Transmission and Survival of *Ralstonia solanacearum* on Tobacco Machinery*

by

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SUMMARY

Bacterial wilt (*Ralstonia solanacearum*) is an extremely damaging disease of flue-cured tobacco in the southeastern USA. It is generally believed that *R. solanacearum* infects field-grown tobacco through the roots. Epidemics of bacterial wilt are so common within South Carolina that the organism must be spread in a more rapid and efficient manner than the movement of soil on equipment. Field trials conducted at the Pee Dee Research and Education Center measured the spread of *R. solanacearum* down a row of tobacco from a point inoculation following machine flower removal (topping), leaf harvest or stalk cutting. Machine topping, leaf harvest and stalk cutting increased disease 879%, 1245%, and 800% respectively within a 20 plant row when compared to hand topping, hand harvesting or a non contaminated stalk cutter (cv. K 326, $P \leq 0.001$). A survey was conducted to determine if *R. solanacearum* is a common contaminate on harvesting equipment in South Carolina. Tobacco harvesting equipment was randomly selected within Horry and Marion counties and sampled for *R. solanacearum* by streaking sterile cotton swabs on the harvester surface, then restreaking the swab onto an enriched Tetrazolium-based selective media (SM3). *R. solanacearum*-like colonies that developed on SM3 were inoculated onto cv. Rutgers tomato to confirm pathogenicity. Bacteria from diseased plants were reisolated on SM3 and *R. solanacearum* identity confirmed with an immuno strip test. Pathogenic populations of *R. solanacearum* were recovered from steel defoliation knives, rubber defoliators and steel guides (51, 50 and 50% of sampled harvesters respectively). The survival of *R. solanacearum* on mechanical steel toppler blades was determined by running the toppler through infected tobacco plants and sequentially sampling the steel

knives over time for viable populations of *R. solanacearum*. Sterile cotton swabs were swept across the blade surface, then streaked onto SM3 media. Confirmed pathogenic populations of *R. solanacearum* could be recovered on SM3 media for up to 6 h after topping *R. solanacearum*-infected tobacco. The efficiency of machine transmission of *R. solanacearum* and its longevity on tobacco machinery may be responsible for the high frequency of bacterial wilt epidemics in flue-cured tobacco occurring in the Southeastern USA and its movement into previously clean fields. [Beitr. Tabakforsch. Int 23 (2008) 137–143]

ZUSAMMENFASSUNG

Die bakterielle Schleimkrankheit (*Ralstonia solanacearum*) an Virginia Tabakpflanzen verursacht ernsthafte Probleme im Südosten der Vereinigten Staaten. *R. Solanacearum* infiziert den Feldbestand normalerweise über die Wurzeln, doch die schnelle Ausbreitung des Pathogens in South Carolina deutet auf einen anderen Ausbreitungsmechanismus hin. Die Ausbreitung des Erregers wurde an der Pee Dee Versuchsstation der Clemson University in South Carolina während des mechanischen Blütenschnitts (topping), der Blätternte, und des Stengelschnitts verfolgt. Im Vergleich zum manuellen Verfahren erhöhten der maschinelle Blütenschnitt, die Blätternte und der Stengelschnitt den Krankheitsbefall um 879%, 1245% bzw. 800% in einer Reihe von 20 Pflanzen (cv. K326, $P \leq 0.001$). Es wurde untersucht, ob *R. solanacearum* wegen seines Vorkommens auf Entemaschinen in South Carolina als Krankheitsüberträger auftritt. Das Vorkommen von *R. solanacearum* wurde an willkürlich ausgewählten Erntemaschinen in den Bezirken Horry and Marion bestimmt.

