Biological Control of crazy root disease on hydroponically grown tomatoes using Pseudomonas strains

Cecilia Chagas de Freitas

Department of Plant Pathology, The Ohio State University, Wooster OH

Abstract

Crazy root disease (CRD) caused by *Agrobacterium rhizogenes* is a problematic disease leading to substantial losses in marketable yield in hydroponically grown cucumbers (*Cucumis sativus*) and tomatoes (*Solanum lycopersicum*). Growers use strict hygiene protocols and sanitation, relying on hydrogen peroxide and other sanitizers to clean the hydroponic system after disease outbreaks, however, this is an expensive and time-consuming process. Biological control is emerging as a possible solution to this troubling problem. Biological control is an environmentally sound and effective means of reducing or mitigating pests and diseases by employing the use of natural enemies. In this work we have tested and identified numerous strains of *Pseudomonas* that can inhibit the growth *A. rhizogenes* under *in vitro* and *in planta* conditions. In our *in vitro* experiments, 14 out of 52 different Pseudomonas strains were able to inhibit pathogen growth. In our *in planta* experiments we identified three different strains (1B1, 48G9 and 93G8) that were able to reduce disease incidence up to 95% on Kalanchoe and soybean. *Pseudomonas* treatments were able to reduce *Agrobacterium rhizogenes* numbers by nearly 1000-

fold in soybean and 100-fold in tomato. On hydroponically grown tomatoes, 1B1 and 93G8 were able to reduce disease incidence by 80%, compared with the non-*Pseudomonas* control while stain 48G9 was able to reduce disease incidence by 50%. Our results suggest that certain *Pseudomonas* strains can inhibit *A. rhizogenes* growth and disease development under hydroponic conditions and can be a potential biocontrol agent for hydroponic growers in the future.

Introduction

Crazy root disease (CRD) (also known as hairy root disease) caused by *Agrobacterium rhizogenes* biovar 1 is a problematic disease which leads to substantial losses in marketable yield of hydroponically grown cucumbers and tomatoes (Weller et al.2006). This disease was first reported in the 1970s in cucumbers fields and was not again detected until 1993 where it appeared in hydroponically grown cucumbers in the United Kingdom (O'Neil and Yarham, 1993). On tomatoes, CRD was first observed in 1997, but the causal agent was not identified as *A. rhizogenes* until 2000 (Weller et al.2000). Today the disease is present in many other countries including France, Belgium, Poland, Switzerland, Netherlands, Russia, Japan, New Zealand, Canada and USA (Ignatov et al.2016, Sawada and Azegami, 2014). In 2017, CRD was the most prevalent disease on hydroponically grown tomatoes in Ontario (Canadian Plant Disease Survey, 2018)

CRD is characterized by the extensive root proliferation with numerous roots containing numerous adventitious root hairs (Gelvin, 1990). The roots are agravitropic and grow upwards out of the top of the hydroponic substrate (rockwool cubes) and can cover the entire cube surface (Bosmans et al. 2017). Root over-proliferation redirects fixed carbon (i.e., sugar) from fruit which leads to reduced yield (Weller et al., 2006). CRD effect on yield include reduced fruit diameter, decrease marketable yield and overall fruit yield by 15% (Weller et al. 2000). Moreover, root overgrowth can block hydroponic tubes that deliver water and nutrients to the plants, which can result in increased prevalence of plant wilting and weakened plants. The dense root environment can also create an optimal environment for other plant pathogens such as *Pythium* to become established and induce root rots that can lead to vascular collapse and death of the plant (Weller et al. 2006).

Disease establishment occurs first upon contact of the bacteria with plant tissue. Upon activation of the virulence pathway, the bacterium transfers a small portion of its DNA that is located on a mega-plasmid (also known as the root-inducing plasmid; Ri plasmid) through a small tube attached to the plant cell. Upon transfer the small piece of DNA (also known as transfer DNA; T-DNA) is incorporated randomly into the plant host's chromosomes. After incorporation, the genes present on the T-DNA are expressed (Hooykaas and Beijersbergen, 1994), leading to hormonal (auxin) imbalance, phenotypic changes and subsequently overproduction of roots (Cardarelli et al. 1987, Nemoto et al. 2009). Once the plant is infected and over-proliferation of roots begins, the disease is not curable. The roots will continue to support further growth of the *A. rhizogenes* which can then spread through the hydroponic system and infect other plants. Growers rely on strict hygiene protocols and sanitation to try and limit initial infection. Sanitation using hydrogen peroxide and other chemical sanitizers is used to clean the hydroponic system after disease outbreaks, but it is an expensive and time-consuming process and does not treat the disease itself but rather reduces biofilm formation. Moreover, the sanitizer concentration may vary with the pathogen strain and sometimes very high concentrations are needed which can be detrimental to both plants and workers alike (Bosmans et al. 2016). The use of antibiotics to control the bacteria is also contra-indicated. Antibiotics use is expensive and highly regulated due to resistance issues (McManus et al. 2002) it is not a viable option in CRD management. Therefore, new alternatives to manage CRD in hydroponic systems are required.

Biological control is emerging as an integral part in managing CRD and other diseases in hydroponic systems. The use of biological control agents (BCAs) is well accepted in agriculture, especially under controlled environment conditions such as those that occur in greenhouses. The high acceptance of biological control in the greenhouse industry is due a lot of factors: the ability

to control the environment to favor BCA establishment (which it is hard to achieve in open fields), the high value of greenhouse crops (allowing growers to spend more with disease control) and the lack of chemicals registered for use in controlled environments which is perceived by the pesticide industry as either too small of a market or increases exposure time for workers (Paulitz and Belanger, 2001). Most BCAs used in greenhouses are for the control of insect pests. Application of predatory mites, parasitic wasp, and beetles are extensively employed to control thrips, aphids, whiteflies and fungus gnats. Although limited, there is research that describes the use of BCA for control of plant diseases in hydroponic systems. Khalil and Alsanius (2010), has shown that *Pseudomonas fluorescens* and other commercial biological control products could decrease root rot severity caused by oomycetes and fungal pathogens on hydroponically grown tomatoes. We hypothesize that the reduced biological complexity encountered in hydroponic substrates such as rockwool can be advantageous for BCAs by reducing the potential growing spaces for competitors that could either compete with or kill the BCAs.

In this described research, we tested strains of *Pseudomonas* spp. for their ability to inhibit *A. rhizogenes* growth and decrease CRD incidence. *Pseudomonas* spp. is a diverse group of bacteria that are ubiquitous in soils. Pseudomonads as a group present many characteristics that make them potent and useful BCAs. They grow quickly, are good colonizers of the rhizosphere, actively compete against other bacterial competitors, can adapt to many types of stresses, produce and secrete a wide range of bioactive compounds, and have been characterized for their biocontrol activity against plant pathogens (Weller, 2007). *Pseudomonas* strains have been shown to exhibit activity against fungal (Yang and Hong, 2018; Cabanas et al. 2018; Khan et al. 2004; D'aes et al. 2011), nematode (Lax et al. 2013; Nam et al. 2018; Norabadi et al. 2013) and bacterial disease causing organisms (Lanteigne, et al. 2012; Sun et al. 2017, Subedi et al. 2019). Some

Pseudomonas strains have been identified for control of related agrobacterial diseases including crown gall disease caused by *Agrobacterium tumefaciens* (Tolba and Soliman, 2013; Dandurishvili et al. 2010) and *Agrobacterium vitis* (Khmel et al. 1998). To our knowledge, this is the first study demonstrating the use of *Pseudomonas* strains to control *A. rhizogenes* in the management of CRD.

