Effect of *Allium fistulosum* Extract on *Ralstonia solanacearum* Populations and Tomato Bacterial Wilt

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Abstract

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To control bacterial wilt (*Ralstonia solanacearum,* phylotype IIB/4NPB), the antimicrobial effect of *Allium fistulosum* aqueous extract was assessed as a preplant soil treatment. Three concentrations of extract (100, 50, and 25%, 1:1 [wt/vol]) were evaluated by in vitro inhibition assay and in vivo experiments in a growth chamber. In vitro, *A. fistulosum* (100 and 50%) suppressed growth of *R. solanacearum.* Preplant treatment of the soil with *A. fistulosum* extract significantly reduced the *R. solanacearum* populations. No pathogen was detected in the soil after treatment with 100% concentrated extract from the third day after application until the end of the experiment. *A. fistulosum* also significantly reduced the incidence of tomato bacterial wilt. In the untreated control, the disease affected 61% of the plants whereas, with 100 and 50% extracts, only 6 and 14% of the plants, respectively, were affected. These results suggest that *A. fistulosum* extracts could be used in biocontrol-based management strategies for bacterial wilt of tomato.

Bacterial wilt caused by *Ralstonia solanacearum* occurs worldwide, affects hundreds of plant species, and is among the most widespread plant diseases in tropical and subtropical regions (17), including the West Indies. It affects both cash and subsistence crops. In Martinique (French West Indies), bacterial wilt is a major disease of vegetable crops, particularly of the most common crop, tomato, which has been affected by *R. solanacearum* phylotype I and IIA since the 1960s (28). After 1999, the situation changed with the emergence of new strains of *R. solanacearum* (positioned in phylotype IIB, sequevar 4NPB) whose hosts include members of the families Solanaceae, Cucurbitaceae, and Anthurium (36,37).

Despite the efforts of several research teams, the efficiency of current strategies for management of bacterial wilt is still limited. Bacterial wilt is a soilborne systemic disease that cannot be efficiently controlled through foliar application of copper pesticides, as reported by M. T. Momol (19). Although host resistance is the most effective control option, it is difficult to obtain cultivars with stable resistance across tropical locations given the high temperatures and humidity that prevail (14). Recent studies reported that the emerging *R. solanacearum* populations affect major resistant sources (23,35), making its control even more difficult. Hence, development of biorational soil treatments for effective management of bacterial wilt is highly desirable.

Natural plant products are important sources of new agrochemicals for the control of some plant diseases (1,8,16,18,21,30). Plant extracts are potentially environmentally safe alternatives and, thus, possible components of integrated pest and disease management programs. Several essential oils and their components have been reported to have a bactericidal effect against *R. solanacearum*; field experiments using thymol and palmarosa oil for preplant soil fumigation showed that it was effective in reducing the incidence of tomato bacterial wilt and resulted in higher yields (18). The use of plant essential oils as biofumigants has also been examined as a component in integrated disease management systems (19). Recently, palmarosa and lemongrass oils were shown to reduce the growth of *R. solanacearum* race 4 (phylotype I) in potting medium and the incidence of bacterial wilt of edible ginger (25). Other authors reported the potential of aqueous extracts of *Hibiscus sabdariffa*, *Punica granatum*, and *Eucalyptus globulus* to protect potato plants against bacterial wilt caused by *R. solanacearum* under greenhouse and field conditions (16).

Allium genus plants have been used since antiquity in traditional medicine and for their antimicrobial properties (15). The properties of *Allium* spp. are attributed to sulfur volatiles produced during degradation of *Allium* tissues (3). The most widely studied substance, allicin (diallylthiosulfinate), present in extract of *Allium sativum*, was shown to have significant antibiosis effects against a wide range of plant-pathogenic bacteria and fungi (12,32). Recently, Balestra et al. (4) demonstrated that *A. sativum* extract was highly effective in protecting tomato plants against three major disease-causing bacteria: *Pseudomonas syringae* pv. *tomato*, *Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*. Other studies showed that *A. sativum* extract reduced *R. solanacearum* populations in in vitro tests and tomato bacterial wilt incidence when applied to soil (2). Hence, we hypothesized that *A. fistulosum,* which is widely cultivated in Martinique and is well adapted to local soil and climatic conditions, could effectively reduce soil populations of *R. solanacearum* and reduce bacterial wilt incidence on tomato.

