td>HI</td>
<td>4/7</td>
<td>3/3</td>
<td>2/3</td>
<td>3/3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>2/3</td>
<td>3/3</td>
<td>1/2</td>
<td>1/1</td>
<td>0/1</td>
<td>NT</td>
</tr>
<tr>
<td>A5450</td>
<td>Tomato</td>
<td>TR</td>
<td>0/4</td>
<td>NT</td>
<td>0/3</td>
<td>0/2</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1</td>
<td>NT</td>
<td>0/4</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>A5345</td>
<td>Tomato</td>
<td>FL</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Ecl</td>
<td>A5149</td>
<td>Edible</td>
<td>HI</td>
<td>0/3</td>
<td>0/3</td>
<td>0/4</td>
<td>0/3</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/2</td>
<td>0/2</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>(0/4)</td>
</tr>
</tbody>
</table>

*Providers of bacterial strains: A4515, E.E. Trujillo; A4679, D.E. Gardner; A5450, D. Cook; A5345, T.M. Momol; and A5192, A5370, and A5149 (our collection).

Zingiberaceae: 1, red ginger; 2, pink ginger; 3, white ginger; 4, red ginger lily; 5, siam tulip; 6, white turmeric; 7, globba; 8, yellow ginger; 9, shampoo ginger; 10, kahili ginger; 11, beehive ginger; 12, torch ginger; 13, edible ginger.

Costaceae: 14, spiral ginger.

The results were recorded 21 d after inoculation (DAI) of the stem of 2- to 3-month-old plants with *Ralstonia solanacearum* strains representing race 4 and race 1. The *Enterobacter cloacae* (Ecl) strain A5149 is the negative control.

*FL, Florida; HI, Hawaii; NT, not tested; Rs, Ralstonia solanacearum; TR, Trinidad.*
strain of *Rs* A4515 was poured into pots (2 L) containing potting medium but no plants. For the second method, 1.0 mL *Rs* was inoculated into each stem (three stems inoculated per plant) with a syringe. For the third method, the rhizome was wounded with a scalpel, followed by pouring 27 mL inoculum into the base of the plant. Two pots (2L) were tested for each method; inoculated plants were 5 months old. The pots were watered daily with 200 mL water for 180 d. Fifty milliliters of effluent water was collected and vortexed each day. A 1-mL subsample was then taken to initiate a 10-fold dilution series (10⁻¹ to 10⁻⁷); 100 μL of each dilution was plated onto modified SMSA medium to assess the viable *Rs* population. We also enumerated the bacterial population in potting medium by removing a 1-g soil sample from the pot, vortexing for 10 s in 10 mL dH₂O, and allowing soil particles to settle for 5 min. One milliliter of the supernatant was then diluted to enumerate viable colonies.

**Results and discussion**

Pathogenicity and host range studies. The race 4 ginger strains affected nearly all ornamental ginger species tested (Table 1). Symptoms ranged from flagging or wilting to plant death. Although the majority of the plants wilted within 10 DAI, the final pathogenicity assessment was recorded 21 DAI, at which time all affected plants were severely wilted or dead. The *Edl* negative control did not wilt any of the plants tested (Fig. 1). Of the three race 1 tomato strains tested, strain A5370 (isolated from tomato in Hawaii) wilted nearly all ginger species, whereas strains A3450 and A5345 (isolated from tomato in Trinidad and Florida respectively) did not wilt any of the ginger species. Most of the infected ginger plants showed moderate to complete wilting of leaves, and none of the ginger species were resistant to the *Rs* race 4 strains, with the possible exception of torch ginger (Table 1), which showed no symptoms. However, only a few plants of this species were available for testing, so results are inconclusive. In contrast to previously published data (Anderson and Gardner, 1999), our results show that the race 4 strains are pathogenic to many ornamental gingers.
Spread of Rs from infected siam tulip to the edible ginger and, subsequently, to mioga fields in the Kochi prefecture of Japan was well documented from 1995 to 1999 (Tsuchiya et al., 2005). Our findings that the Rs race 4 strain from kahili ginger is pathogenic to numerous plant species belonging to the Zingiberaceae and Costaceae families thus has broad implications in Hawaii because of the reported use of this strain as a biocontrol agent for invasive kahili ginger in forest reserves.

