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EFFECT OF CROP ROTATION ON TOMATO BACTERIAL WILT (*RALSTONIA SOLANACEARUM*) AND SURVIVAL OF THE PATHOGEN IN THE RHIZOSPHERES AND ROOTS OF DIFFERENT CROPS IN ETHIOPIA

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A B S T R A C T

Survival of *Ralstonia solanacearum* in a environment or ecosystem depends on many factors, such as the race or strain of the pathogen, physical, chemical, biological soil factors and presence or absence of a host and non-hosts plants. The objectives of this study were to assess the effect of one and two season rotation sequences on the development of tomato bacterial wilt; and the survival ability of *R. solanacearum* in the rhizosphere and roots of presumable hosts and non-host crops in Ethiopia. A one season crop rotation involving tomato- maize-tomato, tomato- common beans - tomato and two season rotations consisting of tomato - maize- common bean-tomato, tomato –common beans – maize-tomato and tomato – tomato- tomato were established at Melkassa in Ethiopia. The effect of the system was evaluated on bacterial wilt of tomato under field conditions using a susceptible tomato cultivar (Marglobe). In one season rotation treatment involving common bean and maize after tomato resulted in a reduction of an average 6% and 16% final wilt incidence, respectively. Similarly, in the two seasons rotation sequence growing tomato after bean-maize and maize-bean resulted in about 29% average final wilt incidence reduction. The onset of wilt incidence was also delayed by one week in the two season rotations with common bean and maize compared to continues tomato growing and one season rotation with non-host crops. Survival of ability of *R. solanacearum,* strain designated as *TomNa3* biovar 1 race 1 was studied under soil rhizosphere and roots of presumably non-host and hosts of different crops under glasshouse conditions. The pathogen was detected in rhizosphere soils and roots of presumable non-host and hosts for the pathogen after 120 days after inoculation. The population of bacterial pathogens was recorded in a declining trend but detectable in the rhizosphere soils and roots of presumable non-host crops at 30, 45, 60, 90 and 120 days after inoculation.

Keywords: Bacterial Wilt, Crop Rotation, Survival, *Ralstonia Solanacearum,* rhizosphere, tomato.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is a serious disease and a major constraint in the production of tomatoes and many other crops in tropical, subtropical, and warm temperate regions of the world (Hayward, 1991). The pathogen is soil-borne and its survival in a particular environment or ecosystem mainly depends on many factors, such as the race or strain of the pathogen, physical, chemical and biological soil factors, presence or absence of hosts and presumed non-hosts (Granada and Sequeira, 1983; Hayward, 1991; Michel and Mew, 1998; Van Elsas *et al*., 2000;). Information on the fate pathogen between cropping seasons and its effective spread to uninfected plants are crucial aspects of the plant disease cycle and management approaches. Bacterial wilt is among the most difficult bacterial diseases to control (Sadler, 2005), and the efficacy of current tactics for management of the disease is highly localized. No conventional bactericides are known to provide effective control of this soil-borne pathogen. Recommended control methods of soil-borne diseases like bacterial wilt include the use of non-chemical methods such as host resistance, organic amendments, crop rotation, soil solarization, and various cultural practices (Michel and

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Mew, 1998; Katan, 2000). The use of resistant varieties, however, is constrained by the high variability of pathogen strains and by environmental factors such as temperature, soil moisture, and the presence of root-knot nematodes (Wang and Lin, 2005).