Material and Methods

1) Bacterial strains and culture conditions

For this study, we used 52 strains of *Pseudomonas* spp. collected from soils in Ohio, Wisconsin, Wyoming and Missouri. Mississippi and Missouri Rivers and from Missouri Botanical Garden frozen material herbarium collection (Table 1). The collection was described previously by Subedi et al.2019, Mavrodi et al.2012, and McSpadden Gardener et al. 2005). The *A. rhizogenes* strain used for this study was the biovar 1 strain designate K599 (also called NCPPB 2659). This strain was originally isolated in 1974 from infected cucumbers in England and has become a significant problem in hydroponic greenhouses. *A. rhizogenes* K599 is highly infective, inducing the formation of large numbers of transgenic roots with abundant lateral root branching. All *Pseudomonas* and *Agrobacterium* strains were grown in LB broth (with the appropriate antibiotics when needed) for 18h at 28 °C, 200 rpm and the final bacterial concentration adjusted for OD600=10^8 CFU/ml.

2) *Agrobacterium rhizogenes* plasmids used for *in planta* assays

For all plant and hydroponic assays, binary plasmids containing left and right border T-DNA sequences flanking reporter gene constructs for the green-fluorescent protein (GFP) or the sweet potato transcription factor (IbMYB1-TF) driven by the pine super ubiquitin promoter

(Collier et al. 2005) were electroporated into the *A. rhizogenes* strain K599. Identification of hairy roots is determined by co-expression of the reporter genes. Expression of GFP in roots was observed using a fluorescence microscope (Excitation 485nm; Emission 505nm) while expression of the IbMYB1-TF induces production of anthocyanin in the transgenic roots, making the transgenic roots to turn purple (Figure 1).

3) *Pseudomonas* strains antagonistic activity *in vitro*

The antagonistic activity *in vitro* was performed by a zone of growth inhibition assay. First, 250 ml of 0.7% LB was inoculated with 250 µl of an *A. rhizogenes* suspension and 7 ml was added to cover an LB plate. Subsequently, sterile 6mm filter paper disks were placed on the top of the plate (2 per plate) and, in each filter, we added 20 µl of the *Pseudomonas* strain. LB only was used as negative control and the antibiotic kanamycin as a positive control. The plates were incubated for 24 to 48 hours at 28^{o}C, and the zones of growth inhibition were measured using a ruler. Strains with significant inhibition were selected for studies in plants. This experiment was replicated seven times.

4) *Pseudomonas* strains antagonistic activity *in planta*

The strains that presented the largest zones for growth inhibition of *A. rhizogenes in vitro* were selected to demonstrate its biocontrol activity in planta against crazy root development. These isolates were tested for their ability to suppress crazy root development in Kalanchoe (*Kalanchoe daigremontiana*). *Kalanchoe daigremontiana* is a model plant to study *Agrobacterium* spp. Each plant stem was pierced with a toothpick (four plants per treatment). Into each plant, 20 µl of saline solution (0.85% NaCl) or *Pseudomonas* suspension was

inoculated. After 10 minutes, the wounds were wrapped in Parafilm. 24 h later, the plants were re-wounded and 20 µl of the GFP-*A. rhizogenes* suspension was inoculated. The stems were wrapped in Parafilms again for 48h. The plants were kept in a greenhouse for three weeks when the disease incidence was accessed. This experiment was repeated ten times in a completely ramdomized design with four plants per treatemnt.

The *Pseudomonas* strains that presented low disease incidence in *Kalanchoe* were selected to demonstrate its biocontrol activity in soybean (*Glycine max*) composite plants (Collier et al. 2005). Soybean is a model plant in our lab for testing *Agrobacterium* spp. genetics. Briefly, soybean (cv. Williams 82) cuttings were placed in a *Pseudomonas* suspension for 3 minutes. After 6h, the same cuttings were placed in a GFP-*A. rhizogenes* suspension for 3 minutes. *Pseudomonas* and pathogen suspensions were prepared in 1/4x Murashige and Skoog (MS) basal medium (pH 5.8) (Sigma-Aldrich, St Louis, MO, USA) to an OD600= 0.3. The MS buffer alone was used as negative control. The cuttings were placed in a plastic bag with a wet paper towel for a week kept at room temperature. After sitting cuttings were transplanted into pots containing vermiculite. The pots were placed in a mist chamber for a week and in a growth chamber (24^0C) for another week. Disease incidence was measure determined by examining shoots for the presence of transgenic roots (GFP positive roots). This experiment was repeated four times in a randomized complete block design with thirty shoots per treatment divided into three groups of ten (blocks). The presence of GFP positive roots on each shoot was recorded and used to calculate disease incidence (# of shoots with GFP roots/total shoots). The results of the three groups per treatment was averaged, to get the final mean for that each treatment in a given experiment.

5) *Agrobacterium rhizogenes* persistence assay in tomato and soybean

Soybean (cv. Williams 82) and tomato (cv. Money Maker) composite plants were inoculated as previously described. Tissue samples (bottom 1 cm of each stem) were collected 0 and 3 days after inoculation. The tissue was weighed and macerated in 200 µl 0.85% NaCl and suspended. For plating, we used 10-fold serial dilutions (8 times) that were prepared in 96-well microplates. For each dilution, 10µl were pipetted onto a plate containing LB agar (in triplicate for each sample). The plates were air dried and then incubated for 48h when the number of colonies was counted. Each experiment was repeated four times in a completely randomized design. For each time point, five plants were collected.

6) Biological Control of CRD under greenhouse/hydroponic conditions

The three most active strains plus a non-active strain were tested under hydroponic conditions. Three to four weeks old tomato seedlings (cv. Money Maker) were transplanted to rockwoll cubes (4"x4"x4") and kept under hydroponic conditions. During the transplant, plants were placed in 500ml beakers and roots were soaked with a *Pseudomonas* solution or water (negative control) for three minutes. Six hours later the rockwool root systems then challenged with an *A. rhizogenes* K599-Myb-TF solution for three minutes. The blank treatment was soaked in water twice. The blank was set up to check for *A. rhizogenes* cross contaminations among treatments. Plants were visually evaluated after four weeks (presence of purple roots) and disease incidence recorded. The experiments were repeated four times in a randomized

complete block design. Each block consists of a hydroponic table with a total of four blocks (each treatment is present once in each table). The experiment unit consists of a tray containing nine rockwool cubes and one tomato plant per cube. We recorded the disease presence in all nine plants in the tray and calculated the disease incidence per tray. In the end, we averaged the results of the four tables together, to get the final mean for that given experiment.