The main objective of this study was to evaluate the in vitro and in vivo antibacterial effects of aqueous extract of *A. fistulosum* on the density of the soil population of *R. solanacearum* and on the incidence of tomato bacterial wilt.

Materials and Methods

One laboratory test and two growth chamber assays were conducted at the Pôle de Recherche Agro-environnementale de Martinique. Laboratory experiment 1 aimed to evaluate the in vitro antibacterial activity of *A. fistulosum* aqueous extract on the growth of *R. solanacearum* on nutrient agar plates. Experiment 2 aimed to assess the effect of an aqueous extract of *A. fistulosum* on *R. solanacearum* population density in artificially infested natural soil. Experiment 3 aimed to evaluate the effect of preplant soil treatments with aqueous extracts of *A. fistulosum* on the incidence of bacterial wilt in a susceptible tomato cultivar.

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Bacterial culture and inoculum preparation. All experiments were performed using the highly virulent *R. solanacearum* strain CFBP6783, belonging to the emerging population phylotype IIB/4NPB (36), which was originally isolated from a diseased *Heliconia* sp. and deposited in the Collection Française de Bactéries Phytopathogènes (CFBP, Angers, France). A single colony of the isolate was grown on casamino acid peptone glucose medium (CPG) (22) for 48 h at 28°C. Bacteria were suspended in sterile distilled water, and cell density was adjusted to 10^8 CFU/ml using a spectrophotometer (optical density at 600 nm).

Plant extracts. Freeze-dried aqueous extracts of *A. fistulosum* L. ('Ciboule blanche') were used in experiment 1. The extract was obtained from fresh commercial plants (bulbs and leaves) and prepared according to Jourand et al. (20). Due to the high level of variability in concentration of active compounds between organs of *Allium* plant species (7) and because it is not known which organ extract from *A. fistulosum* would be most effective, we chose to use an extract including both leaves and bulbs. Dry powder (1 g) was diluted in 1 ml of sterile distilled water to obtain a final concentration of 100% (1:1 [wt/vol]). Crude aqueous extract of *A. fistulosum* was used in experiments 2 and 3. This extract was obtained from 100 g of fresh commercial plants (bulbs and leaves). Plants were washed with running tap water, then with sterile water. Plant materials were cut into small pieces and ground with 100 ml of sterile distilled water (1:1 [wt/vol]) in a blender for 1 min at room temperature and filtered through sterile double-layered cheesecloth. Freeze-dried and crude aqueous extracts at a concentration of 100% were then diluted with sterile distilled water to obtain two additional concentrations of 50 and 25%.

Natural soil. The soil used in these studies was a natural soil obtained from the top layer (first 15 cm, corresponding to the area of the roots of tomato plants) of a field located at the CIRAD station in Rivière Lézarde, Martinique (Nitisol: clay, 86.0% alloyite; silt, 5.3%; sand, 10.9%; organic matter, 4.55%; pH 5.5). The soil was sun dried for 4 days and sieved at 5 mm. Preliminary tests showed, that following soil infestation with *R. solanacearum* at 2.5×10^5 CFU/g of dry soil, disease incidence on the 'Heatmaster' reached 97% within 10 days (*data not shown*).

Experiment 1, in vitro inhibition assay. The antibacterial in vitro assay was carried out according to the agar-well diffusion method (33). Strain CFBP6783 was used at a concentration of 10^7 and 10^8 CFU/ml to obtain useful indications for survival studies in in vivo tests. The agar-well diffusion method was performed on CPG medium. Each bacterial suspension (200 µl) was spread on CPG medium in agar plates. Wells were made in nutrient agar plates using the broad end of a sterile Pasteur pipette (6 mm in diameter), and *A. fistulosum* extracts (20 µl of each concentration tested) were added to each well. Sterile distilled water was used as control. The plates were incubated at 28°C for 24 h and the diameters of inhibition zones were measured in millimeters after the incubation period. The experiments were repeated three times with three replicates in each block. Because there was no block effect, we presented nine replications for each extract concentration and each inoculum concentration for a total of 72 samples.

