

## Limestone rate affects potato bacterial wilt disease and *Ralstonia solanacearum*'s population in the soil in the Western Highlands zone of Cameroon

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Mots clés	Résumé
<p>Pomme de terre ; Flétrissement bactérien ; Dose de calcaire ; <i>Ralstonia solanacearum</i> ; pH du sol.</p>	<p>La bactériose vasculaire causée par <i>Ralstonia solanacearum</i>, est l'une des contraintes majeures à la production de pomme de terre au Cameroun. Afin de réduire les pertes de rendement dues à cette maladie, cette étude visait à étudier l'effet du calcaire sur la bactériose vasculaire et sur la population de <i>R. solanacearum</i> dans le sol. Des tubercules de pomme de terre ont été cultivés en pots sous une ombrière de l'Université de Dschang, de février à mai 2021. Le plan expérimental était un plan en parcelles divisées composé de trois répétitions de deux génotypes de pomme de terre (Dosa et Jacob2005), 05 doses de calcaire (14,7 g, 29 g, 44,1 g, 58,8 g et 73,5 g par plante) et 2 témoins (pas de chaulage sans inoculation et pas de chaulage avec inoculation du pathogène). Les plantes ont été inoculées avec 25 ml de suspension bactérienne, contenant 10<sup>8</sup> UFC/ml. La population bactérienne du sol a été évaluée en comptant les colonies au microscope. Des données ont été recueillies sur l'incidence de la bactériose vasculaire, la population bactérienne dans le sol, le pH du sol, les composantes du rendement et l'infection latente. Les résultats ont montré qu'une faible incidence de la maladie a été associée à un taux élevé de calcaire dans les deux génotypes. Le taux de calcaire a augmenté le pH du sol tout en réduisant la population du pathogène dans le sol. Le poids de tubercule/plante le plus élevé a été obtenu avec un taux de calcaire ≥ 58,8 g/plante. Le chaulage a permis d'éviter les infections latentes lors du stockage. Ainsi, un amendement du sol avec du calcaire à raison de 58,8 g/plant peut réduire la population de <i>R. solanacearum</i> dans le sol et diminuer l'impact de la bactérie au champ ainsi qu'en stockage dans la localité de Dschang.</p>
<p><b>Keywords:</b> Potato; Bacterial wilt; Limestone rate; <i>Ralstonia solanacearum</i>; Soil pH.</p>	<p><b>Abstract</b> Potato bacterial wilt (BW) caused by <i>Ralstonia solanacearum</i>, is one of the major constraints to potato production in Cameroon. In order to reduce yield losses due to BW, this study aimed at investigating the effect of limestone on bacterial wilt disease, and on the <i>R. solanacearum</i>'s population in the soil. Potato tubers were grown in pots in a screen house of the University of Dschang, from February to May 2021. The experimental design was a split plot design made up of three replicates of two potato genotypes (Dosa and Jacob2005), 05 limestone rates (14.7 g, 29 g, 44.1 g, 58.8 g and 73.5 g per plant) and 2 controls (no liming without inoculation and no liming with pathogen inoculation). Plants were inoculated with 25 ml of bacterial suspension, containing 10<sup>8</sup> CFU/ml. The bacterial population in the soil was assessed by counting colonies under microscope. Data were collected on BW incidence, bacterial population in the soil, soil pH, yield components and latent infection. The results showed that low BW incidence was associated with high rate of limestone in both genotypes. Limestone rate increased soil pH while reducing the pathogen population in the soil. The higher tuber weight/plant was obtained with limestone rate ≥58.8 g/plant. Liming enabled to avoid latent infection during storage. Thus, soil amendment with limestone at 58.8 g/plant can reduce <i>R. solanacearum</i> population in the soil and lower the impact of bacterial in the field as well as in the store in Dschang locality.</p>
<p><b>Historic</b> Received : 20 August 2023 Received in revised form : 10 April 2024 Accepted : 04 May 2024</p>	

### 1. Introduction

Potato (*Solanum tuberosum* L.) ranks fourth in world food production after wheat rice and maize. It is currently the leading tuber crop in the

global food system and contributes to the livelihood of millions of populations worldwide [1]. Bacterial wilt is among the most economically important potato diseases in many tropical and sub-tropical regions and can cause losses up to 950 million USD per year [2]. The disease is caused by *R. solanacearum*, a Gram-negative soil borne pathogen. This pathogen can survive in soils and water bodies for long periods [3]. Plant species infected with this pathogen usually show the typical symptoms of wilting and yellowing, which may be followed by

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necrosis and death. The bacteria invade the roots of diverse plant hosts from the soil via wounds or sites of secondary root emergence and disseminate into the xylem vessels [4]. During infection, the bacteria become motile and can rapidly spread throughout the vascular system of the plant, presumably carried along by the transpirational flow [5]. As a diverse complex species, *R. solanacearum* has developed an extremely broad host range throughout the world, including more than 450 host species representing 54 plant families. The pathogen is a severe obstacle to the production of *solanaceous* plants in both tropical and temperate regions [2,6]. Yield losses due to *R. solanacearum* vary from 33 to 90% in potato [7]. The disease is endemic to Cameroon [8,9] where incidence can reach 55% depending on genotype and elevation [10].