7) Statistical analysis

Analysis of variance (ANOVA) was performed to determine the impact of *Pseudomonas* strains using the GLIMMIX procedure of SAS. Means were separated using Dunnett's test (Kalanchoe assay) and Tukey-Krammer test (soybean assay, *A. rhizogenes* persistence assay and hydroponic assay) with SAS software (SAS Institute, Inc., Cary, NC).

Results

1) *Pseudomonas* strains antagonistic activity against *A. rhizogenes in vitro*

Among the 52 screened strains, only fourteen exhibited considerable inhibitory activity in *vitro* (1B1, 15D11, 14B11, 14D6, 48B8, 49G9, 1F2, 15H10, Clinton, Darke, Wayne, 37A11, 93D8, 93F8 and 93G8). The mean values of inhibition halos of *A. rhizogenes* K599 ranged from 15.9 to 25.7 mm (Table 1). 1B1 presented the greater inhibition, followed by Darke and 1F2.

2) *Pseudomonas* strains antagonistic activity in Kalanchoe

Among the fourteen strains that presented the largest inhibition halos for the growth of *A. rhizogenes in vitro*, three were able to reduce crazy root formation in Kalanchoe significantly. 1B1 and 93G8 reduced the CRD incidence up to 100%. 48G9 reduced disease incidence up to

50%. The other strains were not effective in decreasing CRD incidence. All other strains and the saline solution resulted in 100% CRD incidence (Figure 2 and Figure 3).

3) *Pseudomonas* strains antagonistic activity in soybeans

On soybean plants, we tested 1B1, 93G8, 48G9 and a non-active *Pseudomonas* as a control. The control plants (MS buffer and 1F2) presented high levels of disease incidence, 95% on the plants treated with the buffer solution and 70% on plants treated with the strain 1F2. Plants treated with the active strains showed lower disease incidence compared with the controls. 48G9 treated plants presented 41% disease incidence and only 10% on plants treated with 93G8 and 7% of the plants treated with 1B1 presented transgenic roots, representing almost 90% decrease in disease incidence (Figure 4).

4) *A. rhizogenes* persistence on soybeans and tomatoes

The *A. rhizogenes* persistence on soybeans and tomatoes decreased upon the treatment with *Pseudomonas* strains. On soybeans, at day 0, the control plants (water only) and the *Pseudomonas* treated plants presented the same number of *A. rhizogenes* (around 10^8 CFU/gram of plant tissue) (Figure 5A). At day 3, the level of *A. rhizogenes* on control plants increased (10^9 CFU/gram of plant tissue), while plants treated with 48G9, 1B1 and 93G8 *A. rhizogenes* levels decreased (10^6 CFU/gram of plant tissue), representing a reduction of 1000-fold (Figure 5B).

On tomatoes, we observed a similar trend. At day 0, the control plants and the *Pseudomonas* treated plants had similar levels of *A. rhizogenes* (around 10^8 CFU/gram of plant tissue) (Figure 6A). At day 3, the *A. rhizogenes* on control plants held steady (10^8 CFU/gram

of plant tissue), while in the plants treated with 48G9, 1B1 and 93G8 *A. rhizogenes* levels decreased (10^7 CFU/gram of plant tissue), representing a reduction of 10-fold (Figure 6B). These results suggest that the active *Pseudomonas* strains can decrease *A. rhizogenes* growth in plants.

5) Biological Control of CRD under greenhouse/hydroponic conditions

Under hydroponic conditions, the blank treatment presented no disease, indicating that the system was clean with no cross contamination. The disease incidence of the *A. rhizogenes* control (water only) was above 90% while those treated with *Pseudomonas* strains was reduced. Disease incidence in plants treated with *Pseudomonas* strain 1F2 showed 60% disease incidence and 48G9 showed 40% disease incidence. *Pseudomonas* strains 1B1 and 93G8 showed only 20% disease incidence (Figure 7).

Discussion

CRD caused by *A. rhizogenes* biovar 1 strain is a problematic disease in hydroponically grown tomato and cucumber worldwide. Currently, there is no efficient and environmentally safe method available to manage the disease. Despite the increasing importance of CRD in hydroponic greenhouses, information about biological control is still limited. This study is the first reported evidence for *Pseudomonas* biocontrol of CRD. Only one other study describing the efficacy of a possible BCAs against *A. rhizogenes* has been reported. Bosmans et al. 2017 tested the effectiveness of *Paenibacillus* strains against *A. rhizogenes* biovar 1. They found that tomato plants treated with the biocontrol agent showed a reduction of 45% in disease incidence. Together with

our results, this is an indication that the biological control of CRD is possible and it is still a niche to be fully explored.

Pseudomonas spp. are well known as biological control agents against a wide range of plant pathogens. Studies have shown that *Pseudomonas* spp. are promising in controlling diseases caused by fungi, bacteria, and nematodes. Indigenous *Pseudomonas* spp. isolated from olive rhizosphere were effective in reducing olive wilt caused by *Verticillium dahliae* in plants pre-inoculated with them (Cabanas et al. 2018). *Pseudomonas brassicacearum* strain LBUM300 significantly reduce tomato bacterial canker caused by *Clavibacter michiganenses* subsp. *michiganensis* (Lanteigne et al. 2012). Tomato treated with *Pseudomonas protegens* strain CHA0 presented a reduced number of nematodes, egg masses and nematode reproduction factors against the false root-knot nematode *Nacobbus aberrans* under controlled conditions (Lax et al. 2013). Other studies have shown the efficacy of *Pseudomonas* spp. against *A. tumefaciens* causing crown gall in roses and tomato and *A. vitis* in causing crown gall in grapevine. Tolba and Soliman (2013) showed that two strains of *Pseudomonas fragi* were able to reduce crown gall incidence by 88.9% in roses shoots. *Pseudomonas fluorescens* was able to reduce gall masses on tomato stems after four weeks (Dandurishvili et al. 2010). Grapevine plants treated with *Pseudomonas aureofaciens* strains B-4117 and *P. fluorescens* strain CR330D presented reduced disease incidence and severity during seedling root production and grafting (Khmel et al. 1998). From our collection, some strains showed to be effective against *Rhizoctonia* and *Pythium* root rot of wheat (Mavrodi et al. 2012), *Pythium* root rot of soybean (McSpadden Gardener et al. 2005) and *Ralstonia pseudosolanacearum* (Subedi et al. 2019).

After an extensive screening of a collection containing 52 *Pseudomonas* strains, we found that fourteen strains can reduce *A. rhizogenes* growth in vitro. However, only three were effective

against *A. rhizogenes in planta* (*Kalanchoe daigremontiana*, tomato, and soybean). Antagonist strains include 1B1 identified as *P. protegens*, 93G8 identified as *P. brassicacearum* and 48G9 identified as *P. chlororaphis*. Mavrodi et al. (2012) using twenty of our strains, observed the same, where some strains had activity *in vitro*, but not in plants. On the other hand, some strains that exhibited no activity *in vitro*, presented some activity when tested in plants. In a study with *Ralstonia pseudosolanacearum* wilt on tomato plants using our collection, 42 strains were effective *in vitro*, but only three reduced disease incidence and severity on tomato seedlings (Subedi et al.2019). Cabanas et al. 2018 reported that *Pseudomonas* strains that showed better inhibition of *Verticillium dahliae in vitro*, behaved the opposite in olive plants, presenting disease parameters statically equal to the untreated control. Similarly, *P. fluorecens* could inhibit *Ralstonia solanacearum* in vitro but failed to protect *Eucalyptus* against bacterial wilt (Ran et al. 2005*). In vitro* assays based on halo of growth inhibition rely mostly on antibiotics production by the antagonist bacteria (Chen et al. 2003). Since we still don't know the mode of action of the active *Pseudomonas*, we can't rule out other strains as potential BCAs against *A. rhizogenes*.