Simulation of natural infection by Ralstonia solanacearum race 4. The kahili ginger strain of Rs (A4679) wilted all 11 ginger species tested when plants were inoculated without wounding (Fig. 2). Shampoo ginger, beehive ginger, spiral ginger, and kahili ginger were highly susceptible and died within 38 d. Shampoo ginger was the most susceptible and wilted in 26 d, whereas pink ginger was the least susceptible, wilting in 76 d. The presence of the pathogen in stem sections of each species that showed wilting was confirmed by the immunostrip assay. The observation that the pathogen invaded the host even in the absence of a stem or root wound confirms its ability to enter through natural openings. This suggests that the pathogen could spread naturally from the inoculated forest plants to edible ginger and ornamental ginger production areas.

Long-term survival of Ralstonia solanacearum race 4 in potting medium. Ralstonia solanacearum race 4 survived for 180 DAI and was detected at low levels for 150 to 180 DAI in effluent water (Fig. 3). The pathogen was also detected in potting medium 180 DAI (Fig. 4).

In the subsequent experiment, which tested the survival of pathogen after three inoculation methods, the population of the pathogen was no longer recovered from effluent water of plant-free potting medium at 30 d, in comparison with recovery of high populations from effluent water of pots containing stem- and rhizome-inoculated plants (Fig. 5). High populations of Rs were released from infected plants during the early stages of disease development. The pathogen was recovered from effluent water up to 150 DAI with stem- and rhizome-inoculated plants, and 60 DAI from effluent water of plant-free potting medium (Fig. 6). However when potting medium was sampled instead of effluent water, the pathogen survived with stem- or rhizome-inoculated plants for 150 DAI and for 120 DAI in plant-free potting medium (Fig. 7). High populations of Rs were noted when potting medium samples were analyzed instead of effluent water from these pots for all three methods of inoculation. The survival of the pathogen in the absence of host plant debris for 4 months is of particular concern to local growers, because it is an indication that the pathogen may persist in an ecosystem even after infected plants are removed.
Fig. 7. Survival of *Ralstonia solanacearum* (*Rs*) race 4 in potting medium for 180 d after inoculation with three methods: plant-free potting medium ( ), stem inoculation ( ), and rhizome inoculation ( ). Plant-free potting medium represents pots filled with potting media and with no plants that were inoculated with *Rs*; this represents the free-living bacteria. Stem inoculation represents pots with edible ginger, inoculated on the stem with *Rs*. Rhizome inoculation represents pots with edible ginger, and the rhizome was wounded, followed by pouring *Rs* inoculum. Viable cells are reported as log colony-forming units per milliliter (1 cfu/mL = 29.5735 cfu/fl oz). Each point is the average of two samples per treatment and the error bars indicate SD from the mean. The strain tested is *Rs* race 4, A4515.

Pathogen-free edible ginger seed rhizomes are currently produced in potting medium in Hawaii (Hepperly et al., 2004), and many ornamental flower growers use this potting medium for production. The ability of *Rs* to survive in this medium after it is introduced presents risks to both the edible ginger and floriculture ginger industries. Further studies of long-term survival of *Rs* in volcanic soil and ginger field soil are in progress.

**Conclusions**

The results of pathogenicity tests, simulation of natural infection, and long-term survival studies indicate that *R. solanacearum* race 4 can affect negatively both the edible ginger and floriculture ginger industries in Hawaii. Use of *Rs* as a biocontrol agent against an invasive ginger species is a concern to local agriculturalists, and a thorough evaluation of its spread, and survival on weeds and in watercourses is needed before any application in forest reserves.

**Literature cited**