Researchers have made great progress in the areas of biology, molecular biology, geographical distribution, epidemiology, host-pathogen interactions, identification and detection technology of *R. solanacearum* [11]; notwithstanding, how to control bacterial wilt disease caused by this pathogen remains difficult. This is because the pathogen has a wide range of hosts and is able to survive for a long time even in the absence of host plants or in nutrient-depleted bulk soil environments due to its saprophytic ability as well as its ability to survive as epiphyte within the rhizosphere [12,13]. It can also persist at low population in naturally infested soil for years without host plants and the population size can increase to the plant infection threshold within a season when hosts are planted [11]. These characteristics associated with a wide range of host species make the control of bacterial wilt very difficult. The use of resistant genotypes is usually the most widely used method to control diseases. Many genotypes in eggplants (*Solanum melongena* L.), peppers (*Capsicum annuum*), tomatoes (*Solanum lycopersicum* L.), peanuts (*Arachis hypogaea*) or tobacco (*Nicotiana tabacum*) have proven their effectiveness in controlling *R. solanacearum* [7]. However, the high number of races and strains of this pathogen contribute to shorter the plant period of resistance. Chemical control is not effective. Methyl bromide used as a broad-spectrum fumigant for soil sterilization is effective, but has been ban in most countries because of its destructive effect on the ozone layer [14]. Therefore, a plausible method of controlling bacterial wilt is to reduce the inoculum source below the threshold level before a host is planted. This can be done by increasing the soil pH. It has been reported that acidic soils (pH 4.5-5.5) favour the growth of *R. Solanacearum*, while suppressing the growth and the activity of antagonistic bacteria [15]. Several studies have also shown a decrease in bacterial wilt with increase in pH using compost in tomato [16], biochar in tobacco [17] Calcium oxide in ginger [18]. Limestone has been found to increase soil pH and control soil-borne diseases in tobacco [19]. In the Western region of Cameroon, about 80% of the soils are acidic with Ferralsols (pH of 4 to 5) and Nitrosols (pH of 5 to 6) soil types [20]. Solutions have been proposed to buffer excess hydrochloric acid ions in soils; this includes the addition of dolomite, or the application of limestone, which reduces the aluminium and the hydronium toxicity thus improving the hydrogen potential for soil, while releasing the nutrients retained or trapped in the soil colloidal system [21,22]. Moreover, it has been reported that, calcium released by limestone applied to the soil can improve plant resistance to many pathogens [23]. The limestone rate required to increase pH while reducing pathogen population in Cameroonian soils is not known. The only study performed in the area with the aim to increase pH focused on the yield of green bean [24] This study aimed at investigating the effect of limestone rate on the bacterial wilt disease in potato genotypes and on the *R. solanacearum* population in the soil.

## 2. Material and methods

### 2.1. Experimental site

The study was carried out at the University of Dschang. Dschang is a city in the Western Highlands zone of Cameroon and is located 1400 m above the sea level, between latitudes 5° 25' and 5° 30' North, longitudes 10° 00' and 10° 05' East. It has an equatorial climate which is characterized by an average annual temperature of 20.8°C. The average annual rainfall is 1785 mm. There are two seasons: a rainy season from mid-March to mid-October and a dry season from mid-October to mid-March. The hottest months of the year are February and March while the coldest months are July and August [24].

Pot experiments were conducted in a screen house from February to May 2021, and repeated in 2022. The soil used was acidic, had sandy clay texture, 12.30 mEq/100 g total exchange capacity, 129 N/acre estimated nitrogen release and 14.34% organic matter. Sulfur, phosphorus and sodium were respectively 12, 6 and 11 mg/kg. Calcim, magnesium and potassium were respectively 918, 122, 130 mg/kg.

### 2.2. Plant material

The plant material used were disease free tubers of two potato genotypes (Dosa and Jacob2005), provided by *TOWA structure of seed Production*. Dosa and Jacob2005 are two of the most commonly grown potato genotypes cultivated by farmers in the Western Highlands of Cameroon.

### 2.3. Experimental design, liming and cropping

The experimental design was a split plot design, made up of three replicates of two potato genotypes (Dosa and Jacob2005) and 5 rates of limestone (14.7 g, 29 g, 44.1 g 58.8 g and 73.5 g/plant has to make respectively 1 t/ha, 2 t/ha, 3 t/ha, 4 t/ha and 5 t/ha in the field using a planting distance of 35 cm between plants and 70cm between rows) and 2 controls (no liming without pathogen and no liming with pathogen inoculation). Each experimental unit contained a genotype and a rate of limestone in 9 pots containing sterilized soil. Limestone from Figuil was applied and thoroughly mixed with the soil in pots of 44.5 cm height and 37.2 cm width. The pots were watered at two days interval, for four weeks (for better incorporation of the limestone into the soil) before planting the potato tubers. Single tuber was planted in each pot. Plants were watered as needed to keep the soil moist until the plants grew to the inoculation stage. Inoculation was done at 35 days after planting.