Experiment 2, population density after soil treatment. This experiment was performed in a growth chamber with an average relative humidity of 80%, in darkness, and a 30 to 26 ± 2°C temperature regime. Plastic bags were filled with 1.5 kg of dried soil, and 120 ml of sterile water were added to moisten the soil. The soil from each bag was then artificially infested by adding 40 ml of the *R. solanacearum* inoculum solution at 10^7 CFU/ml to reach a final estimated population of 2.7×10^5 CFU/g of dry soil. Two hours after soil contamination, bacterial populations, including *R. solanacearum* and "no RS bacteria" (bacterial populations other than *R. solanacearum* and growing on semi-selective medium [SMSA] medium), were enumerated using the method described below. Six hours after inoculation, treatments were applied by drenching the soil with 200 ml of *A. fistulosum* aqueous extracts at 100, 50, and 25% concentrations (three treatments), whereas the same amount of sterile water (200 ml) was used for the untreated control. Bags

were then vigorously shaken to homogenize their contents and closed (but not hermetically) to limit the escape of major volatiles and contamination. The bacterial population was enumerated at 3, 7, 10, 14, 21, 28, 35, 52, and 59 days after treatment. At each enumeration date, 5 g of soil from each bag was sampled and suspended in 25 ml of buffered soil dispersion solution (0.1 M NaCl, 0.01% sodium dodecyl sulfate, and 0.1% sodium pyrophosphate; pH 7.2) (34), shaken at 100 rpm for 30 min in a mixer-rotator REAX2 (Heidolph; Fisher Scientific Inc.), and left to settle for 5 min. The supernatant (1 ml) was used to start a 10-fold dilution series (from 10^{-1} to 10^{-4}) and 50 µl of each dilution was plated on duplicate plates of modified SMSA medium (13). After incubating for 3 days at 28°C, *R. solanacearum* colonies and no RS bacteria colonies that appeared on the plates were counted, and the number of viable cells in the soil at the time of sampling was estimated from the count. The *R. solanacearum* colonies isolated from the soil were further confirmed by Pmx-polymerase chain reaction (PCR) and Mmx-PCR analysis as described by Prior and Fegan (27). To enumerate the soilborne population as CFU per gram of dry soil, soil moisture was recorded at each sampling date. Two 10-g samples of soil were removed from each bag and oven dried at 130°C for 24 h to determine soil moisture. For each date and each treatment, the experiments have been repeated five times with two replicates per bag, giving a total of 400 samples.

Experiment 3, disease control. This experiment was performed in a growth chamber, with the climatic conditions and soil described above. Pots (0.4 liter) were filled with 400 g of dry soil, and 50 ml of sterile water was added to each pot to moisten the soil. The soil from each pot was then artificially infested by adding 10 ml of the *R. solanacearum* inoculum solution at 10^7 CFU/ml to obtain a final estimated population of 2.5×10^5 CFU/g of dry soil. Two hours after inoculation, treatments were applied by drenching the soil with 50 ml of *A. fistulosum* aqueous extracts at 100 and 50%, whereas controls received the same amount of sterile water (50 ml). To detect possible phytotoxicity problems on tomato, two durations of preplant treatment (15 and 30 days) were tested before the tomato plants were transplanted. During these periods (15 and 30 days), the pots were covered with aluminum sheets to limit the escape of volatiles and contamination. Two days before the tomato plants were transplanted, the aluminum sheets were removed to allow excess volatiles to escape. The susceptible tomato Heatmaster (Seminis Seeds) was used to assess the incidence of bacterial wilt disease. One 3-week-old tomato seedling was transplanted in each pot. Bacterial wilt incidence was calculated as the percentage of wilted plants. For each treatment, the experiments have been repeated three times with 12 replicates (individual pots) in each block. Each plant was checked separately three times a week for 4 weeks (total of 2,808 samples).

Wilted tomato plants were first sampled for isolation of *R. solanacearum* on modified SMSA medium. Presumptive colonies of *R. solanacearum* were confirmed by Pmx-PCR and Mmx-PCR analysis as described by Prior and Fegan (27).