*Received: 4th January 2008 – accepted: 2nd June 2008

Proben wurden mit keimfreien Wattestäbchen gesammelt und anschließend auf spezifischen und angereicherten Tetrazolium Nährboden (SM3) übertragen. *R. solanacearum*-ähnliche Kolonien, die sich auf dem Nährboden entwickelt hatten, wurden auf Pathogenität mit Tomatenpflanzen (cv. Rutgers) untersucht. Das Bakterium selbst wurde mittels 'Immuno-Strip test' nachgewiesen. Pathogene *R. solanacearum* Populationen wurden an 50% der Stahlmesser der Blatterntemaschine, an 51% der Gummi-Blatternter und 50% der Stahlführungen gefunden. Die Überlebensdauer der *R. solanacearum* Populationen an den mechanischen Stahlschneidern wurde anhand schrittweiser Probenahmen bestimmt, nachdem die Schneiden durch infizierte Tabakpflanzen gefahren wurden. Proben wurden von den Stahlschneiden mit keimfreien Wattestäbchen genommen und auf SM3 Nährboden ausgestrichen. Pathogene *R. solanacearum* Populationen wurden bis zu 6 h nach dem Schneiden der infizierten Pflanzen gefunden. Die Effektivität der Übertragung durch Erntemaschinen von *R. solanacearum* und die Überlebensdauer an den Erntegeräten könnte das erhöhte Aufkommen der Schleimkrankheit an 'flue-cured' Tabakpflanzen und dessen Ausbreitung in bisher krankheitsfreie Felder im Südosten der Vereinigten Staaten erklären. [Beitr. Tabakforsch. Int. 23 (2008) 137–143]

RESUME

La pourriture brune (*Ralstonia solanacearum*) est une maladie importante du tabac de type Virginie dans le sud-est des Etats-Unis. En générale cette bactérie infecte la plante à travers les racines. Des épidémies de pourriture brune sont si communes en Caroline du Sud que l'organisme doit être répandu par un mécanisme plus rapide et plus efficace que le simple mouvement de sol sur les équipements. Des essais au champ ont été conduits à la Pee Dee Research and Education Center sur la propagation de *R. solanacearum* le long d'un rang de tabac à partir d'un point d'inoculation après écimage mécanique de la fleur, la récolte mécanique des feuilles ou la coupe mécanique des tiges. L'écimage mécanique de la fleur, la récolte mécanique des feuilles et la coupe mécanique des tiges a augmenté la maladie de 879%, 1245%, et 800% respectivement au sein d'un rang de 20 plantes par rapport à l'écimage manuel de la fleur, la récolte manuelle ou l'utilisation d'équipement à couper la tige non contaminé (cv. K 326, $P \leq 0.001$). Une étude a été réalisée pour documenter si *R. solanacearum* était un contaminant commun en Caroline du Sud. Des équipements de récolte du tabac ont été sélectionnés au hasard dans les régions de Horry et Marion. Des échantillons ont été prélevés sur la surface de moissonneuses puis soumis à un milieu Tetrazolium sélectif. Les colonies de type *R. solanacearum* qui se sont développées sur ce milieu ont été inoculées sur une plante de tomate (cv. Rutgers) pour confirmer leur pathogénicité. Les bactéries prélevées sur des plantes malades ont été de nouveau isolées sur le milieu Tetrazolium sélectif et l'identité de *R. solanacearum* a été confirmée par un test immunologique. Des populations de *R. solanacearum* ont été récupérées sur 51% des lames défoliateurs en acier, 50% des défoliateurs en caoutchouc,

et 50% des guides en acier des moissonneuses échantillonnées. La survie de *R. solanacearum* sur les lames en acier d'une machine à écimer a été déterminée en utilisant la machine sur des plantes infectées, et puis examinant les lames au cours du temps pour des populations viables de *R. solanacearum*. Des populations pathogènes de *R. solanacearum* ont été retrouvées jusqu'à 6 heures après l'écimage des plantes infectées. L'efficacité de la machine à transmettre *R. solanacearum* et la longévité de la bactérie sur la machine à écimer sont peut-être responsables de la haute fréquence d'épidémies de pourriture brune sur le tabac Virginie cultivé dans le Sud-est des Etats-Unis et son mouvement dans des champs auparavant non-infectés. [Beitr. Tabakforsch. Int. 23 (2008) 137–143]

INTRODUCTION

Bacterial wilt is an extremely damaging disease of flue-cured tobacco (*Nicotiana tabacum* L.) in the Southeastern USA (2, 4, 7, 8, 10). This disease is commonly known as Granville wilt in the United States, named for its devastating effect on the tobacco-dependent economy of Granville County, North Carolina at the turn of the 20th century. Early research conducted by personnel at the North Carolina Experiment Station developed a management scheme including crop rotation, host resistance and soil fumigation, which provided excellent disease control for this disease in tobacco for nearly a half century.