### 2.4. Preparation of *Ralstonia solanacearum* inoculum and inoculation of plants

Bacterial inoculum was prepared by placing 20 ml of distilled water into Petri dishes containing pure cultures of the bacterium. The pure cultures were carefully brushed with a fine brush and the mixture was filtered with a Whatman filter paper. The resulting cell concentration of bacterial suspensions was determined at 600 nm with a DU 800 spectrophotometer. The turbidity of the bacterial solution was adjusted with distilled water or with the bacterial culture depending of the density in order to obtain a turbidity equivalent to that of a McFarland standard. Cell concentrations of about 10<sup>8</sup> CFU/ml corresponding to 0.3 optical density were prepared for this study. Each plant of each treatment except one of the control treatments were inoculated with 25 ml of bacterial suspension, containing 10<sup>8</sup> CFU/mL. The inoculum was poured on a ring created in the soil around the potato plant and allowed to slowly penetrate the roots. The potatoes plants were watered as needed to keep the soil moist until the plants grew to the inoculation stage. Inoculation was done when the plants were very vulnerable to attack (35 days after planting of potato), and in the morning to prevent interruption through evapotranspiration. Soil samples were collected twelve weeks after limestone application and analyzed for pH measurement.

## 2.5. Identification of *Ralstonia solanacearum*

*R. solanacearum* was isolated from potato stems showing bacterial wilt symptoms. The pathogen was then cultured on Nutrient Agar plates according to [25]. After two days when the colonies were well formed on the Nutrient agar medium, showing a bold, cream-white colour [26], the identification test was performed to confirm that the culture was that of *R. solanacearum*. This was done using the gram staining method [27] which consisted of spreading a culture of bacteria on a clean slide, then drying it. The preparation was stained with Gention violet and left to dry for 20 minutes. It was rinsed with distilled water and the slide was stained with Lugol and left to act for 1 minute, then rinsed again with distilled water and covered with 95% ethanol and left for 30 seconds. Furthermore, it was washed with water, stained with basic Fushsine and left for 10 seconds. Finally, it was washed again with water and allowed to dried. This was followed by observation under the microscope at the 100 objectives with immersion oil. A pink colour was observed, confirming that the bacteria present were Gram-negative. The smooth circular shape of the pathogen confirmed that it was *R. solanacearum* [28].

## 2.6. Data collection

**Days to onset wilting and Bacterial wilt incidence:** Plants were observed for wilt symptoms and days to onset wilting was collected. Bacterial wilt incidence was assessed at three-day intervals from the date of first symptom appearance by counting the number of plants attacked and expressed as a percentage of total plants in the plot to obtain wilt incidence. The final bacterial wilt incidence was collected 26 days after inoculation.

**Potato yield components:** In order to evaluate the effect of bacterial wilt on potato yield, tubers were harvested at maturity (90 days after planting), and yield components such as the number of tubers per plant and weight per plant of healthy tuber were collected.

**Quantification of *Ralstonia solanacearum* in the soil:** Soil samples (10 g), from pooled samples of soil collected from the root region (10–15 cm soil depth) of infected potato plants, were suspended in 90 mL sterile water and shaken for 20 min. The supernatant collected from the settled suspension was subjected to serial dilution. This was followed by the spreading of aliquots (0.1 ml each) of supernatants from the dilution series, on Nutrient Agar plates for 48 hours, following the method of [25]. After identification and confirmation of this pathogen as the causal agent of wilting, quantification of *R. solanacearum* population done through plate counts under a photonic microscope. The number of colonies forming units obtained was divided by the dilution factor, then by the sample weight.

**Latent infection:** Potato tubers from each treatment were incubated at  $30 \pm 2$  °C. This was done by monitoring symptom development after a period of 3 weeks [29]. During this phase, potato tubers showing bacterial ooze from the eyes were counted and the number of attacked tubers was expressed as a percentage of the total tubers incubated to obtain the percentage of latently infected tubers.

## 2.7. Data Analysis

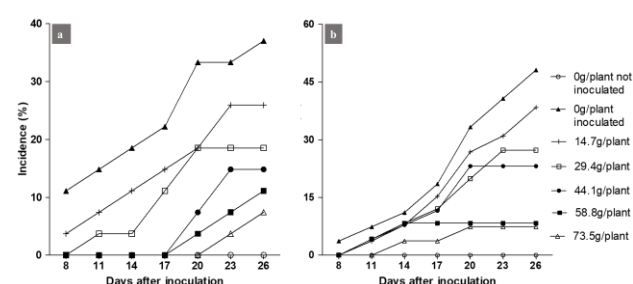
Mean values of both years for disease incidence, yield parameters, pH, and pathogen population were subjected to analysis of variance in order to compare treatments using GenStat 21<sup>st</sup> Edition [30]. When the ANOVA test showed significant probability of difference between means ( $p < 0.05$ ), means were separated using the Fisher's protected least significant difference (LSD) test.