At the end of the experiment (4 weeks after transplantation), the presence of *R. solanacearum* on remaining and asymptomatic plants was assessed on the basal part of the stem for the calculation of colonization index.

Statistical analyses. *Experiment 1.* Diameters of the inhibition zone were analyzed by analysis of covariance as a function of the concentration of extract (covariable), the concentration of the inoculum (factor), and their interaction. Means of inhibition diameters were then compared using Tukey's honestly significant difference test.

Experiment 2. Counts of *R. solanacearum* and no RS bacteria were analyzed using a generalized linear model (GLM) with Poisson error as a function of the concentration of the extract, time after treatment, and interaction between these two variables (fitted as quantitative variables). Differences in deviances (ΔDev) were used to assess the effects of factors when using the GLMs (24). Overdispersion was accounted for using Quasi-Poisson instead of Poisson models in R (11).

Experiment 3. The incidences of bacterial wilt (percentage of wilted plants) were analyzed using a generalized linear mixed-effects model (GLMM; 6) with a binomial error as a function of the duration of the preplant treatment (factor), the concentration of *A. fistulosum* extract (quantitative variable), time after transplantation (quantitative variable), and first-order interactions between these three variables. We included "plant identifier" and "block" as crossed (nonhierarchical) random effects. We treated them as random effects because we assumed that they contained unobserved heterogeneity that we could not model, and considered them as pseudo-replication. We used standard model simplification procedures to remove nonsignificant terms from the model (39). The significance of each term was assessed through the change in deviance between models with and without that term. We used the *glmer* function in the "lme4" library (5) in the statistical program R 2.12.1 (29).

Results

Experiment 1, in vitro inhibition assay. The concentration of the extract ($F = 387.6$; df = 1,68; $P < 0.0001$), the concentration of the inoculum ($F = 7.7$; df = 1,68; $P = 0.007$), and their interaction ($F = 4.6$; df = 1,68; $P = 0.034$) all had strong effects on the diameter of inhibition zone.

A. fistulosum extract at concentrations of 50 and 100% exhibited antimicrobial activity against *R. solanacearum* strain CFBP6783 at both bacterial concentrations (10^7 and 10^8 CFU/ml) used in vitro (Table 1). The best inhibition effects of the extracts were observed at a bacterial concentration of 10^7 CFU/ml. Growth of *R. solanacearum* was significantly lower when the concentration of the *A. fistulosum* was 100 rather than 50%, regardless of the bacterial concentration ($P < 0.0001$). At a concentration of 25%, no effects of *A. fistulosum* were observed on growth of *R. solanacearum* ($P = 1$).

Experiment 2, population density after soil treatment. The concentration of the extract ($\Delta Dev = 9038$; df = 1,396; $P < 0.0001$), time after treatment ($\Delta Dev = 89417$; df = 1,396; $P < 0.0001$), and their interaction ($\Delta Dev = 7797$; df = 1,396; $P < 0.0001$) had strong effects on the population of *R. solanacearum* in soil. The same variables also had significant effects on the population of no RS bacteria (concentration of the extract: $\Delta Dev = 1033233$, df = 1,396, $P < 0.0001$; time after treatment: $\Delta Dev = 45681$, df = 1,396, $P < 0.0001$; and their interaction: $\Delta Dev = 11176$, df = 1,396, $P = 0.0025$).

Soil treated with *A. fistulosum* extract reduced populations of *R. solanacearum* strain CFBP6783 and increased populations of no RS bacteria compared with the untreated control soil over a period of 60 days after soil treatment (Fig. 1). The initial population of *R. solanacearum* in the untreated control was 5.0 log CFU/g of dry soil. The population of *R. solanacearum* in the control remained stable, with similar counts of *R. solanacearum* (5.0 log CFU/g of dry soil) 60 days later (Fig. 1A). The effect of *A. fistulosum* extract was apparent 3 days after soil treatment. *A. fistulosum* extract at a concentration of 100% reduced the population of *R. solanacearum* faster than other concentrations (50 and 25%) and populations