In hydroponic systems, only 1B1 and 93G8 were effective. The activity of the tested *Pseudomonas* strains was reflected mainly due to a substantial decrease in CRD incidence. While the untreated plants showed nearly 100% disease incidence, plants treated with 1B1 and 93G8 showed 20% disease incidence. Several other studies on hydroponic systems have demonstrated the effect of *Pseudomonas* against other pathogen, mostly fungi and oomycetes causing root rots. A *P. protegens* related strain was effect reducing *Rhizoctonia* root rot on lamb's lettuce (Moruzzi et al. 2017). The bacterial strain *Pseudomonas fluorescens* 5.014 reduced *Pythium ultimum* and *P. aphanidermatum* root rot on tomatoes (Khalil and Alsanius, 2010). *P. chlororaphis* reduced root

rot caused by *Pythium ultimum* by 50% on lettuce (Lee et al.2015). Therefore, *Pseudomonas* have potential to be incorporated as a tool in managing diseases during hydroponic cultivations.

This study demonstrates that *Pseudomonas* strains can inhibit *A. rhizogenes* growth *in vitro* and *in planta*. Pseudomonas strains 1B1 and 93G8 are effective reducing disease incidence by up to 80% in hydroponically grown plants. Therefore, they are promising candidates to be incorporated in a biological control product against CRD. Further studies are necessary to elucidate their modes of action and survival in the hydroponic system. This way we can improve their performance and develop application recommendations for hydroponic growers.

Acknowledgments

We thank the help of Edwin Navarro and Angel Leon during data collection. The IR4 project for funding this work. An additional thanks to CAPES and the Brazil Science Without Border Program for the financial support to Cecilia Chagas de Freitas

References

1. Bosmans L., Van Calenberge B., Paeleman A., Moerkens R., Wittemans L., Van Kerckhove S., De Mot R., Lievens B., and Rediers H. 2016. Efficacy of hydrogen peroxide treatment for control of hairy root disease caused by rhizogenic agrobacteria. J. Appl. Microbiol. 121 2:519-527.

2. Bosmans L., De Bruijn I., Gerards S., Moerkens R., Van Looveren L., Wittemans L., Van Calenberge B., Paeleman A., Van Kerckhove S., De Mot R., Rozenski J., Rediers H., Raaijmakers J. M., and Lievens B. 2017. Potential for Biocontrol of Hairy Root Disease by a *Paenibacillus* Clade. Frontiers in Microbiology 8:447.

3. Cabanas C Gomez-Lama., Legarda G., Ruano-Rosa D., Pizarro-Tobias P., Valverde-Corredor A., Niqui J. L., Trivino J. C., Roca A., and Mercado-Blanco J. 2018. Indigenous *Pseudomonas* spp. Strains from the Olive (*Olea europaea* L.) Rhizosphere as Effective Biocontrol Agents against *Verticillium dahliae*: From the Host Roots to the Bacterial Genomes. Frontiers in Microbiology 9:277.

4. Canadian Plant Disease Survey, 2018.

5. Cardarelli M., Spano L., Mariotti D., Mauro M., Vansluys M., and Costantino P. 1987. The Role of Auxin in Hairy Root Induction. Molecular & General Genetics 208 3:457-463.

6. Chen W., Morgan D., Felts D., and Michailides T. 2003. Antagonism of *Paenibacillus lentimorbus* to *Botryosphaeria dothidea* and biological control of panicle and shoot blight of pistachio. Plant Dis. 87 4:359-365.

7. Collier R., Fuchs B., Walter N., Kevin Lutke W., and Taylor C. 2005. *Ex vitro* composite plants: an inexpensive, rapid method for root biology. Plant Journal 43 3:449-457.

8. D'aes J., Gia Khuong Hoang Hua, De Maeyer K., Pannecoucque J., Forrez I., Ongena M., Dietrich L. E. P., Thomashow L. S., Mavrodi D. V., and Hofte M. 2011. Biological Control of Rhizoctonia Root Rot on Bean by Phenazine- and Cyclic Lipopeptide-Producing Pseudomonas CMR12a. Phytopathology 101 8:996-1004.

9. Dandurishvili N., Toklikishvili N., Ovadis M., Eliashvili P., Giorgobiani N., Keshelava R., Tediashvili M., Vainstein A., Khmel I., Szegedi E., and Chernin L. 2011. Broad-range antagonistic rhizobacteria *Pseudomonas fluorescens* and *Serratia plymuthica* suppress Agrobacterium crown gall tumours on tomato plants. J. Appl. Microbiol. 110 1:341-352.

10. Gelvin S. 1990. Crown Gall Disease and Hairy Root Disease - a Sledgehammer and a Tackhammer. Plant Physiol. 92 2:281-285.

11. Hooykaas P., and Beijersbergen A. 1994. The Virulence System of *Agrobacterium-tumefaciens*. Annu. Rev. Phytopathol. 32:157-179.

12. Ignatov A. N., Khodykina M. V., Vinogradova S. V., Polityko V. A., Pluschikov V. G., and Kornev K. P. 2016. First Report of Rhizogenic Strains of *Agrobacterium radiobacter* Biovar 1 Causing Root Mat of Cucumber and Tomato in Russia. Plant Dis. 100 7:1493-1493.

13. Khalil S., and Alsanius B. W. 2010. Evaluation of biocontrol agents for managing root diseases on hydroponically grown tomato. Journal of Plant Diseases and Protection 117 5:214-219.

14. Khan M. R., Khan S. M., and Mohiddin F. A. 2004. Biological control of Fusarium Wilt of chickepea through seed treatment with the commercial formulation of *Trichoderma harzianum* and/or *Pseudomonas fluorescens*. Phytopathol. Mediterr. 43 1:20-25.

15. Khmel I., Sorokina T., Lemanova N., Lipasova V., Metlitski O., Burdeinaya T., and Chernin L. 1998. Biological control of crown gall in grapevine and raspberry by two *Pseudomonas* spp. with a wide spectrum of antagonistic activity. Biocontrol Sci. Technol. 8 1:45-57.

16. Lanteigne C., Gadkar V. J., Wallon T., Novinscak A., and Filion M. 2012. Production of DAPG and HCN by *Pseudomonas* sp LBUM300 Contributes to the Biological Control of Bacterial Canker of Tomato. Phytopathology 102 10:967-973.

17. Lax P., Marro N., Agaras B., Valverde C., Doucet M. E., and Becerra A. 2013. Biological control of the false root-knot nematode *Nacobbus aberrans* by *Pseudomonas protegens* under controlled conditions. Crop Protection 52:97-102.