## 3. RÉSULTATS

### 3.1. Days to onset wilting and disease incidence

Onset of wilting occurred 8 days after inoculation (DAI) for both Dosa and Jacob2005 genotypes. Days to onset wilting increased with limestone rate. In the Dosa genotype, the disease appeared 8 DAI for limestone rate  $\leq 14.7$  g/plant, 11 DAI for 29.4 g, 20 DAI for 44.1 g and 58.8 g of limestone per plant and 23 DAI for 73.5 g of limestone per plant. In Jacob2005, the disease onset was 8 DAI in plants without liming, 11 DAI in plants limed with 14.7 to 44.1 g of limestone and 14 DAI in plants limed with 73.5 g of limestone per plant. Plants without inoculation and no limestone showed no symptom in both genotypes (Fig. 1).

Bacterial wilt incidence decreased with limestone rate during potato growth in both Dosa and Jacob2005 genotypes. Final bacterial wilt incidence was recorded 26 DAI. In the Dosa genotype, final bacterial wilt incidence ranged from 7.41 % in plants grown in soil limed with 73.5 g of limestone to 37.03 % in inoculated plants grown in non-limed soil. In Jacob 2005, final bacterial wilt incidence ranged from 7.41 % in plants limed with 73.5 g of limestone, to 48.15 % in inoculated plants without liming (Fig. 1)



**Figure 1:** Bacterial wilt incidence in Dosa (a) and Jacob2005 (b) genotypes as a function of time and limestone rate

### 3.2. Effect of genotype and limestone rate on potato yield components

Table 1 shows the total number of tubers per plant and the weight of marketable tubers per plant as influenced by the limestone rate and genotype. The total number of tubers was higher in genotype Dosa (4.91) than in genotype Jacob2005 (4.13). There was no effect of limestone on the number of tubers. Genotype Jacob2005 showed higher weight of marketable tubers (137.60 g/plant) as compared to Dosa (102.9 g/plant).

**Table 1:** Total number of tubers in potato genotypes as affected by limestone rate

Limestone rate (g/plant)	Genotype		Rate mean
	Dosa	Jacob2005	
0 without inoculation	6.30 ± 1.40	5.32 ± 0.84	5.81 ± 0.76
0 with inoculation	4.78 ± 0.17	4.37 ± 0.35	4.57 ± 0.20
14.7	5.26 ± 0.83	4.59 ± 0.38	4.92 ± 0.43
29.4	4.49 ± 0.56	3.70 ± 0.20	4.10 ± 0.32
44.1	3.42 ± 0.83	3.00 ± 0.45	3.21 ± 0.43
58.8	5.32 ± 0.31	3.77 ± 0.53	4.55 ± 0.44
73.5	4.78 ± 1.22	4.18 ± 0.50	4.48 ± 0.61
<b>Genotype mean</b>	<b>4.91 ± 0.33<sup>α</sup></b>	<b>4.13 ± 0.22<sup>β</sup></b>	
	F	p-value	
Genotype	6.72	0.021	
Limestone rate	1.64	0.219	
Genotype * Limestone rate	0.25	0.949	

<sup>α,β</sup>: Genotype mean followed by different symbols for each parameter are significantly different.

The weight of marketable tubers in inoculated soils increased with limestone rate and stopped increasing when 73.5 g of limestone were applied per plant. No significant difference was observed between the

weight of marketable tubers obtained in the non-inoculated soil (141.6 g) and that obtained in soils where limestone rate applied was  $\geq 58.8$  g/plant. Interaction between genotype and limestone rate for both the total number of tuber and the weight of marketable tubers was not consistent (Table 2).

**Table 2:** Weight of marketable tubers in potato genotypes as affected by limestone rate

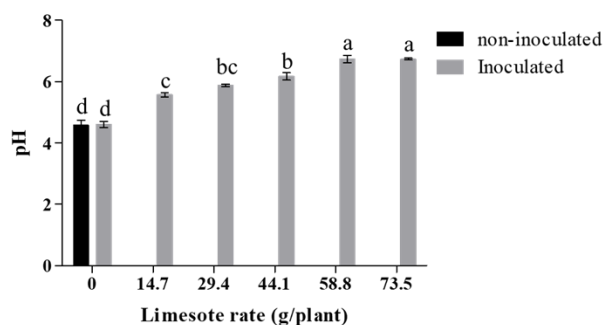
Limestone rate (g/plant)	Genotype		Rate mean
	Dosa	Jacob2005	
0 without inoculation	129.20 $\pm$ 3.37	154.10 $\pm$ 9.70	141.60 $\pm$ 7.22 <sup>a</sup>
0 with inoculation	71.30 $\pm$ 10.37	96.90 $\pm$ 13.23	84.10 $\pm$ 9.44 <sup>c</sup>
14.7	80.70 $\pm$ 4.73	115.70 $\pm$ 2.04	98.20 $\pm$ 8.17 <sup>bc</sup>
29.4	91.40 $\pm$ 13.95	130.40 $\pm$ 9.10	110.90 $\pm$ 11.46 <sup>b</sup>
44.1	98.50 $\pm$ 17.44	126.90 $\pm$ 7.89	112.70 $\pm$ 10.65 <sup>b</sup>
58.8	142.10 $\pm$ 3.20	168.00 $\pm$ 9.30	155.00 $\pm$ 7.26 <sup>a</sup>
73.5	106.80 $\pm$ 8.26	171.40 $\pm$ 13.29	139.10 $\pm$ 16.06 <sup>a</sup>
<b>Genotype mean</b>	<b>102.90 <math>\pm</math> 6.17<sup>b</sup></b>	<b>137.60 <math>\pm</math> 6.55<sup>a</sup></b>	
		F	p-value
Genotype		68.54	0.014
Limestone rate		15.62	<0.001
Genotype * Limestone rate		1.19	0.343

<sup>a,b,c</sup>: Rate mean followed by different letters for each parameter are significantly different.

