

## An Overview of Bacterial Wilt Disease of Tomato in Nigeria

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### INTRODUCTION

Tomato contains high levels of the antioxidants vitamin C, lycopene and  $\beta$ -carotene. Tomato is best adapted to warm and dry environments; in hot-wet environments, yields are low due to poor fruit-setting caused by high temperatures and disease problems. Among diseases, bacterial wilt caused by *Ralstonia solanacearum* (Smith), formerly *Pseudomonas solanacearum* has been reported to be the most damaging, causing reduction in yield and economic loss (Elphinstone, 2005).

Different control strategies have been suggested but no single strategy has shown 100% efficiency in control of the disease. Bacterial wilt in commercial tomato fields may result in significant yield reductions up to complete loss under favorable disease conditions (Boshou, 2005).

**Tomato world production statistics:** The world's average tomato yield in 2001 was 27 Mt ha<sup>-1</sup> but in tropical Africa only 8 Mt ha<sup>-1</sup> of fruit are harvested from field production. Some average yields per country are: Nigeria 7 Mt ha<sup>-1</sup>, Kenya 12 Mt ha<sup>-1</sup>, Egypt 35 Mt ha<sup>-1</sup> and France 120 Mt ha<sup>-1</sup>. Tomato production occurs in 144 countries, China is a major producer in area (1,255,100 ha) and yield (30,102,040 Mt). The leading countries in fruit yield per hectare are the Netherlands (4,961,539 Mt ha<sup>-1</sup>) and Belgium (4,166,667 Mt ha<sup>-1</sup>). Present world production is about 100 million ton fresh fruit produced on 3.7 million ha (Elphinstone, 2005).

The top 5 tomato producers in the world (Table 1) in 2008 accounted for about 129 million ton. China, the largest producer, accounted for about one quarter of the global output, followed by United States and Turkey. For plum or processing tomatoes, California accounts for 90% of U.S. and 35% of world production (Hartz *et al.*, 2008). Extremely high tomato yields, 450-500 Mt ha<sup>-1</sup> can

Table 1: Top producers of tomatoes (2008)

Countries	Production (Mt)
China	33,811,702
United States	12,575,900
Turkey	10,985,400
India	10,260,600
Egypt	7,550,000
Italy	5,976,912
Total	129,649,883

(FAO, 2008)

be obtained in heated and lighted glasshouses in the Netherlands with a single crop being supported for 9 months (Hartz *et al.*, 2008). Seed yields are 100-150 kg ha<sup>-1</sup> for F1 hybrids and up to 300 kg ha<sup>-1</sup> for open pollinated cultivars. Tomato has been in cultivation in Nigeria for a very long time and it is available year round in different agroecological zones (Adebayo and Ekpo, 2005).

### Life cycle, epidemiology and dissemination of *Ralstonia solanacearum*

The bacterium *R. solanacearum* has been reported to be primarily a soilborne and waterborne pathogen (Adebayo and Ekpo, 2005). It infects host plants primarily through roots, entering through wounds formed by lateral root emergence or by damage caused by soilborne organisms (Adebayo and Ekpo, 2006). The bacterium can also enter plants by way of stem injuries caused by insects, handling or from mechanical damage. Once inside roots or stems, the bacterium colonizes the plant through the xylem in the vascular bundles (Denny, 2006).

The race 3 biovar 2 (R3b2) of the bacterium is most severe on plants when temperatures are between 25 and 35°C and decreases in aggressiveness when temperatures exceed 35°C or fall below 18°C (Ciampi and Sequeira, 1980). Active disease at temperatures below 18°C is rare (Ciampi and Sequeira, 1980; Hayward, 1991). The pathogen could be spread from infested to healthy fields by soil transfer on machinery and surface run off water and it could be disseminated from infested surface water

to uninfested fields by flooding or irrigation (Adebayo and Ekpo, 2006). Plant-to-plant infection can occur when bacteria shed from infected roots move to roots of nearby healthy plant; McCarter (1991) reported that long distance spread of the pathogen can occur with transportation of latently infected transplants. The bacterium could survive for days and up to years in infested water, wet soils or in soil layers >75 cm (Denny, 2006) from where it can be dispersed and only antagonist microorganisms and environmental factors, mainly temperature, soil type and soil moisture can affect *R. solanacearum* survival.

**Distribution and host range:** The race 1 as reported by Denny (2006) is widely distributed in tropical and subtropical regions and it infects over 50 families including those in the Solanaceae i.e., eggplant (*Solanum melongena* (L.)), pepper (*Capsicum* sp.), potato (*S. tuberosum* (L.)) and tomato (*S. lycopersicum* (L.)). One subgroup R3b2 as reported by Lemay *et al.* (2003), attacks plants at higher altitudes in tropical, subtropical and warm-temperate areas.

