

Recent Widespread Damage of Commercial and Landscape Roses In Florida To Crown Gall Disease: Characterizing the Bacterial Strains and Establishing Management Strategies

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ABSTRACT

The bacterial strains associated with aerial and crown gall of roses with recent outbreaks were characterized and confirmed as *Agrobacterium tumefaciens* by biochemical and molecular assays. Ethanol (70% and 90%) and sodium hypochlorite (bleach; 0.1% and above) are highly effective in killing *Agrobacterium* cells within 10 seconds, while hydrogen peroxide at 3% killed all cells within 10 seconds.

Field studies on roses on effectiveness of compounds are in progress and have exhibited slower progression of galls and are currently being evaluated. The findings so far have been shared with through an EDIS extension article in press and a training program for producers, extension agents and regulators.

OBJECTIVES

1. To characterize the bacterial strains using cultural, biochemical and molecular assays, and compare to previously identified strains in the U.S and worldwide.
2. To assess pathogenicity and aggressiveness of the strains on carrot, tomato and commercially grown rose varieties.
3. To identify antibacterial sanitizers, effective concentrations and exposure times to minimize the risk of contamination through pruning equipment.
4. To develop a field management strategy using Systemic Acquired Resistance (SAR) inducers, antibiotics, bio-control agents and chemical control.
5. To disseminate the developed strategy for combatting crown gall through field trial demonstration (one training program to be conducted in Fall 2018 for rose producers, extension agents and industry personnel).

METHODS

Relevant to objective 1:

- a) Bacterial strains were isolated from the samples collected from many wholesale and retail nurseries, and landscapes in 2017-2018.
- b) Biochemical characterization of the strains were conducted and c) Molecular identification of the isolates was used to identify the strains by using conventional Polymerase Chain reaction (PCR) using 16S primers.

Relevant to objective 2:

- a) Carrot Disc Bioassay: Collected carrot samples were sterilized with commercial bleach followed by washing with sterile deionized water for three times. Each disc was overlaid with 100 µL of log 8 CFU/mL of the strains. The petri dish was sealed by parafilm and incubated in growth chamber at 25-30C. During 7 days, the disks were checked for young galls (tumors) developing from the tissue around the central vascular system.
- b) Tomato Stem Bioassay: Pathogenicity of the strains was confirmed on five-week-old tomato transplants with needle inoculation of bacterial suspensions containing 108 CFU/mL. Control plants (injected with water) was maintained in the growth chamber for 10-12 d at 25C and 70% RH. After 2 weeks, the stems were checked for young galls (tumors) developing from the stem tissue

Relevant to objective 3:

Hydrogen peroxide (0.1%, 0.5%, 1% and 3%), household bleach (0.1%, 0.5%, 10%, and 20%), ethanol (70% and 90%) and CuSO4 (1%, and 3%) were tested against a well-characterized *A. tumefaciens* strain isolated from rose in Florida under in-vitro conditions in NA media. The exposure times tested were 10 sec., 30 sec., 1 min., and 5 min.

Relevant to Objective 4:

Gallex (2,4-xyleneol, meta-cresol), Galltrol (bio-control agent, *A. radiobacter* Strain K84), and Nogall (bio-control agent, *A. radiobacter* Strain K1026) and Actigard (SAR inducer) are being tested by foliar application and also by painting on young galls. To determine the effectiveness of each chemical and concentration, the evaluations include number of galls, gall sizes, overall plant vigor, and bloom count for 4-6 months.

RESULTS

Relevant to objective 1:

Crown gall on roses was widely presently present in nurseries in Florida and in FNGLA member nurseries in Georgia. A total of 67 samples from 19 plants from 6 grower locations, which included mature and young galls from aerial parts or from the crown region of the plant, were collected and plated on a semi-selective medium for *Agrobacterium*. The pure culture of the isolates strains were characterized biochemically, which confirmed all strains as Gram negative, and oxidase positive. PCR and sequencing of a representative set of samples confirmed 8 strains as *Agrobacterium tumefaciens* (Table 1).

Table 1. Biochemical and molecular characterization of a representative set of purified strains confirmed as *Agrobacterium tumefaciens* isolated from galls on roses.

a - Negative

b + Positive

Strain number	Gram reaction	Oxidase	Carrot disk assay	Tomato assay	16S sequencing ID
1	- ^a	+ ^b	-	-	<i>Agrobacterium tumefaciens</i> strain 6N2
2	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain 6N2
3	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain IAM 12048 or strain NCPPB2437
4	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain 6N2
5	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain BGRI-SK8
6	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain IAM 12048 or strain NCPPB2437
7	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain TH05
8	-	+	-	-	<i>Agrobacterium tumefaciens</i> strain TH05

Relevant to objective 2:

The strains confirmed as *Agrobacterium tumefaciens* did not produce galls in carrot disk assay or tomato assay, which was unexpected and atypical for *Agrobacterium tumefaciens* (Table 2). Studies on roses are in progress.