<sup>a,b</sup>: Genotype mean followed by different symbols for each parameter are significantly different

### 3.3. Effect of limestone application rate on soil pH

The pH of the limestone, soil before and soil after limestone application was measured and recorded. The pH of the limestone was 8.3 and the pH of the soil before limestone application was 5.6. Fig. 2 shows the soil pH values after application of the different rates of limestone. There was a significant increase in pH with limestone rate. The highest value of pH (6.73) was recorded with limestone rate  $\geq 58.8$  g/plant whereas the lowest values were obtained in the non-limed soils whether inoculated (4.60) or not (4.57).



**Figure 2:** Soil pH as affected by limestone rate. <sup>a,b,c</sup>: bars with different letters in each column are significantly different

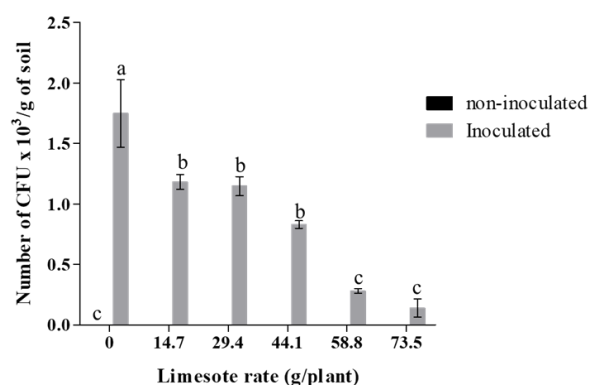
### 3.4. Effect of limestone application rate on soil population of *Ralstonia solanacearum*

The mean number of *R. solanacearum* colonies in the soil are shown in Fig. 3. From this figure, the population of *R. solanacearum* significantly decreased with limestone rate in the inoculated soil from 1750 UFC/g of soil in the non-limed soil 140 CFU/g of soil with 73.5 g of limestone per plant. The number of *R. solanacearum* colonies obtained with limestone rates  $\geq 58.8$  g/plant was not different from that obtained on non-inoculated soil.

### 3.5. Latent infection in stored tubers

After 3 weeks of incubation at  $30 \pm 2$  °C latently infected tubers were not observed in limed soils whatever the limestone rate. Only the tubers harvested on plants grown on non-limed soils and inoculated with the

pathogen showed latently infected tubers with 10% in genotype Jacob2005 and 2% in genotype Dosa.



**Figure 3:** *R. solanacearum* population in the soil as affected by limestone rate. <sup>a,b,c</sup>: bars with different letters in each column are significantly different

## 4. Discussion

The present study showed the influence of limestone rate on bacterial wilt incidence and yield components in potato genotypes. It also reported the influence of limestone on the soil pH and on the pathogen population in the soil.

Results showed that, higher rate of limestone increased days to onset wilting and reduced bacterial wilt incidence in all genotypes. Likewise, limestone application increased soil pH as well as it reduced the pathogen population in the soil. Similar results were obtained with tobacco [12,31] and tomato [32,33]. It has been reported that soil pH directly influences plant disease infection by affecting the survival of soil-borne pathogens and soil microbes [34]. Recent studies showed that soil pH improvement after lime and wood ash application reduces the occurrence of bacterial wilt [15].

High soil pH is especially important for controlling bacterial wilt as it was previously done using biochar [17], lime and wood ash [15]. As a soil-borne pathogen *R. solanacearum* abundance can be reduced and bacterial wilt suppressed by improving soil properties [35,36]. In the Western region of Cameroon where this study was carried out, pH ranges from 4 to 6 [20]. It has been reported that, acidic conditions (pH 4.5 - 5.5) favor the growth of *R. solanacearum*, the causal agent of bacterial wilt disease [15]. Previously authors studying the effect of limestone on soil properties and soil-borne disease management focused mainly on particle sizes and reported that smaller particles were most effective to enhance soil properties [38-40]. One of the management decisions related to liming is the lime application rate: "how much lime is needed to neutralize soil acidity?" [40]. The pH of the soil used in the current study was 4.57 and the particle size of limestone applied was <0.25 mm. Results showed that, increase in limestone rate contributed to increase the soil pH while reducing the pathogen colonies, suggesting that limestone inhibited the growth of *R. solanacearum* by changing the soil pH. Higher limestone rate ( $\geq 58.8$  g/plant) resulted in pH 6.73 and very low number of pathogen colonies. [41] reported that, soils with very low acidity (pH 6.45) suppressed the growth of *R. solanacearum* and alleviated the occurrence of bacterial wilt. Moreover, soil acidic level significantly affects the soil bacterial community structure as a consequence of changes in soil elements availability, leading to the enrichment of the potentially beneficial bacteria and the suppression of bacterial wilt. However, [38] reported that, the optimum pH for *R. solanacearum* growth is 6.5, suggesting that the effect of limestone on *R. solanacearum* may be