Sudden outbreaks of bacterial wilt in previously uninfested areas as well as a general increase in disease intensity across North and South Carolina since the mid to late 1980's provides evidence of a breakdown in the management system used to control this tobacco disease (3). Newer cultivars, such as K 149 and Ox 207, have improved bacterial wilt resistance but have failed to reduce disease losses (2, 10). The introduction of *Ralstonia solanacearum*, the causal organism, into previously uninfested land in South Carolina is an ecological disaster that will affect production of all solanaceous crops in these affected areas across generations. Disease losses in some locations are disturbingly reminiscent of the losses in the earlier part of the last century in Granville County where entire fields were lost to bacterial wilt and farm families financially ruined. The rapid increase in disease occurred concurrently with drastic changes in the federally operated flue-cured tobacco quota and support program. A rapid decline in the number of allotment holders and average support price changed patterns of production. Farmers mechanized production and expanded production acreage both within and across counties to form economically viable production units. Farm machinery was moved from farm to farm violating principals of pathogen quarantine. The rapid spread of bacterial wilt within South Carolina suggests that the organism is being spread in a more rapid and efficient manner than would be expected solely by the movement of soil on equipment. Data suggests that mechanical flower removal has been a factor in the rapid spread of this pathogen; a preliminary report on mechanical transmission of the pathogen has been published (3).

The objectives of these studies were to determine: the efficiency of machine toppers, harvesters and stalk cutters to spread *Ralstonia solanacearum* from an inoculated,

diseased plant to adjacent healthy plants within a row, the incidence of *R. solanacearum* on farm machinery within our production areas, and evaluate the longevity of *R. solanacearum* on farm machinery.

MATERIALS AND METHODS

Inoculum and field preparation

The test sites were located at the Pee Dee Research and Education Center, Florence, South Carolina, on Norfolk sandy loam soils (75% sand, 17% silt, 8% clay, 0.08% organic matter, pH 5.8–6.0). The fields were planted in soybean the year preceding the trials. Multipurpose soil fumigant 1,3-D (78%) + chloropicrin (17%), [98 liters/ha (11.7 mL/m)] was applied with a positive pressure electric pump system and injected 15 cm deep with a single chisel placed in the center of a 60-cm-wide bed. Bedding disks were used to seal the chisel opening and form a 36-cm-high bed with fumigant placement 40 cm from the top of the bed. Plots consisted of a single row of plants, 12.2 m long, bordered by untreated rows with 1.2-m row spacing. All treatments were replicated four times and arranged in a randomized complete block design. The tobacco cultivars were selected based on their level of resistance to bacterial wilt and were classified as susceptible (K 326) or resistant (K 346) and were grown using standard agronomic practices (4). Bacterial cells (isolate NC 132) from 48-hr nutrient agar plates were suspended in sterile water at $OD_{600} = 0.2 = 10^8$ cells/mL. A 10^6 cfu/mL dilution was used as inoculum.

Mechanical flower removal (topping)

Tobacco plants were inoculated with *R. solanacearum* (10^6 cfu/mL) by misting the bacteria onto a scalpel and cutting into the stem cambial layer 30 cm below the top of the inflorescence. The first two plants within a 22-plant plot were inoculated. *R. solanacearum* can occur naturally in the upper portion of a diseased tobacco stalk (personal observation). In the machine-topped plots, the first two inoculated plants would be machine topped prior to topping the remaining 20 healthy plants. In adjacent hand topped plots, the inoculated plants would be hand topped immediately prior to hand topping the remaining healthy plants within the row. An untopped row served as a control. Plots were arranged in a randomized complete block 2×2 factorial design where main blocks were topping method (hand vs. machine) and subplots were tobacco cultivar. Inoculations were conducted 8 days prior to the hand/machine topping operations. Plants were assessed for disease symptoms 14 days following topping and at weekly intervals following the first evaluation. Plants were assessed for disease symptoms on a 0–5 scale where: 0 = no foliar or stem necrosis and 5 = completely dead. At the completion of the trial each stalk was split from the top of the plant to the soil line and vascular tissue rated for necrosis on a 0–5 scale where: 0 = no necrosis and 5 = 100% of the vascular tissue necrotic with extensive pith decay. Representative plants expressing disease symptoms were assayed for *R. solanacearum* using an

Agdia Immuno Strip Test (Agdia Elkhart, IN 46514). Two trials were conducted in each of 2004 and 2005 (four trials).