Other solanaceous and non-solanaceous plants also may act as alternative hosts for R3b2. On potato alone, R3b2 was reported to be responsible for an estimated US\$1 billion in losses each year (Elphinstone, 2005).

Tomato bacterial wilt is mostly caused by race 1 which has a wide host range and can survive in soil for a long period and they are highly variable in genotype and aggressiveness. Some highly aggressive strains can cause severe symptoms, even to tomato varieties classified as resistant (Denny, 2006).

**Description of the pathogen:** *R. solanacearum* was considered a species complex due to significant variation within the group (Fegan and Prior, 2005). It was historically subdivided into five races, arranged loosely on host range and 5 biovars, based on the ability of *R. solanacearum* strains to produce acid from a panel of 5-8 carbohydrate substrates, including disaccharides and sugar alcohols.

No laboratory test has been reported to define the race of an isolate because host ranges of strains are broad and often overlap but a phylogenetically meaningful classification scheme was developed based on DNA sequence analysis (Fegan and Prior, 2005). They further used this scheme to divide species complexes into 4 major groups called phylotypes that broadly reflect ancestral relationships and geographical origins of strains. The *R. solanacearum* R3b2 strain belongs to phylotype II and sequevars 1 and 2 (Fegan and Prior, 2005).

**Culture, identification and conservation:** *R. solanacearum* is a gram-negative, rod-shaped, largely aerobic bacterium that is 0.5-0.7×1.5-2.0 µm in size. Liquid and solid agar growth media are commonly used for culture. For most strains optimal growth temperature is between 30 and 35°C (Denny and Hayward, 2001; Hayward, 1991).

The optimal growth temperature for R3b2 is 30°C (Denny and Hayward, 2001). On solid agar media, individual colonies are usually visible after incubation for 36-48 h 30°C; colonies of the normal or virulent, type are white or cream colored, irregularly shaped, highly fluidal and opaque. Occasionally, colonies of the mutant or non-virulent type appear; these are uniformly round, smaller and butyrous (dry) and Tetrazolium Chloride (TZC) medium can differentiate colony types. On TZC, virulent colonies appear white with pink centers and non-virulent colonies are a uniform dark red. *R. solanacearum* can be stored for many years at room temperature in sterilized tap, distilled or deionized water. It will also survive long-term at -80°C in liquid culture broth containing 40% glycerol (Denny and Hayward, 2001).

**Symptoms and diagnosis of *Ralstonia solanacearum*:** Bacterial streaming is a common sign of *R. solanacearum*. When cut stem sections from infected plants are placed in water, threads of a viscous white slime stream from the cut end of the stem within minutes. These threads are bacterial ooze exuding from the infected xylem and the streaming test is a valuable diagnostic tool for quick detection of bacterial wilt diseases in the field. However, it may not be useful early in disease development (McCarter, 1991).

**Detection and identification:** Pathogen identification consists of observation of signs and disease symptoms (Denny, 2006). Symptomatic plants in the field or greenhouse can be tested for *R. solanacearum* using screening tests that can allow early detection. These screening tests include bacterial streaming, plating on a semi-selective medium (Elphinstone, 2005) and immuno-diagnostic assays using species-specific antibodies. Denny (2006) reported that the USDA-APHIS-PPQ has tested and recommends use of inexpensive commercially available immunostrips for rapid detection of *R. solanacearum* in the field or laboratory which are fast and require minimum equipment. However, he stated that they cannot be used to identify the race or biovar. Several microbiological and molecular methods can be used to identify *R. solanacearum* at the sequevar, race and biovar level following recovery from asymptomatic plants or from water or soil samples. These include immunodiagnostic

assays using species-specific antibodies, polymerase chain reaction with species-specific primers and biovar tests (Denny, 2006).

**Condition favorable for development:** The disease develops slowly when soil temperature is <20°C or soil moisture is low. The pathogen prefers acidic soils (soil pH <7.0). The disease can occur on all types of soil including sandy and clay types. The presence of root damaging microorganisms will accelerate disease development (Adebayo and Ekpo, 2006).

**Review of research work on *Ralstonia solanacearum* (Smith) in Nigeria:** Oyedun *et al.* (1997) conducted a survey of bacterial wilt of tomato caused by *R. solanacearum* in the tomato growing states of Kano, Kaduna, Oyo and Ogun in Nigeria. Yield loss of over 70% was observed in the rainforest zone while the savanna zone had yield loss of between 10-30%. There was no significant correlation between yield loss and cropping systems employed. Osuinde and Ikediugwu (2002) examined the microflora associated with the root-surface of tomato cultivars and *R. solanacearum* was frequently isolated. The increase in population of *R. solanacearum* plays an important role in relation to pattern of occurrence of the disease. Bell pepper, eggplant and tomato, the crops most susceptible to the pathogen are grown from April to November in the south and February to June in the north (Adebayo and Ekpo, 2006).