18. Lee S., Ge C., Bohrerova Z., Grewal P. S., and Lee J. 2015. Enhancing plant productivity while suppressing biofilm growth in a windowfarm system using beneficial bacteria and ultraviolet irradiation. Can. J. Microbiol. 61 7:457-466.

19. Mavrodi O. V., Walter N., Elateek S., Taylor C. G., and Okubara P. A. 2012. Suppression of Rhizoctonia and Pythium root rot of wheat by new strains of Pseudomonas. Biological Control 62 2:93-102.

20. McManus P., Stockwell V., Sundin G., and Jones A. 2002. Antibiotic use in plant agriculture. Annu. Rev. Phytopathol. 40:443-+.

21. McSpadden Gardener B., Gutierrez L., Joshi R., Edema R., and Lutton E. 2005. Distribution and biocontrol potential of phlD(+) pseudomonads in corn and soybean fields. Phytopathology 95 6:715-724.

22. Moruzzi S., Firrao G., Polano C., Borselli S., Loschi A., Ermacora P., Loi N., and Martini M. 2017. Genomic-assisted characterisation of *Pseudomonas* sp strain Pf4, a potential biocontrol agent in hydroponics. Biocontrol Sci. Technol. 27 8:969-991.

23. Nam H. S., Anderson A. J., and Kim Y. C. 2018. Biocontrol Efficacy of Formulated *Pseudomonas chlororaphis* O6 against Plant Diseases and Root-Knot Nematodes. Plant Pathology Journal 34 3:241-249.

24. Nemoto K., Hara M., Suzuki M., Seki H., Oka A., Muranaka T., and Mano Y. 2009. Function of the *aux* and *rol* genes of the Ri plasmid in plant cell division *in vitro*. Plant Signaling & Behavior 4 12:1145-1147.

25. Norabadi T. M., Sahebani N., Etebriam H. R. 2013. Biological control of root-knot nematode (*Meloidogyne javanica*) disease by *Pseudomonas fluorescens* (Chao). Arch of Phytopathology and Plant Protection Journal 5 47:615-621

26. O'Neill TM, Yarham D, 1993. The cucumber root mat mystery. Grower 118, 31–3.

27. Paulitz T., and Belanger R. 2001. Biological control in greenhouse systems. Annu. Rev. Phytopathol. 39:103-133.

28. Ran L., Liu C., Wu G., van Loon L., and Bakker P. 2005. Suppression of bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. in China. Biological Control 32 1:111-120.

29. Sawada H., Azegami K. 2014. First report of root mat (hairy root) of tomato (*Lycopersicon esculentum*) caused by *Rhizobium radiobacter* harboring cucumopine Ri plasmid in Japan. Japanese Journal of Phytopathology 2 80: 98– 114.

30. Subedi N., Taylor C. G., Paul P.A., Miller S. A. 2019. Combining partial host resistance with bacterial biocontrol agents improves outcomes for tomatoes infected with *Ralstonia pseudosolanacearum*. Crop Protection (In press).

31. Sun D., Zhuo T., Hu X., Fan X., and Zou H. 2017. Identification of a *Pseudomonas putida* as biocontrol agent for tomato bacterial wilt disease. Biological Control 114:45-50.

32. Tolba I. H., and Soliman M. A. 2013. Efficacy of native antagonistic bacterial isolates in biological control of crown gall disease in Egypt. Annals of Agricultural Sciences 58 1:43-49.

33. Weller D. M. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: Looking back over 30 years. Phytopathology 97 2:250-256.

34. Weller S., Stead D., ONeill T., Hargreaves D., and McPherson G. 2000. Rhizogenic *Agrobacterium* biovar 1 and cucumber root mat in the UK. Plant Pathol. 49 1:43-50.

35. Weller S., Stead D., and Young J. 2006. Recurrent outbreaks of root mat in cucumber and tomato are associated with a monomorphic, cucumopine, Ri-plasmid harboured by various Alphaproteobacteria. FEMS Microbiol. Lett. 258 1:136-143.

36. Weller S., Stead D., and Young J. 2004. Acquisition of an Agrobacterium Ri plasmid and pathogenicity by other alpha-Proteobacteria in cucumber and tomato crops affected by root mat. Appl. Environ. Microbiol. 70 5:2779-2785.

37. Yang X., and Hong C. 2018. Biological control of boxwood blight by *Pseudomonas protegens* recovered from recycling irrigation systems. Biological Control 124:68-73.

Table 1: *Pseudomonas* strains used on this study, halo size of the *in vitro* test and source of origin of each strain.

Strain	*Pseudomonas* specie	Halo size (mm)	Source of origin
15D11	*P. vranovensis*	17.6 ± 2.6	Mississippi River
14B11	*P. chlororaphis*	17.3 ± 2.6	Missouri River
14D6	*P. chlororaphis*	0	Mississippi River
48B8	*P. chlororaphis*	18 ± 1.2	Wisconsin Soil
48G9	*P. chlororaphis*	18.7 ± 3.5	Wisconsin Soil
1B1	*P. protogens*	25.7 ± 3.8	Mississippi River
1C5	*P. protogens*	0	Mississippi River
1F2	*P. protogens*	19.1 ± 1.6	Mississippi River
12H11	*P. protogens*	0	Missouri River
14B2	*P. protogens*	0	Missouri River
15G2	*P. protogens*	0	Missouri River
15G6	*P. protogens*	0	Missouri River
15H3	*P. protogens*	0	Missouri River
15H10	*P. protogens*	16.7 ± 3.1	Missouri River
38G2	*P. protogens*	0	Wyoming Soil
Clinton	*P. protogens*	18.9 ± 3.3	Ohio Soil
Darke	*P. protogens*	21 ± 3.1	Ohio Soil
Wayne	*P. protogens*	17.9 ± 3.1	Ohio Soil
29G9	*P. poae*	0	Herbarium
36C8	*P. poae*	0	Wyoming Soil
88A6	*P. rhodesiae*	0	Missouri Soil
90F12-1	*P. rhodesiae*	0	Missouri Soil
36B7	*P. brassicacearum*	0	Wyoming Soil
36D4	*P. brassicacearum*	0	Wyoming Soil
93D8	*P. brassicacearum*	15.9 ± 1.9	Missouri Soil
93F8	*P. brassicacearum*	16.1 ± 1.9	Missouri Soil
93G8	*P. brassicacearum*	18 ± 1.6	Missouri Soil
Delaware	*P. brassicacearum*	0	Ohio Soil
Wood 3	*P. brassicacearum*	0	Ohio Soil
37D10	*P. brassicacearum*	0	Wyoming Soil
48H11	*P. brassicacearum*	0	Wisconsin Soil
38D4	*P. brassicacearum*	0	Wyoming Soil
38D7	*P. brassicacearum*	0	Wyoming Soil
36C6	*P. frederiksbergensis*	0	Wyoming Soil
37A10	*P. frederiksbergensis*	0	Wyoming Soil
37A11	*P. frederiksbergensis*	17.6 ± 2.9	Wyoming Soil
38F7	*P. frederiksbergensis*	0	Wyoming Soil
39A2	*P. frederiksbergensis*	0	Wyoming Soil
94G2	*P. frederiksbergensis*	0	Missouri Soil
48C10	*P. lini*	0	Wisconsin Soil
2F9	*P. fluorescens*	0	Missouri River
24D3	*P. fluorescens*	0	Herbarium
28B5	*P. fluorescens*	0	Herbarium

36B3	*P. fluorescens*	0	Wyoming Soil
36F3	*P. fluorescens*	0	Wyoming Soil
36G2	*P. fluorescens*	0	Wyoming Soil
48D1	*P. fluorescens*	0	Wisconsin Soil
48D5	*P. fluorescens*	0	Wisconsin Soil
89F1	*P. fluorescens*	0	Missouri Soil
90D7A	*P. fluorescens*	0	Missouri Soil
90F12-2	*P. fluorescens*	0	Missouri Soil

Figure 1: IbMyb1-TF induces the expression of anthocyanin on the transgenic roots.