Mechanical leaf removal

A multi-pass tobacco harvester (Carolina Tobacco Services, Bennettsville, SC), utilizing spiral rubber leaf defoliators, was driven through tobacco to evaluate mechanical transmission of *R. solanacearum* from a contaminated harvester to healthy plants within the row. In 2005, inoculum (1×10^6 cfu of *R. solanacearum* isolate NC 132) was applied directly to the tobacco harvester leaf defoliators immediately prior to leaf removal. In 2006, the first two plants at the beginning of the row were inoculated with 1×10^6 cfu of NC 132 by stab inoculation 15 cm above the soil line 8 days prior to leaf removal. The bottom four leaves were removed and the remaining leaves were not harvested.

Plots were arranged in a randomized complete block 2×2 factorial design where main blocks were harvesting method (hand vs. machine) and subplots were tobacco cultivar. Plants were assessed for disease symptoms 14 days following leaf removal and at weekly intervals following the first evaluation. Plants were assessed for disease symptoms on a visual rating as previously described. At the completion of the trial each stalk was split from the top of the plant to the soil line and vascular tissue rated for necrosis as previously described. At the completion of the trials, representative tobacco plants showing diseased symptoms were assayed for *R. solanacearum* using an immuno strip test as previously described.

Mechanical stalk destruction

A steel cutter blade was infested with *R. solanacearum* inoculum by misting a bacteria suspension (5×10^7) directly to the steel cutter blade immediately prior to cutting the stalks in a 12.1 m row of healthy tobacco plants. The leaves were removed from the plants by hand and the stalks mechanically cut 15 cm above the soil line. Stalks from control plots were cut with a steel cutter that was surface sterilized with 75% ethanol. Plots were arranged in a randomized complete block 2×2 factorial design where main blocks were cutting method (contaminated vs. sterilized cutter) and subplots were tobacco cultivar. Each treatment was replicated four times and the experiment was repeated in time (2000, 2002). Plants were assessed for disease symptoms by removing each root system from the soil and rating crown (tissue located at the soil line) and root (roots branching from the tap root) vascular necrosis on a 0 to 5 scale where: 0 = no vascular discoloration and 5 = 100% of the root vascular tissue necrotic. The presence of *R. solanacearum* in plant roots showing signs of vascular necrosis was confirmed by isolation of *R. solanacearum* on SM3 media (5), and pathogenicity on a tomato bioassay. Representative populations were reisolated from the tomato bioassay and confirmed for *R. solanacearum* with an immuno strip test as previously described. Data were analyzed using analysis of variance and factorial techniques (15). All calculations were performed with JMP Analysis System (SAS Institute, Cary, NC).

Figure 1. Bacterial wilt in susceptible (K 326) and resistant (K 346) tobacco cultivars following mechanical transmission from inoculated plants to adjacent healthy plants during flower removal. The first two plants within a 22 plant plot were inoculated with *R. solanacearum*. Data are the average of two trials (2004). The bars illustrate the standard error within a sampling date. The least significant differences ($P = 0.05$) for the 3, 4 and 5 week sampling was 12%, 12% and 13%, respectively.

Incidence of R. solanacearum on farm machinery

A survey was conducted to determine if *R. solanacearum* is a common contaminate on tobacco harvesting equipment in South Carolina. Horry and Marion counties South Carolina were selected since bacterial wilt is a common tobacco disease in these counties. Sampling of tobacco harvesting machinery in Horry and Marion counties were conducted in 2001 and 2002 by driving through the counties on a grid pattern and sampling all harvesters found within tobacco fields. Each sampling site was at least 2 km apart. If machinery was in use, we requested the farmer to stop the machinery while samples were collected. Some machinery was parked adjacent to recently harvested fields. Harvesting equipment was sampled for *R. solanacearum* by streaking sterile cotton swabs first on the harvester surface, then transferring the swab to 5 mL of sterile distilled water. The swab was agitated within the sterile water and streaked onto SM3 media (5). *R. solanacearum* like colonies that developed on the SM3 media were individually inoculated onto Rutgers tomato. All plants producing bacterial wilt like disease were re-isolated on SM3 media and the presence of *R. solanacearum* confirm using an immuno strip test as previously described.

Longevity of R. solanacearum on farm machinery

The survival of *R. solanacearum* on mechanical tobacco toppler blades were determined by running the toppler through *R. solanacearum* infected plants and sequentially sampling the steel knives for viable populations of *R. solanacearum*. The toppler was placed under a steel shelter to avoid direct sunlight on the cutter blades. The trials were conducted on 25 July and 1 August, 2000 for trial 1 and 2 respectively.