mainly related to the role of  $\text{Ca}^{2+}$ . These authors found direct correlation of  $\text{Ca}^{2+}$  with the suppressive effect on the growth of *R. solanacearum*. According to [42], limestone influence porosity and pore size distribution of soil, changing the rate of water absorption and the extent of air pressure build-up within aggregates. Moreover, our previous study showed that the impact of bacterial wilt disease was lower in higher elevation where the soil was more acidic than in the lower elevations with higher acidity. This means that, the survival of soil-born pathogen requires suitable conditions which are not limited to soil pH.

As regarding the inoculated potato grown on non-limed soils final bacterial wilt incidence was higher in genotype Jacob2005 than in Dosa. This is consistent with the findings of [10] who showed that genotype Dosa was more tolerant to bacterial wilt disease than genotype Jacob2005. In general, Genotype Dosa showed higher number of tubers per plant, but lower weight of tubers per plant as compared to genotype Jacob2005. Previous studies performed in the same area showed positive correlation between bacterial wilt incidence and the potato number of tubers indicating that genotype with lower disease incidence produced more tubers [10]. Higher weight of marketable tubers although higher bacterial wilt incidence observed in genotype Jacob2005 indicates that weight of tubers was mainly affected by the genotype than by disease. Although Jacob2005 is a high yielding potato genotype as [10] reported loss of performance due to bacterial wilt. In this study, limestone addition increased the weight of marketable tubers by suppressing bacterial wilt incidence and increasing the soil pH. It has been reported that Liming, through soil pH optimization and better availability of nutrients, improves potato yield in acidic soil conditions [42,43]. According to [44], increase in crop yields due to lime application was attributed to the reduction of exchangeable aluminum, increase in soil pH and greater concentrations of basic nutrients such as Ca, Mg and K. It has also been reported that lime addition increased the phosphorus uptake which can be otherwise unavailable to crops as a result of phosphorus fixation in acidic soils [45–47].

No latently infected tuber was found in limed soils. This implies that, by reducing wilt incidence, limestone addition prevented harvested potato tubers from being infected by *R. solanacearum*, hence preventing post-harvest losses. Latent infection was observed only in the inoculated and non-limed soil. The percentage of latently infected tubers was higher in genotype Jacob2005 than in Dosa. The genotype Dosa was previously reported to be more tolerant than Jacob2005 to bacterial wilt [10]. The presence of latently infected tubers could be explained by the fact that host plants infected with *R. solanacearum* may not show any disease symptoms which appear but during tubers storage.

## 5. Conclusion

These findings showed that increasing rate of limestone contributed to increase soil pH hence reducing soil acidity. Limestone applied at a rate of 58.8 g/plant as an equivalent of 4 t.ha<sup>-1</sup> was effective in reducing the population of *R. solanacearum* in the soil and controlling potato bacterial wilt disease in Dschang locality. This rate can be used to alleviate bacterial wilt incidence, suppress latent infection in stored tubers and increase yield even in the most susceptible potato genotypes. These results showed that limestone can serve as a sustainable soil amendment to control bacterial wilt, as it is effective and environmentally friendly. Limestone application can prevent post-harvest losses in potato due to bacterial wilt.

## Acknowledgments

The authors are grateful to the *TOWA Structure of seed production* who provided potato seeds.