Adebayo and Ekpo (2005) reported that wilt disease caused by *R. solanacearum* in 80% of tomato fields in parts of Nigeria between May and November 1998. Affected plants exhibited initial wilting of terminal leaves followed (within 2 days) by sudden and permanent wilting. For further identification of the causal organism, 10 tomato plants showing wilt symptoms were collected from each of five fields at Ibadan and 20 farmers' fields. Creamy bacterial sap from these samples was plated on tetrazolium chloride media and plates incubated at 30°C for 48 h (Adebayo and Ekpo, 2006) for biovar determination. Basal media was prepared to include one of the following: cellobiose, lactose, maltose, dulcitol, mannitol or sorbitol.

All isolates utilized the three disaccharides and 3 hexose alcohols and according to Hayward's classification, all isolates were biovars and the isolates caused rapid wilting of test plants. *R. solanacearum* was reisolated from the vascular bundles of test plants (Adebayo and Ekpo, 2005). This was the first time a biovar of *R. solanacearum* affecting tomato crops was reported in Nigeria. Taiwo *et al.* (2007) examined bacterial and fungal wilts causing yield loss in

tomato and which required sustainable control strategies to reduce their incidence. They inoculated tomato with the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicolson and Gerdemann) Gerd. et Trappe and treated plants with manure and inorganic fertilizer. It was found that *G. mosseae* slightly reduced wilt severity relative to the control. Also, year and fertilizer significantly affected numbers of fruit and fertilizer and mycorrhizae increased vitamin C content.

Adebayo *et al.* (2009) examined integrated crop management strategies for control of bacterial wilt disease. Effects of rotation of tomato with other crops on soil populations of *R. solanacearum* and on bacterial wilt disease incidence of tomato were evaluated. Monocropped Cassava (*Manihot esculenta* Crantz), *Mucuna puriens* L., *Crotalaria juncea* L. and intercrops of Cassava/Crotalaria, Cassava/Mucuna and a natural grass mix (control) were rotated with tomato cvs. Mira, Ronita, Roma VFN and Ibadan Local. Monocropped Mucuna significantly reduced soil populations of *R. solanacearum* by the end of the rotation period; the natural grass rotation had the highest population of the pathogen. The wilt incidence was delayed in cvs. Mira, Roma VFN and Ronita compared to Ibadan Local but all were 80% or more infected after 8 weeks.

**Management of bacterial wilt of tomato:** Because *R. solanacearum* is a soilborne pathogen and host resistance is limited, bacterial wilt is difficult to control (Hayward, 1991; Saddler, 2005). Moreover, *R. solanacearum* is widely distributed and has a broad host range (Denny, 2006; Hayward, 1991). In locations where *R. solanacearum* is established, some level of bacterial wilt control is possible by using a combination of methods that include.

**Host resistance:** Resistant or moderately resistant, tomato cultivars, such as FL7514 and BHN 466 should be used. Resistance may vary with location and temperature because of bacterial strain differences (Hanson *et al.*, 1996; Wang *et al.*, 1998). Grafting susceptible tomato cultivars onto resistant tomato or other solanaceous rootstocks is effective against Asian strains of *R. solanacearum* and is used commercially in different locations worldwide (Saddler, 2005). Effectiveness of grafting for use against R3b2 has not been tested.

**Chemical control and soil treatment:** Chemical soil fumigation or application of phosphoric acid is effective for controlling bacterial wilt of tomato in the field (Chellemi *et al.*, 1994; Ji *et al.*, 2005). Similarly, soil treatments, including modification of soil pH, solarization

and application of stable bleaching powder reduced bacterial populations and disease severity on a small scale (Saddler, 2005). Drawbacks of these methods may include environmental damage, cost and high labor input. Additionally, most of these methods still have to be tested against R3b2 strains of *R. solanacearum*. Chemical control should be integrated with other methods to reduce selection pressure for pathogen resistance. Recently, use of a Thymol derived volatile biochemical (currently not commercially available) reduced disease incidence and increase yield in field experiments in Florida (Ji *et al.*, 2005). Similarly, application of the plant resistance inducer, acibenzolar-S-methyl (Actigard, Syngenta, Greensboro, NC) in combination with moderately resistant cultivars, enhanced resistance against bacterial wilt Pradhanang *et al.*, 2005).