The toppler was maintained in ambient temperature (30 °C and 33 °C, respectively for trial 1, and 2) and was sampled for *R. solanacearum* by streaking sterile cotton swabs first on the cutter blade, then transferring the swab to 5 mL of sterile distilled water. The swab was agitated within the sterile water and streaked onto a selective media (SM3) as previously described. The toppler was sampled at 0, 5, 10, 15, 30 and 45 min, and 1, 2, 3, 4, 5, 6, 7, 8 h after topping diseased tobacco. *R. solanacearum* like colonies that developed on the SM3 media were individually inoculated onto Rutgers tomato. All plants producing bacterial wilt like disease were re-isolated on SM3 media. If *R. solanacearum* like colonies could be reisolated from the tomato, the tobacco toppler was considered contaminated with *R. solanacearum*. Representative *R. solanacearum* like colonies were confirmed for *R. solanacearum* using immunino assay strips as previously described.

RESULTS

Mechanical transmission during flower and leaf removal, and mechanical stalk cutting

Mechanical flower removal of a *R. solanacearum* infected tobacco plant contaminated the steel cutter blades and allowed for mechanical transmission of *R. solanacearum* from a diseased plant to adjacent healthy plants within the row. Plants were symptomless for 2–3 weeks after which bacterial wilt developed rapidly with typical bacterial wilt symptoms. Percent infection increased following mechanical flower removal 879% and 506% when averaged over years and locations for the susceptible (K 326) and resistant (K 346) cultivars respectively (Figures 1, 2). The resistant cultivar K 346 had less disease ($P \leq 0.05$) than the susceptible cultivar K 326 when averaged over trials for the 3, and 4 week sampling date in (2004) (Figure 1) and the 3–6 week sampling period for site 1 (2005) (Figure 2). The resistant cultivar K 346 did not differ in percent infection for site 2, 2005.

Bacterial wilt increased ($P = 0.001$) in tobacco that was machine harvested when compared to tobacco that was hand harvested. *R. solanacearum*, when applied to the rubber defoliators as a bacterial suspension, was transmitted from the contaminated rubber defoliators to healthy tobacco plants by direct contact with stem tissue (Table 1). Plants were symptomless for 2–3 weeks after which bacterial wilt developed rapidly with typical bacterial wilt symptoms. In 2005, percent infection increased following mechanical leaf removal 864% and 12,750% for the susceptible (K 326) and resistant (K 346) cultivars, respectively when compared to hand harvesting. In 2006, *R. solanacearum* was transmitted from diseased plants, inoculated 8 days prior to leaf removal, to healthy plants within the row. Percent infection increased following mechanical leaf removal 1972% and 976% for the susceptible (K 326) and resistant (K 346) cultivars, respectively when compared to hand harvesting (Table 1). A cultivar $\times$ harvest method interaction was observed for stem necrosis (2006). Contamination of a steel stalk cutter with *R. solanacearum* increased percent infection, and crown and root necrosis ($P = 0.001$) when compared to tobacco stalks cut with a non-

Figure 2. Bacterial wilt in susceptible (K 326) and resistant (K 346) tobacco cultivars following mechanical transmission from inoculated plants to adjacent healthy plants during flower removal. The first two plants within a 22-plant plot were inoculated with *R. solanacearum*. The test was repeated in space (2005). The bars illustrate the standard error within a sampling date. The least significant differences ($P = 0.05$) for the 3,4,5 and 6 week sampling was 48, 56, 46 and 46% respectively for trial 1 and 24, 19, 19, 20 and 18% respectively for trial 2.

Table 1. Incidence of bacterial wilt in resistant and susceptible tobacco cultivars following mechanical leaf removal using a multipass tobacco harvester (ns = not significant)

Treatment ^{a,b}	2005		2006	
	% Infection	Stem necrosis ^c	% infection	Stem necrosis
<i>Tobacco cv. 326</i>				
Hand harvested	7.4	0.2	3.7	1.4
Machine harvested	64	1.9	73	3.4
<i>Tobacco cv. 346</i>				
Hand harvested	0.4	0	4.3	1.7
Machine harvested	51	1.3	42	3.0
$P > F$				
cv. 326 vs cv. 346	0.03	0.01	0.001	ns
Hand vs machine	0.001	0.001	0.001	0.001
cv. × harvest method	ns	ns	ns	0.02

^a Multipass tobacco harvester (Carolina Tobacco Services, Bennettsville, SC) was used to remove the lower four leaves of each plant within the row.