## References

1. Devaux A, Goffart J, Kromann P. The Potato of the Future : Opportunities and Challenges in Sustainable Agri-food Systems Introduction : The Current Situation of Global Food Security. *Potato Res.* 2021;64(4):681–720.
2. Elphinstone JG. The current bacterial wilt situation: A global overview. In: Allen C, Prior P, Hayward AC, editors. *Bacterial Wilt Disease and the Ralstonia solanacearum Species Complex*. St. Paul, USA: *American Phytopathological Society*; 2005. p. 9–28.
3. Louws FJ, Rivard CL, Kubota C. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Sci Hortic (Amsterdam)* [Internet]. 2010;127(2):127–46. Available from: <http://dx.doi.org/10.1016/j.scienta.2010.09.023>
4. Kerr J. *Plants de pomme de terre, Guide de la CEE-ONU sur les maladies, parasites et défauts des plants de pomme de terre*. New York Genève. 2014;18.
5. Denny TP. *Ralstonia solanacearum* - a plant pathogen in touch with its host. *Trends Microbiol.* 2000;8(11):486–489.
6. Janse JD. Potato brown rot in western Europe – history, present occurrence and some remarks on possible origin, epidemiology and control strategies. *EPPD Bull.* 1996;26(3–4):679–695.
7. Lopes CA, Buso JA, Melo PE. Breeding for Resistance to Bacterial Wilt of Potatoes in Brazil. *Mol Ecol Asp.* 1998;290–300.
8. Priou S, Aley P, Chujoy E, Lemaga B, French ER. Integrated control of bacterial wilt of potato [Internet]. CIP Series IV-III; 1999. p. 30. Available from: <http://cipotato.org/wp-content/uploads/2014/10/guiaing.pdf>
9. Toukam GMS, Gilles C, Wicker E, Guilbaud C, Kahane R, Allen C, et al. Broad diversity of *ralstonia solanacearum* strains in Cameroon. *Plant Dis.* 2009;93(11):1123–1130.
10. Anoumaa M, Kanmegne G, Suh K, Kouam EB, Djomo SH, Deloko TCD, Fonkou T., Resistant genotypes combined with high elevation level provide bacterial wilt control in potato (*Solanum tuberosum* L.) in the Western Highlands zone of Cameroon. *Crop Prot.* 2022;160(6):106046.
11. Dittapongpitch V, Surat S. Detection of *Ralstonia solanacearum* in soil and weeds from commercial tomato fields using immunocapture and the polymerase chain reaction. *J Phytopathol.* 2003;151(4):239–246.
12. Wu K, Yuan S, Xun G, Shi W, Pan B, Guan H, et al. Root exudates from two tobacco cultivars affect colonization of *Ralstonia solanacearum* and the disease index. *Eur J Plant Pathol.* 2015;141(4):667–677.
13. Carvalho ADF, Lopes CA, Ragassi CF. Desempenho de cultivares de batata sob diferentes espaçamentos em solo naturalmente infestado com *Ralstonia solanacearum*. *Hortic Bras.* 2017;35(4):507–511.
14. Salgon S. Déterminisme génétique de la résistance au flétrissement bactérien chez l' aubergine et applications en sélection variétale. ARMEFLHOR. 2017. 218 p.
15. Li S, Liu Y, Wang J, Yang L, Zhang S, Xu C, et al. Soil acidification aggravates the occurrence of bacterial wilt in south China. *Front Microbiol.* 2017;8(April):1–12.