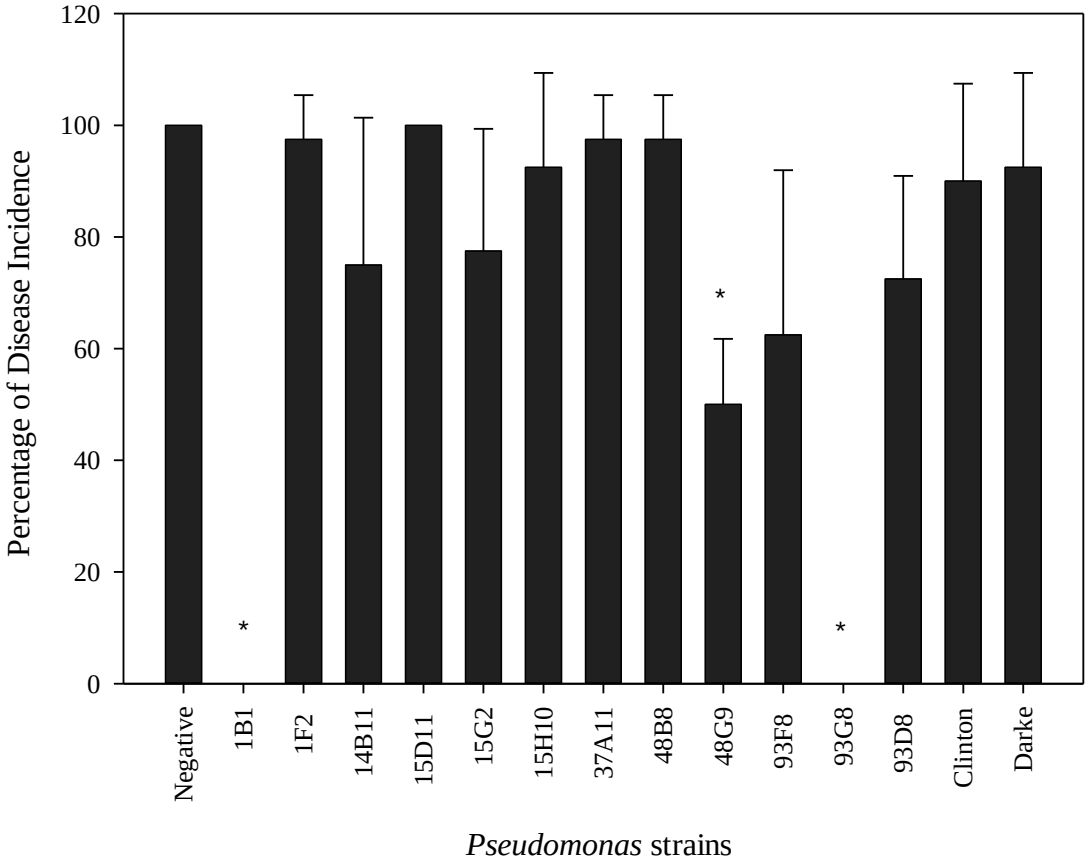

Figure 2: Percentage of disease incidence on *Kalanchoe daigremontiana* three weeks after inoculation with *Pseudomonas* strains and *A. rhizogenes*. Treatments followed by an asterisk (*) are significantly different according to a Dunnett's test ($P \leq 0.05$). Error bars represent the standard deviation of the mean.

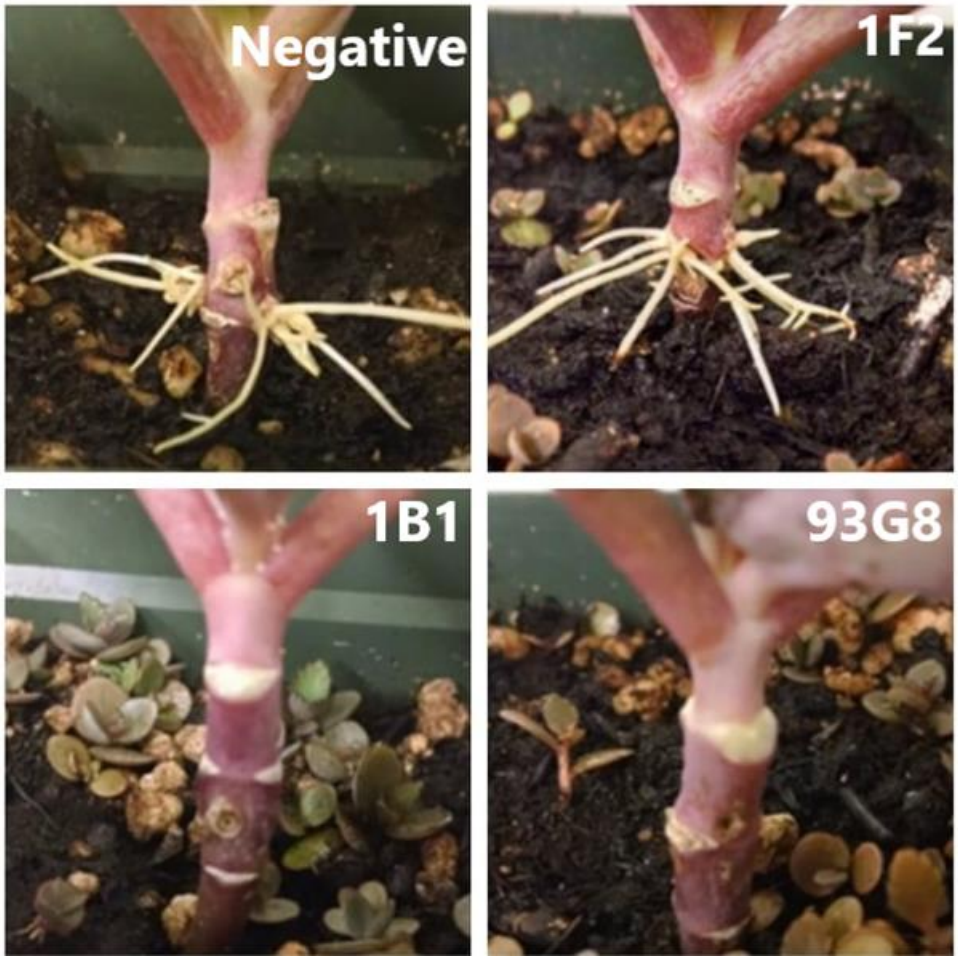

Figure 3: Disease incidence on *Kalanchoe daigremontiana* three weeks after inoculation with *Pseudomonas* strains and *A. rhizogenes.* Negative control and strains 1F2, 1B1 and 93G8.