^b In 2005, inoculum (1×10^6 cfu of *R. solanacearum* isolate NC 132) was applied directly to the tobacco harvester defoliators immediately prior to leaf removal. In 2006, the first two plants at the beginning of the row were inoculated with 1×10^6 cfu of SC 06 by stab inoculation 15 cm above the soil line 8 d prior to leaf removal.

^c Stem necrosis rated on a 0 to 5 scale where: 0 = no vascular discoloration and 5 = 100% of the stem vascular tissue necrotic.

contaminated steel cutter. Averaged over trials, 100% of the root systems were infected with *R. solanacearum* following stalk cutting with a *R. solanacearum* contaminated cutter (Table 2).

Incidence of R. solanacearum on farm machinery

Pathogenic populations of *R. solanacearum* were recovered from steel defoliator knives, rubber defoliators and steel guides (51%, 50% and 50% of sampled tobacco

harvesters, respectively) (Table 3). Of 33 mechanical harvesters sampled, 17 contained viable populations of *R. solanacearum* as determined by a tomato bioassay. Some harvesters were parked within the field and were not actively harvesting tobacco. Since the harvesters were physically located within a tobacco field it was assumed that the harvester was used to process tobacco within the same day of sampling. Data are the cumulative number of samples collected over the 2001 and 2002 growing season in approximately equal proportions.

Table 2. Incidence of bacterial wilt in crown and root tissue following mechanical stalk cutting (ns = not significant)

Treatment ^a	% infection ^b	Crown necrosis ^c	Root necrosis
<i>Tobacco cv. 326</i>			
Surface sterilized cutter blade	12.5	0.7	0.7
Inoculated cutter blade	100	4.3	4.3
<i>Tobacco cv. 346</i>			
Surface sterilized cutter blade	7.5	0.9	0.9
Inoculated cutter blade	100	4.3	3.7
<i>P</i> > <i>F</i>			
cv. 326 vs cv. 346	ns	ns	ns
Untreated vs inoculated cutter blade	0.001	0.001	0.001
cv × inoculation	ns	ns	ns

^a Data are the average of two trials (2000 and 2002). Inoculum was applied directly to the steel cutter blade immediately prior to stalk cutting.

^b % Infection = number of plants with vascular discoloration within the crown tissue.

^c Crown (tissue located at the soil line) and root (roots branching from the tap root) necrosis was rated on a 0 to 5 scale where: 0 = no vascular discoloration and 5 = 100% of the vascular tissue necrotic.

Longevity of *R. solanacearum* on a tobacco topper

Pathogenic populations of *R. solanacearum* were recovered from steel topping knives from 0 to 3–6 hours after topping tobacco plants infected with *R. solanacearum* (Table 4). In trial 1, *R. solanacearum* could be recovered up to 5 h after topping diseased tobacco. In trial 2, which was conducted later in the year with a different source of infected tobacco plants, *R. solanacearum* could be recovered up to 3 h after topping diseased tobacco. Failure to recover culturable populations of *R. solanacearum* on enriched media does not exclude transmission of *R. solanacearum*. *R. solanacearum* can be in a viable state but not culturable on an enriched media (VBNC) (1). These trials did not study VBNC populations of *R. solanacearum*.

DISCUSSION

The major components in a tobacco disease management program for bacterial wilt include: crop rotation, host plant resistance, and soil fumigation with a multipurpose fumigant containing chloropicrin. Integration of these principals into a comprehensive management system provided

Table 4. Survival of *Ralstonia solanacearum* on mechanical topper blades following topping of *R. solanacearum* infected tobacco

Sampling interval	<i>R. solanacearum</i> positive	
	Trial 1 ^a	Trial 2
Immediately prior to topping	–	–
Immediately after topping infected tobacco	+	+
5 min	+	+
10 min	+	+
15 min	+	+
30 min	+	+
45 min	+	+
1 h	+	+
2 h	+	+
3 h	+	+
4 h	+	–
5 h	+	–
6 h	–	–
7 h	–	–
8 h	–	–