Table 2. Carrot and tomato assay to test for the pathogenic characteristic of the strains

Strain number	Carrot disk assay	Tomato assay
1	-	-
2	-	-
3	-	-
4	-	-
5	-	-
6	-	-
7	-	-
8	-	-

Relevant to objective 3:

The in-vitro study demonstrated that sodium hypochlorite (household bleach; 0.1%, 0.5%, 10%, and 20%), ethanol (70% and 90%) and hydrogen peroxide at 3% killed all *Agrobacterium tumefaciens* cells within 10 seconds of exposure to the compound (Fig. 1). Some minor contaminants were noted in some of these plates, which were confirmed not to be *Agrobacterium* sp. by PCR. Hydrogen peroxide showed inconsistent responses at lower than 3% concentrations with 0.1% of hydrogen peroxide showed minimal effect on the cells, 0.5% and was not effective at 10 seconds exposure, but was effective at higher exposure times. The control CuSO4 had no effect on the cells in comparison to non-treated cells.

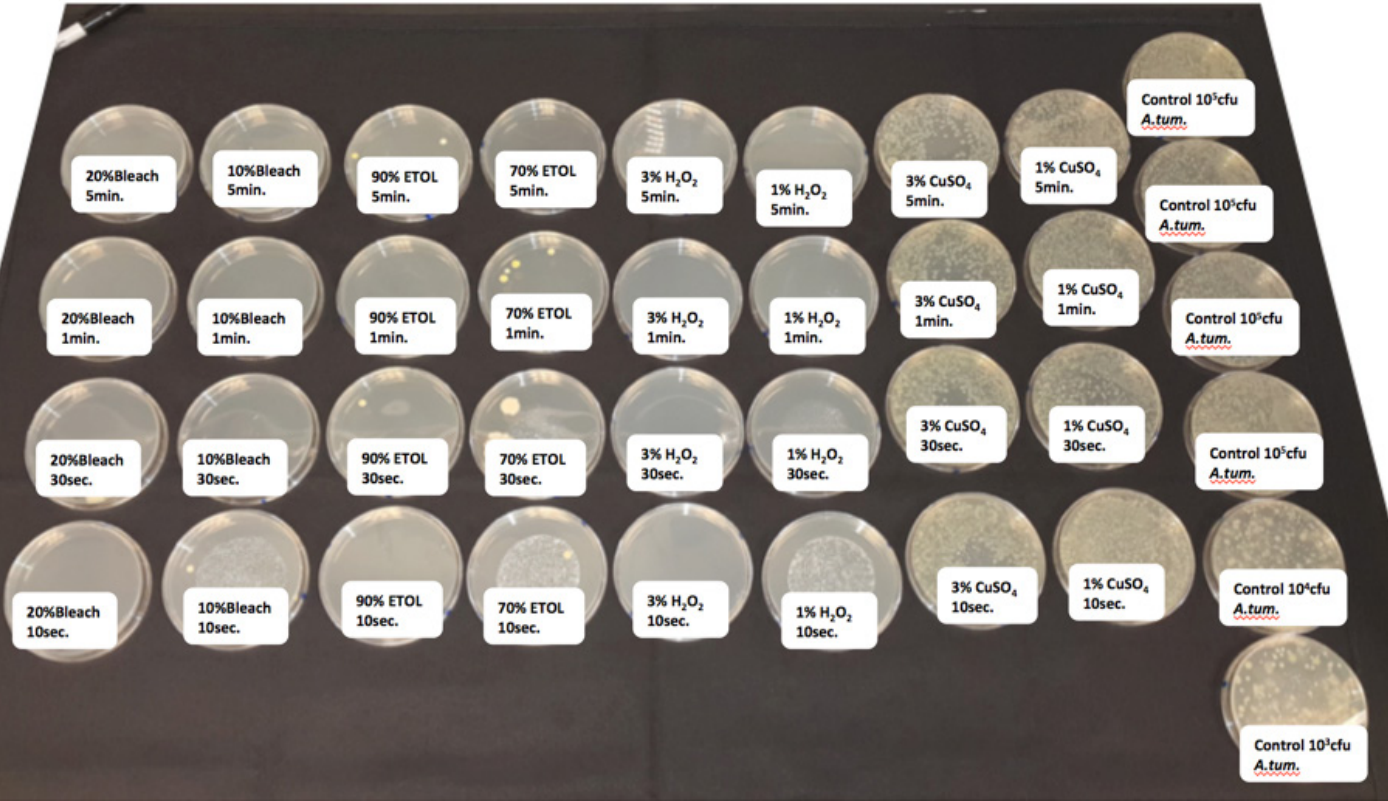
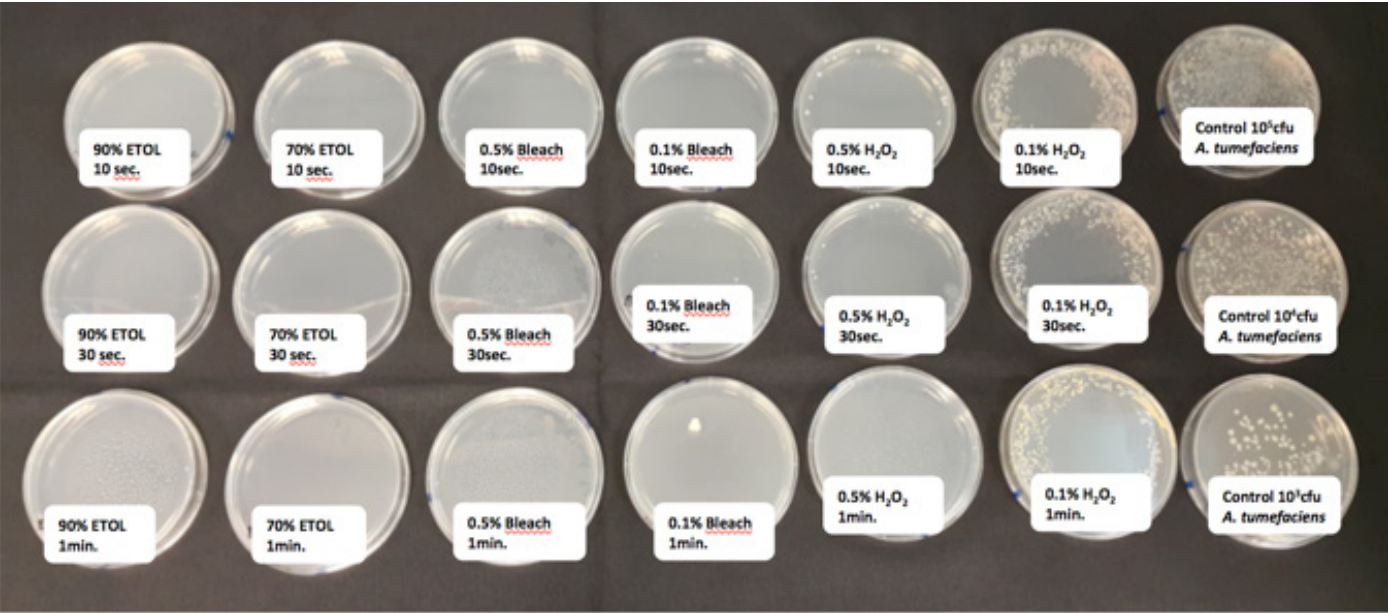