were undetectable 3 days after soil treatment whereas, in soils treated with *A. fistulosum* extract at concentrations of 50 and 25%, the populations declined to 2.6 and 4.6 log CFU/g of dry soil, respectively, 3 days after soil treatment. At 60 days after soil treatment, population densities continued to be undetectable with *A. fistulosum* extract at a concentration of 100%; with *A. fistulosum* extract at a concentration of 50%, *R. solanacearum* populations declined from 5.0 to 0.8 log CFU/g of dry soil whereas, with *A. fistulosum* extract at a concentration of 25%, *R. solanacearum* populations remained high (4.3 log CFU/g of dry soil). The initial bacterial population of no RS bacteria in untreated control was undetectable at the beginning of the experiment and increased slightly to reach 0.3 log CFU/g of dry soil after 60 days (Fig. 1B). The effect of *A. fistulosum* extract on bacterial populations of no RS bacteria was apparent 3 days after soil treatment. With *A. fistulosum* extract at a concentration of 100%, bacterial populations of no RS bacteria increased considerably faster than with extract at other concentrations (50 and 25%) until reaching 8.0 log CFU/g of dry soil 3 days after soil treatment. In parallel, the no RS bacteria populations in soils treated with *A. fistulosum* extract at concentra-
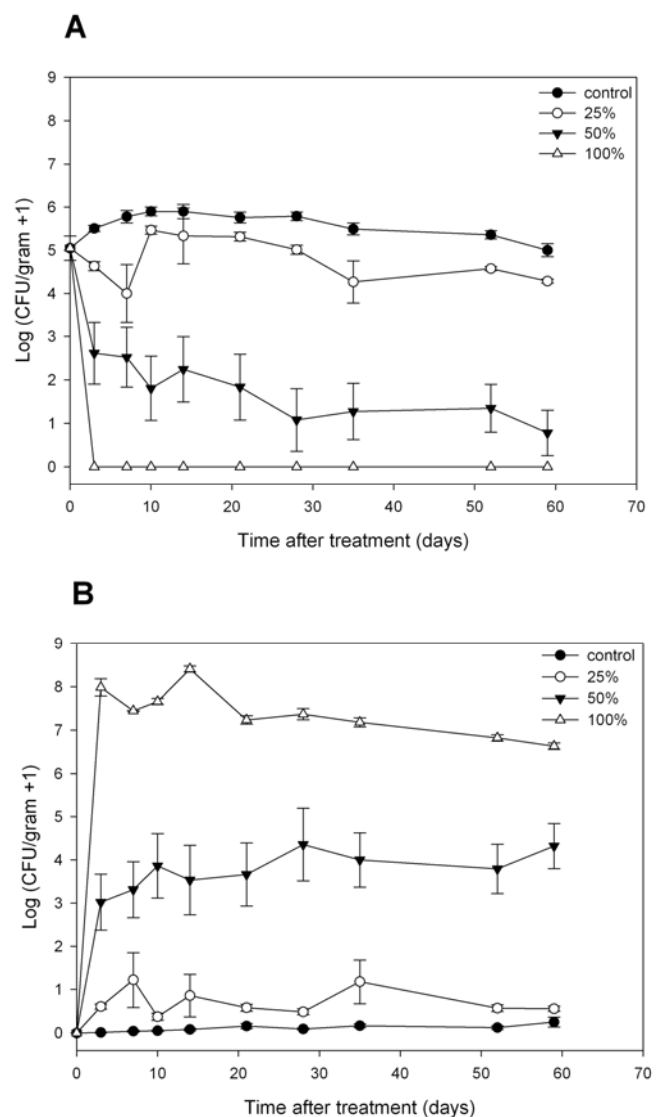

Fig. 1. A, Population of *Ralstonia solanacearum* phylotype IIB/4NPB and **B,** bacterial populations other than *R. solanacearum* in artificially infested soil after treatment with different concentrations (100, 50, and 25%) of *Allium fistulosum* aqueous extracts. Viable cells grown on semi-selective medium (South Africa) are reported as log CFU per gram of dry soil +1. Infested soil without extract served as control. Each data point is an average of 10 replications. Standard errors are shown by vertical bars.