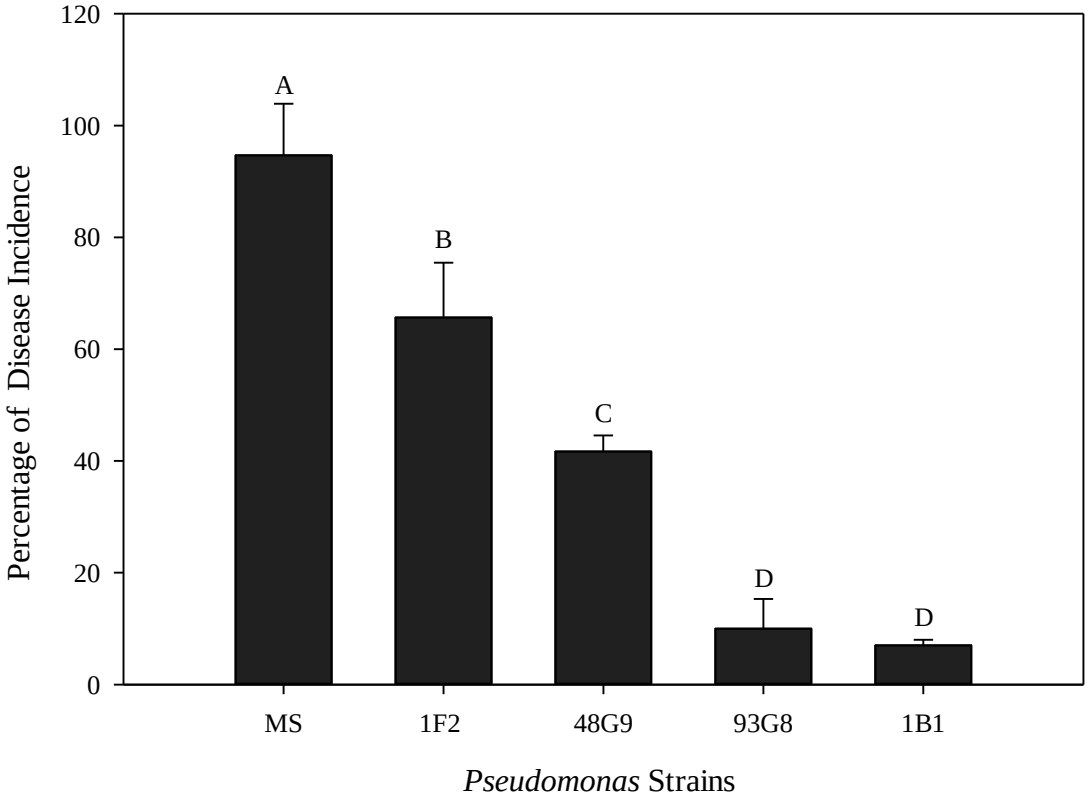

Figure 4: Percentage of disease incidence on soybean composite plants. Means with the same letter are significantly equal according to a Tukey-Kramers test ($P \leq 0.05$). Error bars represent the standard deviation of the mean.

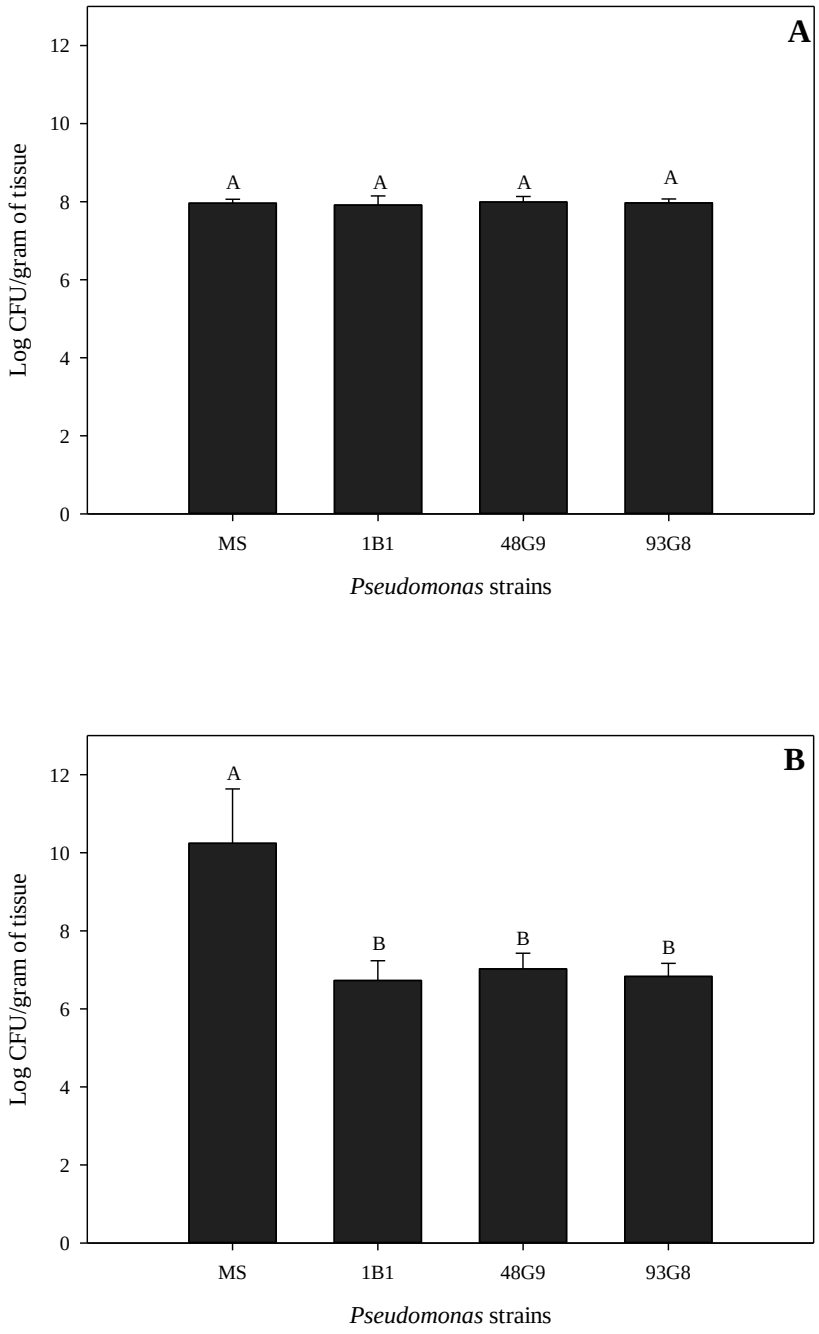

Figure 5: A. *A. rhizogenes* population on soybean shoots at day 0 after inoculation. **B**. A. *rhizogenes* population on soybean shoots at three days after inoculation. Means with the same letter are significantly equal according to a Tukey-Kramers test ($P \leq 0.05$). Error bars represent the standard deviation of the mean.