Fig. 1. Antibacterial properties of sodium hypochlorite (bleach), ethanol and hydrogen peroxide on a *Agrobacterium tumefaciens* isolated from rose.

Relevant to objective 4:

Field trials are in progress at NFREC, Quincy and the findings will be reported once the study is completed in Fall 2018 and potentially in Spring 2019 depending upon symptom development as progression of gall formation in roses is much more delayed than we expected.

Relevant to objective 5:

An EDIS document on *Agrobacterium* sp. on rose was prepared and now accepted for publication and should be available for all rose producers (wholesale, retail), extension agents, master gardeners in the next few weeks in the UF-IFAS extension website. The information pertaining to disease management approaches, strain biology, sanitation approaches for which the studies were completed was shared with Florida and Georgia producers at the Florida Georgia Green Industry update in June 2018. The findings were also individually shared with producers especially pertaining to sanitation practices for reducing the risks associated with crown gall.

CONCLUSIONS & RECOMMENDATIONS

The cause of crown gall in FNGLA associated nurseries were confirmed as strains of *Agrobacterium tumefaciens*. These strains are atypical in nature with no gall formation in carrot disk or tomato assay. The effective sanitizers for effectively killing *Agrobacterium tumefaciens* has been studied and duration of exposure required for this activity were confirmed. Field trials are in progress to evaluate compounds for effectiveness against crown gall disease. An extension article was developed and training program has been conducted for producers and other clientele.

Ethanol and bleach are effective sanitizers in reducing risks associated with *Agrobacterium tumefaciens*. Hydrogen peroxide is effective at the highest concentration tested in this study. Further recommendations based on field trials will be issued once the studies are completed.