Table 1. Antibacterial effects of *Allium fistulosum* extracts on growth of *Ralstonia solanacearum* phylotype IIB/4NPB on casamino acid peptone glucose medium after 24 h of incubation at 28°C

Concentration (%)[z]	Diameter of inhibition zone (mm) (means ± SE)[y]	
	10^7 CFU/ml	10^8 CFU/ml
100	19.2 ± 0.5 a	15.5 ± 0.6 a
50	14.1 ± 0.5 b	9.7 ± 1.0 b
25	0 ± 0 c	0 ± 0 c
Control	0 ± 0 c	0 ± 0 c

[y] Values are the mean of nine replicates. SE = standard error. Values within columns followed by different letters are significantly different at $P \le 0.05$ according to Tukey's honestly significant difference test.

[z] Concentration of *A. fisuolum* extracts (1:1 [wt/vol]); 1 g of extract diluted in 1 ml of distilled water corresponds to a concentration of 100%.

tions of 50 and 25% increased to 3.0 and 0.6 log CFU/g of dry soil, respectively, 3 days after soil treatment. At 60 days after soil treatment, densities of no RS bacteria populations reached 6.6 log CFU/g of dry soil with *A. fistulosum* extract at a concentration of 100%; with *A. fistulosum* extract at a concentration of 50%, bacterial populations increased to 4.3 log CFU/g of dry soil whereas, with *A. fistulosum* extract at a concentration of 25%, populations remained low (0.6 log CFU/g of dry soil).

Experiment 3, disease control. *A. fistulosum* extracts at concentrations of 100 and 50% were the most effective in the in vitro and soil population survival studies; therefore, these concentrations were selected for disease assessment. Mixed effects models (Table 2) showed a significant effect of the duration of the preplant treatment, the concentration of the extract, and interaction between these two variables on the incidence of bacterial wilt. The effect of time after transplanting was not significant but the effects of first-

order interactions between this variable and the two others (duration of the preplant treatment and concentration of *A. fistulosum* extract) on the incidence of bacterial wilt were significant.

Typical bacterial wilt symptoms were observed 5 days after the tomato plants were transplanted (Fig. 2). The cause of the wilt was confirmed to be *R. solanacearum* phylotype IIB/4NPB by isolating the bacterium from wilted plants followed by Pmx-PCR and Mmx-PCR analysis of the bacterial culture obtained.

A. fistulosum extracts provided effective protection against tomato bacterial wilt. At an extract concentration of 100%, only 6 and 3% of the plants were wilted at the end of experiment in the 15- and 30-day preplant treatments, respectively, which was significantly less than the untreated control (Fig. 2). *A. fistulosum* extracts at a concentration of 50% also significantly reduced the final incidence of bacterial wilt compared with the untreated control; 14 and 11% of plants were wilted in the 15- and 30-day preplant treatments, respectively. Both concentrations of *A. fistulosum* extract significantly protected tomato plants from bacterial wilt disease, regardless of the duration of preplant treatment.

At the end of the experiment, no asymptomatic tomato plants were infected at the basal part of the stem, suggesting the absence of latent infections in remaining Heatmaster tomato plants, regardless of the concentration of the *A. fistulosum* extract and the duration of preplant treatment.

Discussion

Due to the lack of commercially available resistant cultivars of tomato for the management of diverse plant-pathogenic organisms, including *R. solanacearum*, the use of plant-derived compounds to control bacterial diseases has been the subject of increasing interest (4,18,19,26). Because emerging *R. solanacearum* populations were reported to affect international major resistant sources (23), *A. fistulosum*-derived extract was evaluated as alternative biocontrol in this study.