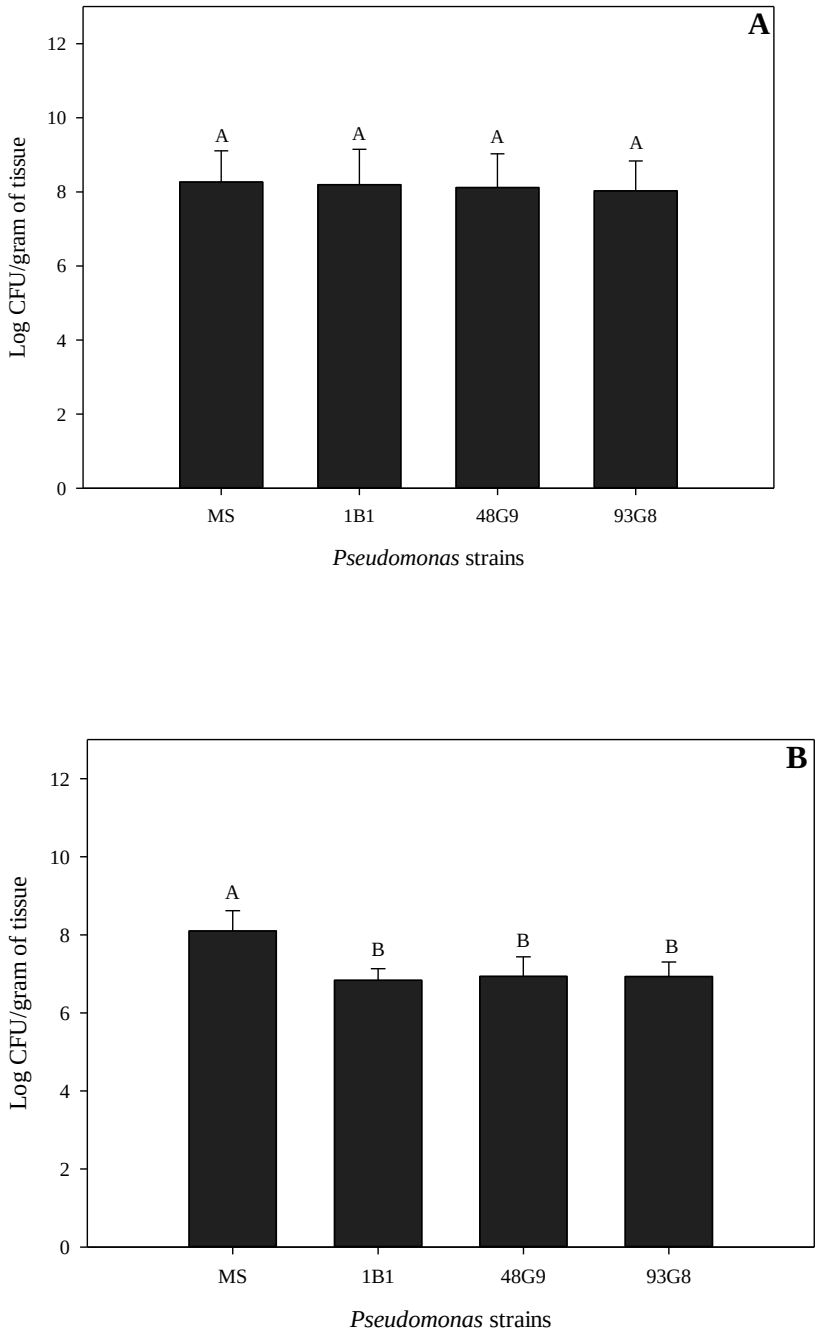

Figure 6: A. *A. rhizogenes* population on tomato cuttings at day 0 after inoculation. **B.** A. *rhizogenes* population on tomato cuttings at three days after inoculation. Means with the same letter are significantly equal according to a Tukey-Kramers test ($P \leq 0.05$). Error bars represent the standard deviation of the mean.

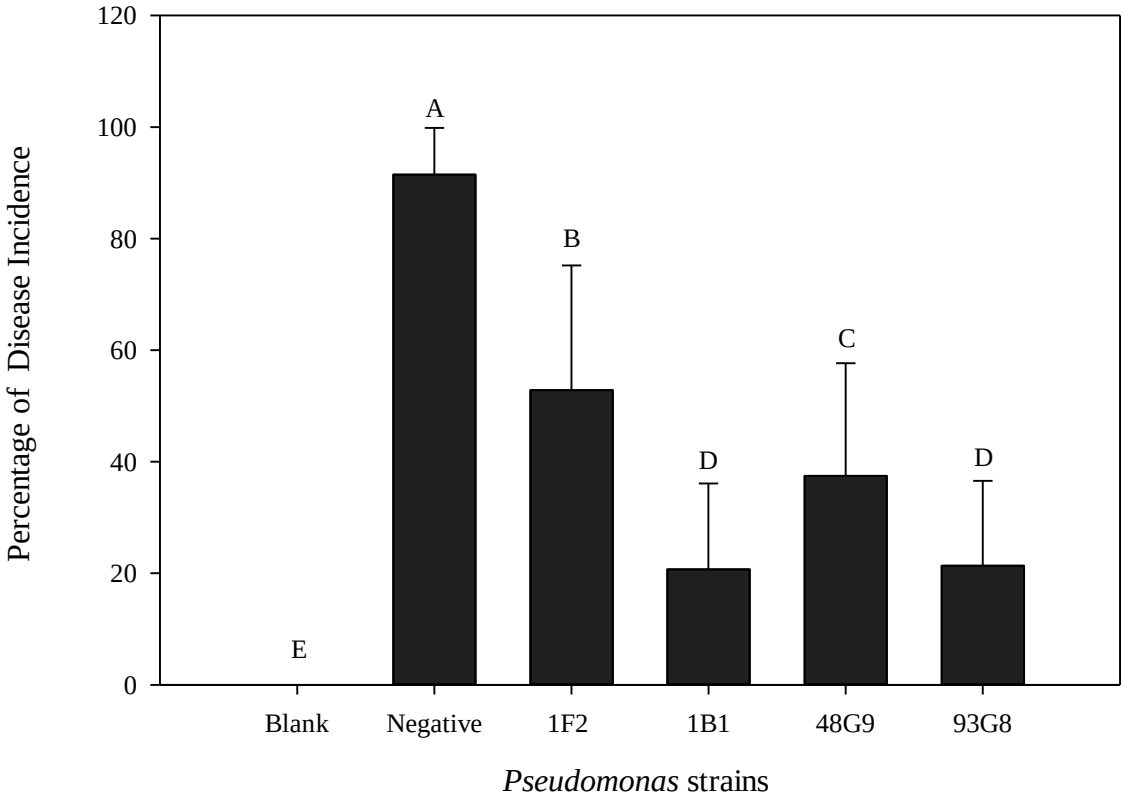

Figure 7. Crazy root disease incidence on hydroponic system. Means with the same letter are significantly equal according to a Tukey-Kramers test ($P \leq 0.05$). Error bars represent the standard deviation of the mean.