**Biological control:** Biological control based on suppressive soils or known *R. solanacearum* antagonists, has shown promising results in small scale experiments but needs to be validated on a larger scale and against R3b2 (Saddler, 2005). Akira *et al.* (2009) reported that *Pythium oligandrum* (Drechsler) suppresses bacterial wilt caused by *R. solanacearum*.

**Phytosanitation and cultural practices:** The best strategy for controlling bacterial wilt in the field consists primarily of phytosanitation and cultural practices. In regions where bacterial wilt of potato is endemic or in locations where *R. solanacearum* is present but not yet established, these methods may be effective.

These practices include crop rotation with non-host plants such as grasses, intercropping, control of weed and root-knot nematode populations, planting at non-infested production sites, removal of weeds or crop residue where inoculum persists, selection of appropriate planting time to avoid heat, deep plowing of crop residues, satisfactory soil drainage and early- and late-season irrigation management (Hayward, 1991; Saddler, 2005). In locations where the pathogen is not present it is important to prevent introduction and if inadvertently introduced, subsequent movement of race 1 of *R. solanacearum* from infested to uninfested locations or fields.

Effective cultural sanitation practices are critical to keep non-infested areas clean. Sanitation efforts include planting only certified disease free plantlets, disinfecting equipment before moving between fields, controlling floodwater flow and never using surface water for irrigation. In greenhouses, sanitary practices for tomato transplant production may include avoidance of sub-irrigation, wide separation of greenhouses from field

production areas, disinfestation of frames, trays and tools, use of pathogen-free soils or potting mixes, weed control and limited handling of plants (McCarter, 1991). A zero tolerance level includes reinforcement of quarantine regulations, exclusionary practices, sanitary protocols and inspections designed to prevent introduction of infected plants. Pest response guidelines for *R. Solanacearum* R3b2, presents current information for detection, control, containment and eradication of this pathogen (Floyd, 2008).

In addition to establishing exclusionary strategies, growers should monitor potentially infested sites for early detection and subsequent eradication of R3b2. Key sites for monitoring include soils in which R3b2-infected plants have been identified, rivers and other surface water used for irrigation, particularly when infected weed hosts may be present and tomato production fields in the vicinity of geranium production greenhouses (APHIS-PPQ, 2005). Host removal and destruction is required along with disinfestation as well as several years of non-host production in infested fields or associated growing areas before the quarantine can be removed. In case of contamination water treatments such as filtration or chemical disinfection may be applied under control of legal authorities (APHIS-PPQ, 2005). Recommended management strategies for bacterial wilt on tomato caused by *R. solanacearum* (Momol, 2005) include:

**Before planting:**

- Effective weed control in and around tomato fields and aquatic weed control around irrigation ponds
- Application of 3-4 years rotation and cover crops for infested fields
- Rotation and cover crops should not be irrigated with *R. solanacearum* contaminated pond or surface water
- Well drained and leveled fields should be used and for cultivation
- The soil pH should be raised to 7.5-7.6 and available calcium increased by liming

**During production:**

- The pathogen should be excluded by applying strict sanitation practices
- Chlorination of irrigation water continuously if surface water or *R. solanacearum* infested pond water is used
- Effective weed control in and around tomato fields and irrigation ponds
- Application of plant resistance inducer such as Actigard (Syngenta) for moderately resistant cultivars (i.e., FL 7514). Actigard enhances resistance against this disease if it is used in combination with moderately resistant cultivars

#### After harvest:

- Crop residue should be plowed under immediately
- Suitable rotation should be practiced and cover crops used to avoid weeds that support *R. solanacearum* populations

In areas where *R. solanacearum* R3b2 is not known to be established strict phytosanitary and regulatory procedures should be established to prevent introduction and if inadvertently introduced, subsequent movement of the pathogen.

**Exclusionary practices:** Includes quarantine, testing and visual inspection of imported material of host plants, regulation and establishment to prevent introduction of the pathogen.

Practices such as cleaning and sanitizing field and handling equipment and application of good sanitary cultural practices will hinder movement of the pathogen from infested to pathogen-free fields in case of inadvertent introduction.

#### CONCLUSION

At the greenhouse, sanitary practices for tomato transplants production may include avoidance of sub-irrigation, wide separation of greenhouses from field production areas, disinfection of all frames, trays and tools, use of pathogen-free soils or potting mix, control of weeds and limited handling of plants. The occurrence of *R. solanacearum* in fields represents the possibility of the total loss of tomato yields. The disease can be controlled to a degree but eradication if at all possible is expensive and long-term. It may be that the best alternative for future control of the disease will be through development of resistant varieties.

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