16. Popoola AR, Ganiyu SA, Babalola OA, Ayo-John EI, Fajinmi AA, Kehinde IA, Adegboye TH. Impact of soil amendments and weather factors on bacterial wilt and yield of two tomato cultivars in Abeokuta, Nigeria. *S. Afr. J. Plant Soil*. 2014;31(4):195–201.
17. Zhang C, Lin Y, Tian X, Xu Q, Chen Z, Lin W. Tobacco bacterial wilt suppression with biochar soil addition associates to improved soil physiochemical properties and increased rhizosphere bacteria abundance. *Appl Soil Ecol*. 2017;112:90–96.
18. Vudhivanich S. Effect of soil amendment with urea and calcium oxide on survival of *Ralstonia solanacearum*, the causal agent of bacterial wilt or rhizome rot of ginger. *Kasetsart J (Nat Sci)*. 2002;36(3):242–247.
19. Wu K, Yuan S, Wang L, Shi J, Zhao J, Shen B, et al. Effects of bio-organic fertilizer plus soil amendment on the control of tobacco bacterial wilt and composition of soil bacterial communities. *Biol Fertil Soils*. 2014;50(6):961–971.
20. Ngachie V, Ngachie 1992. *Tropicicultura*. 1992;10(2):61–63.
21. Antoniadis V, Hatzis F, Bachtsevanidis D, Koutroubas SD. Phosphorus Availability in Low-P and Acidic Soils as Affected by Liming and P Addition. *Commun Soil Sci Plant Anal*. 2015;46(10):1288–1298.
22. Matsuo K, Ae N, Vorachit S, Thadavon S. Present soil chemical status and constraints for rice-based cropping systems in vientiane plain and neighboring areas, Lao PDR. *Plant Prod Sci*. 2015;18(3):314–22.
23. Fageria NK, Baligar VC. Chapter 7 Ameliorating Soil Acidity of Tropical Oxisols by Liming For Sustainable Crop Production. In: SPARKS DL, editor. *Advances in Agronomy*. Academic Press; 2008. p. 345–399.
24. Tamfuh Azinwi P, Kamga Pangop C, Douanla Tapindje D, Boukong A, Tabi F, Cho-Ngwa F, Bitom D. Effect of dolomite amendment of acid Andosols on the performance of two green beans varieties in the Cameroon Western Highlands. *Int J Adv Geosci*. 2019;7(1):1–9.
25. Kenfack J, Fogue M, Hamandjoda O, Tamo Tatietse T. Promoting Renewable Energy and Energy Efficiency in Central Africa: Cameroon Case Study. *Proc World Renew Energy Congr – Sweden*. 8–13 May, 2011, Linköping, Sweden. 2011;57:2602–9.
26. Li P, Shi L, Gao MN, Yang X, Xue W, Jin L-H, Hu D-Y, Song B-A. Antibacterial activities against rice bacterial leaf blight and tomato bacterial wilt of 2-mercapto-5-substituted-1,3,4-oxadiazole/thiadiazole derivatives. *Bioorganic Med Chem Lett*. 2015;25(3):481–484.
27. Sharma DK. Morphological and biochemical characterization of *Ralstonia solanacearum* (smith) in brinjal (*Solanum melongena* L.) in Rajasthan (India). *Adv Plants Agric Res*. 2018;8(3):284–288.
28. Collins CH. Counting microorganism, microbiological methods. 8<sup>th</sup> ed. *Microbiological methods*. London ; Arnold; 1993. 140 p.
29. Schaad NW. Laboratory guide for identification of plant pathogenic bacteria. *Minnesota: American Phytopathological Society*. 1980. 72 p.
30. Ciampi L, Sequeira L, French ER. Latent infection of potato tubers by *Pseudomonas solanacearum*. *Am Potato J*. 1980;57(8):377–386.
31. VSN International. *Genstat for Windows 21<sup>st</sup> Edition*. VSN International, Hemel Hempstead, UK. 2020.
32. Shen G, Zhang S, Liu X, Jiang Q, Ding W. Soil acidification amendments change the rhizosphere bacterial community of tobacco in a bacterial wilt affected field. *Appl Microbiol Biotechnol*. 2018;102(22):9781–9791.
33. Michel V V, Wang J, Midmore DJ, Hartman GL. Effects of intercropping and soil amendment with urea and calcium oxide on the incidence of bacterial wilt of tomato and survival of soil-borne *Pseudomonas solanacearum* in Taiwan. *Plant Pathol*. 1997;46(4):600–610.
34. Michel V V., Mew TW. Effect of a soil amendment on the survival of *Ralstonia solanacearum* in different soils. *Phytopathology*. 1998;88(4):300–305.
35. Ghorbani R, Wilcockson S, Koocheki A, Leifert C. Soil management for sustainable crop disease control : a review. *Env Chem Lett*. 2008;6:149–162.
36. Tafesse S, Braam C, van Mierlo B, Lemaga B, Struik PC. Association between soil acidity and bacterial wilt occurrence in potato production in ethiopia. *Agronomy*. 2021;11(8):1541.
37. Hu Y, Li Y, Yang X, Li C, Wang L, Feng J, et al. Effects of integrated biocontrol on bacterial wilt and rhizosphere bacterial community of tobacco [Internet]. Vol. 11, *Scientific Reports*. Nature Publishing Group UK; 2021. Available from: <https://doi.org/10.1038/s41598-021-82060-3>
38. He K, Yang SY, Li H, Wang H, Li ZL. Effects of calcium carbonate on the survival of *Ralstonia solanacearum* in soil and control of tobacco bacterial wilt. *Eur J Plant Pathol*. 2014;140(4):665–75.
39. Viadé A, Fernández-Marcos LM, Hernández-Nistal J, Alvarez E. Effect of particle size of limestone on Ca, Mg and K contents in soil and in sward plants. *Sci Agric*. 2011;68(2):200–208.
40. Anderson NP, Hart JM, Sullivan DM, Christensen NW, Horneck DA, Pirelli GJ. *Applying Lime to Raise Soil pH for Crop Production (Western Oregon)*. Oregon State University Extension publication. 2013.
41. Zhang S, Liu X, Zhou L, Deng L, Zhao W, Liu Y, Ding W. Alleviating soil acidification could increase disease suppression of bacterial wilt by recruiting potentially beneficial rhizobacteria. *Microbial Spectr*. 2022;10(2):1–14.
42. Al-Ani AN, Dudas MJ. Influence of Calcium Carbonate on Mean Weight Diameter of Soil. *Soil Tillage Res*. 1988;11:19–26.
43. Marquez WL, Baucas M. Effect of liming on the yield and nutrient uptake of potato, carrot, cabbage, and garden pea [1990]. *Northern Philippines*; 1990.
44. Jovovic Z, Dolijanovic Z, Spalevic V, Dudic B, Przulj N, Velimirovic A, et al. Effects of liming and nutrient management on yield and other parameters of potato productivity on acid soils in montenegro. *Agronomy*. 2021;11(5):1–13.
45. Caires EF, Haliski A, Bini AR, Scharr DA. Surface liming and nitrogen fertilization for crop grain production under no-till management in Brazil. *Eur J Agron* [Internet]. 2015;66:41–53. Available from: <http://dx.doi.org/10.1016/j.eja.2015.02.008>
46. Smyth TJ, Sanchez PA. Effects of Lime, Silicate, and Phosphorus Applications to an Oxisol on Phosphorus Sorption and Ion Retention. *Soil Sci Soc Am J*. 1980;44(3):500–505.
47. Ejigu W, Selassie YG, Elias E, Molla E. Effect of lime rates and method of application on soil properties of acidic Luvisols and wheat (*Triticum aestivum*, L.) yields in northwest Ethiopia. *Heliyon* [Internet]. 2023;9(3):e13988. Available from: <https://doi.org/10.1016/j.heliyon.2023.e13988>
48. Getachew A, Temesgen D, Tolessa D, Ayalew A, Geremew T, Chelot Y. Effect of lime and phosphorus fertilizer on acid soil properties and barley grain yield at Bedi in Western Ethiopia. *African J Agric Res*. 2017;12(40):3005–312.