In the current study, *A. fistulosum* extract was found to be effective in reducing *R. solanacearum* growth in an in vitro assay and,
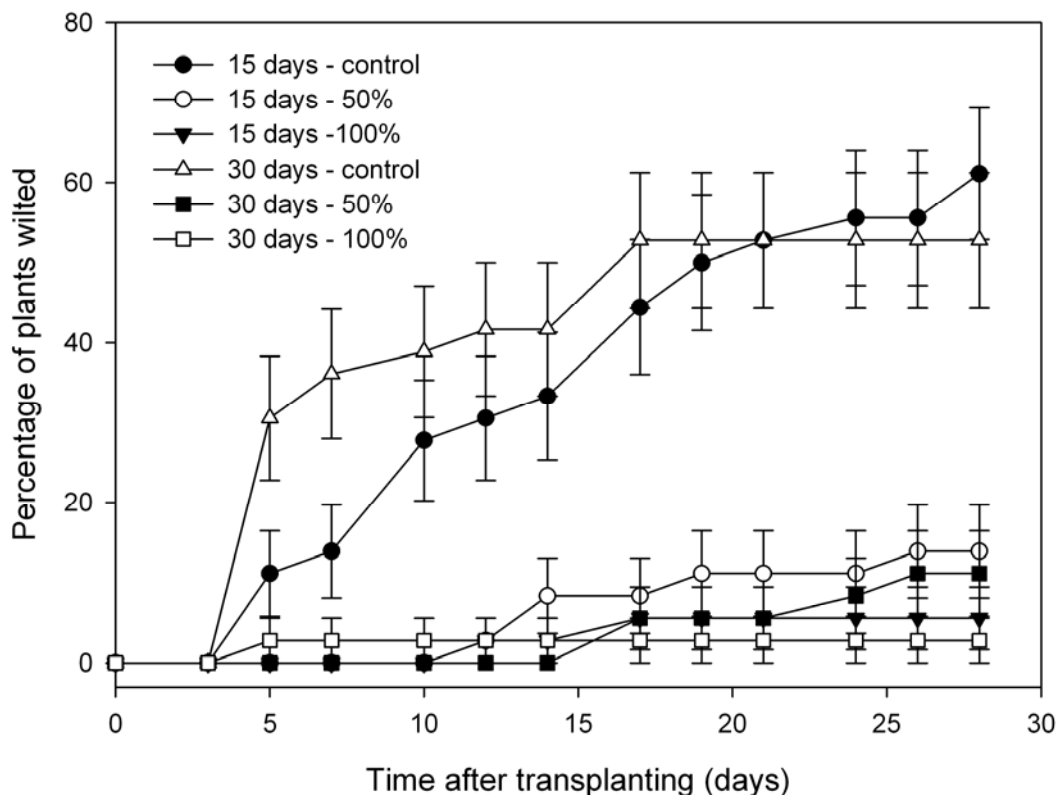
Table 2. Analyses of bacterial wilt incidence by generalized mixed-effect models with binomial error (total of 2,808 samples)

Model[z]	df	AIC	Log-likelihood	P value
d, t, c, c:t, d:t, c:d	9	805	−393	…
−c:t	8	862	−399	0.0005
−d:t	8	813	−398	0.0013
−c:d	8	810	−397	0.0054
d, t, c	6	832	−410	…
−d	5	1,179	−586	<0.0001
−t	5	831	−411	0.3548
−c	5	1,634	−812	<0.0001
Null	3	1,822	−908	…

[z] All models include two random effects: "plant identifier" and "block". Codes for effects: d = duration of preplant treatment, t = time after transplanting, c = concentration of *Allium fistulosum* extract. A null model with no variables was included for comparative purposes. The significance of each effect was tested by removing the variable from one of the two complete models.

Fig. 2. Incidence of bacterial wilt on tomato 'Heatmaster' with two durations of preplant treatments (15 and 30 days) at two concentrations (100 and 50%) of *Allium fistulosum* aqueous extracts. Infested soil without extract served as control. Values in the figure represent means of 36 replicates. Errors bars indicate standard errors of the means.

also, *R. solanacearum* populations and bacterial wilt incidence of tomato grown in infested soil.

Our studies, based on an in vitro assay using well diffusion, showed the antibacterial properties of *A. fistulosum* against the highly virulent *R. solanacearum* strain CFBP6783, belonging to the emerging population phylotype IIB/4NPB (experiment 1). The antibacterial effect was confirmed in in vivo assays using artificially infested soil. The *R. solanacearum* population decline effect was significant, rapid (3 days), and persistent (2 months) independent of the *A. fistulosum* extract concentration (experiment 2). *R. solanacearum* was not recovered from the soil treated with *A. fistulosum* extract at a concentration of 100% whereas several tomato plants wilted after transplantation in the same soil treated with *A. fistulosum* extract at a concentration of 100% (experiment 3). In this case, the pathogen may have survived at very low levels in the soil, making it undetectable by culturing. Residual inoculum was able to infect and colonize roots of tomato but not to any great degree. In the same experiment, growth of no RS bacteria populations on SMSA medium indicate that *A. fistulosum* extracts might increase microbial activity in the soil. However, no adverse effects were observed and no symptoms from major bacterial diseases developed on tomato plants.