^a Topper placed under shelter in ambient air temperatures (30 °C and 33 °C, respectively for trial 1, and 2) to avoid direct sunlight on steel topper blades. *R. solanacearum* like colonies isolated from SM3 media were inoculated onto “cv. Rutgers” tomato. (+) = *R. solanacearum* recovered from bioassay test; (–) = *R. solanacearum* was not recovered from bioassay plant. Positive samples were confirmed for *R. solanacearum* using immunino assay strips. The trials were conducted on 25 July and 1 August, 2000 for trial 1 and 2, respectively.

excellent bacterial wilt control in tobacco for nearly a half century in the southeastern USA. Declining profit in tobacco production has resulted in farmers expanding their production in order to produce sufficient cash flow to support a farm operation. To process additional leaf, tobacco farmers have increased their reliance on mechanization in flower and leaf removal. The steady increase of bacterial wilt in South Carolina coinciding with the increased role of mechanization in tobacco management suggests *R. solanacearum* is being transmitted on contaminated equipment. The trials in these studies show that *R. solanacearum* can be efficiently spread from a point inoculation to the next 20 plants with a single pass of a topper, harvester or stalk cutter. The probability of spreading the bacterium from diseased tobacco plants to healthy plants would increase with each pass of a topper, or machine harvester. Farmers mechanically remove tobacco flowers and leaf with multiple passes down a crop row. In areas where bacterial wilt is prevalent and machine flower removal and leaf harvesting has been conducted,

Table 3. Incidence of *R. solanacearum* on mechanical harvesters in Marion and Horry counties, SC

Harvester type ^a	Number sampled	<i>R. solanacearum</i> positive	% contaminated
Steel defoliator knives	27	14	51
Rubber defoliator blades	4	2	50
Steel guides	2	1	50
Total number	33	17	51

^a Harvesters were randomly selected by drive through a county and sampling on a grid pattern with a minimum of 2 km distance between sampling sites. Data are the cumulative number of samples collected over the 2001 and 2002 growing season.

stalk infection reaches 100% of all plants within the field (personal observation based on grid sampling, data not presented). Host resistance to bacterial wilt reduced machine transmission in most experiments, however if conditions were favorable for bacterial colonization of stem tissue (high soil moisture) resistance was overwhelmed by the pathogen and little difference was observed between a resistant and susceptible cultivar (Figure 2). Greater stem necrosis was observed in hand harvested resistant tobacco (K 346) vs. the susceptible cultivar (K 326) at one location (Table 1, 2006). *R. solanacearum* was naturally present in the field at low levels and the level of stem colonization was small when compared to the susceptible variety K 326.

Normally, infection of susceptible crops by *R. solanacearum* occurs through the root system. However, *R. solanacearum* can be transmitted mechanically in other crop systems. *R. solanacearum* can be spread from diseased to healthy Perilla (*Perilla crispa* Tanaka) plants during harvesting where leaves are removed with a mechanical steel cutter (5). Likewise tomato can be inoculated with *R. solanacearum* during sucker removal and mechanical clipping (10, 11). In banana, *R. solanacearum* infects roots slowly through root wounds; however, disease increases rapidly when bacteria are mechanically transmitted from diseased plants to healthy banana plants by pruning tools. Early reports on tobacco suggest that *R. solanacearum* can be spread during hand topping (13, 15) however, our data suggests this is a very inefficient method of transmission. Presumably the bacteria would be on workers hands. Disease symptoms in tobacco may be misidentified as hollow stalk due to severe disintegration of the pith in some plants following machine flower removal (8).

The efficacy of transmission, and longevity of *R. solanacearum* on tobacco machinery allows for the rapid increase in losses to bacterial wilt. More alarming, farm machinery is often transported from farm to farm potentially moving the organism to previously uncontaminated fields. The rapid increase in bacterial wilt in South Carolina tobacco, along with disease occurring in fields with no history of bacterial wilt is an ecological disaster that will affect production of solanaceous crops within this area for generations to come.

Sterilization of contaminated tools or cutters is difficult during a topping or harvesting operations and would pose a serious challenge to producers. Although the movement of contaminated machinery will be difficult to control, recognition of the threat of machine transmission of *R. solanacearum* will encourage implementation of sound disease management strategies including equipment sanitation which will minimize the impact of cross contamination of fields with *R. solanacearum*. Improved design of mechanical tobacco toppers and harvesters, and chemical or biological controls (12) are needed to minimize machine transmission of *R. solanacearum*.

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