The reduction in the *R. solanacearum* populations in the soil treated with *A. fistulosum* extracts at 100 and 50% concentrations resulted in a marked decrease in disease incidence on tomato Heatmaster in a growth chamber. Although much of the activity of *A. fistulosum* extracts may be inherent in the active compounds and extract concentration, the duration of preplant treatment determine effectiveness for disease control, as shown in our study.

Our finding that *A. fistulosum* extract greatly reduced *R. solanacearum* in soil within 3 days suggests that it could be used under field conditions with a short period between soil treatment and planting. Although it is expected that *R. solanacearum* populations in most field conditions are not as high as those used in this study, a hindrance to field application of *A. fistulosum* extract is the huge quantity of fresh plant material required. Thus, large-scale application of *A. fistulosum* extract to treat *R. solanacearum*-infested fields in Martinique is not yet possible considering that at, 100 and 50% concentrations, 40 and 20 kg of fresh plant material, respectively, would be needed to treat 1 m^2 to a 30-cm depth. To reduce the quantity required, application at field scale could be through drip irrigation lines, as already tested by Ji et al. (19) for application of thymol. However, further studies are needed to optimize the dosage of application to achieve maximum disease control efficacy and economic benefit.

Three different mechanisms may explain the observed disease control by *A. fistulosum* extracts: (i) direct antimicrobial activity of active compounds contained in the extract or liberated by the extract, (ii) indirect antimicrobial activity induced by the stimulation of antagonistic or competitive microorganisms, and (iii) induction of systemic resistance in host plants resulting in reduction of disease development. The first mechanism is well documented (3,30). Several molecules, including allicin (9,10), have been identified and their antimicrobial properties against a wide range of plant pathogens demonstrated (12,30,32). To demonstrate the bactericidal properties of *A. fistulosum* extract, epifluorescence microscopy has to be performed, as described by Paret et al. (25). Our results suggest that the second mechanism could play a role in the control of bacterial wilt induced by *A. fistulosum* extract but we were unable to confirm that hypothesis because we used SMSA to evaluate no RS bacteria populations in soil, and this medium only allows certain bacteria to grow. Thus, this hypothesis thus needs to be critically tested using nonselective media such as Levure-peptone-glucose agar (31). The third mechanism, induction of systemic resistance, has already been demonstrated by Hassan et al. (16) in potato plants against bacterial wilt disease by plant extracts. The induction of systemic resistance in tomato against bacterial wilt by *A. fistulosum* extract can be evaluated using a split-root system or by measuring the activity of defense-related enzymes (16).

Cover cropping followed by mulching of *A. fistulosum* in order to release active compounds of the crop into pathogen-infested fields is also an interesting approach. With this approach, two additional suppression mechanisms might occur: (i) disruption of the infectious cycle of *R. solanacearum*, leading to a reduction in its population in the soil, and (ii) release of allelopathic compounds in the soil via root exudates of crops with antibacterial properties against *R. solanacearum*, as reported by Yu (38) for *A. tuberosum*.

A. fistulosum extracts showed promising results in our study. The extract inhibits growth of *R. solanacearum* phylotype IIB/4NPB in vitro. Its application to soil drastically reduced *R. solanacearum* populations and the incidence of tomato bacterial wilt in growth-chamber experiments. Plant-derived compounds have the advantage of limited negative impacts on human health and on the environment. Due to the volatile property and broad-spectrum functions of *Allium* spp., *A. fistulosum* products could be used in the integrated management of soilborne disease in tomato if an increase in the yield of the tomato crop can be shown. Further studies are also required to determine the effectiveness of *A. fistulosum* products in the field and to compare cultivars with different levels of sulfur-based volatiles to manage bacterial wilt of tomato.

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