Crop rotation is among the various cultural practices that can be used both for soil- borne pathogens (Katan, 2000). Several kinds of grass and other crops such as sweet potato have been reported for their effect in reducing bacterial wilt incidence (Akiewand Trevorrow, 1994; Lemaga *et al*., 2001; Saddler, 2005). The number of seasons that a rotation crop should be grown depends on the level of infestation, the survival capacity of the pathogen in local soils and prevailing climate, and other factors. In some cases, a single year of rotation can significantly reduce bacterial wilt (at least in the short term), but the usual recommendation is for a two or more years rotation schedule (Abdullah and Sijam, 1992). Bare fallowing and flooding can also reduce the pathogen soil population (Hartman and Elphinstone, 1994), but generally are not feasible. Unfortunately, farmers often do not adopt effective rotations or intercropping because of limited land availability and pressure to produce a subsistence crop or one with high cash value (Michel *et al*., 1997; Lemaga *et al.,* 2001;). However, it is generally considered that crop rotation with a non-host crop is one of the recommended control options (Lemaga *et al.,* 2001, Michel *et al*., 1997). Nevertheless, use of crop rotation for the control of bacterial wilt has been reported to be a strain, crop or region specific (Akiew and Trevorrow, 1994; Saddler, 2005). Due to the wide range of crop and weed hosts of the pathogen of bacterial wilt (Hayward, 1994), it is essential to identify the crops, which can best fit for such purposes. At the same time, it is important to provide information on the extent of pathogen survival under prevailing local conditions.

Therefore, the objectives of this study were to assess the effects of one and two seasons rotation sequences on the development of tomato bacterial wilt; and the survival ability of *R. solanacearum* in the rhizosphere and roots of presumable hosts and non-host crops at Melkassa condition in Ethiopia.

MATERIALS AND METHODS

Study site and field conditions: The experiments were carried out at Melkassa Agriculture Research Center (MARC), Ethiopia located at $8^0$24'985 N latitude and $39^0$19.529 'E longitudes, an altitude of 1,550 meters above seas level. The total average annual rainfall at the centre is 763 mm, about 70% of which is received during the main rainy season from June to September. The annual average maximum temperature is 28.4^0C and the minimum average temperature is 14^0 C. The soil texture of the experimental field plot is loamy type with 32% sand, 42% silt and 26% of clay, pH = 7.9, organic matter (1.18%), available phosphorous (P) (14.22 ppm), cation exchange capacity (CEC) of 32 milliequivalent (meq/100 g of soil). Carbon to nitrogen ratio (C/N) is 10 and exchange cation (meq/100) of 0.72, 4.1, 18.5 and 3.37 for Na^{+1}, K^{+1}, Ca^{++2} and Mg^{++2}, respectively. The experimental field plots were observed to be free of bacterial wilt disease caused by *R. solanacearum.*

Bacterial strain and field inoculation: A highly virulent bacterial strain, designated as *TomNa3* isolated from tomato in Ethiopia belonging to biovar 1/race 1 phylotype III, resistant to the rifampicin antibiotic (50ppm) was used for artificial field inoculation. The bacterial strain was maintained as suspension in sterile distilled water in an Eppendorf tube at room temperature and revived by streaking on triphenyl tetrazolium chloride (TTC) agar medium (Kelman, 1954) containing (10 g Bacto peptone, 5 g D-glucose, 1 g casamino acids, 15 g Bacto agar, 1000 ml distilled water and 10 ml of filter-sterilized solution of 0.5% (w/v) 2,3,5-triphenyl tetrazolium chloride (Sigma, Germany) was added to the medium after autoclaving. A single colony of the strain was further multiplied on nutrient glucose agar (NGA) medium containing 0.3% beef extract, 0.5% Bacto peptone, 0.25% D-glucose and 1.5% agar incubated for 48 h at 30 °C. Inoculum suspension of an optical density (OD_{620nm} = 0.06 corresponding to approximately 10^8 CFU/ml (colony forming units per milliliter) was inoculated as soil drench at a rate of 1 liter/m^2) to freshly field transplanted tomato seedlings as described by Michel *et al*. (1997) on same day of transplanting. Seedlings of the susceptible tomato cultivar (Marglobe) were raised on seedbed and transplanted to field plots on June 18, 2007. To maintain soil moisture and enhance the development of bacterial wilt, all plots were watered through furrow irrigation by taking precaution and avoiding over the flow of water from one plot to the others. The incidence of bacterial wilt was monitored starting 4 days post inoculation (dpi) up to 30 dpi where no further disease development occurred. The average final wilt incidence reached 85%±4 in inoculated plots after 30 days post inoculation

and then the plants were chopped and mixed with the soil and beds were re-cultivated manually and ready for rotation sequence treatments.

Experimental design and rotational sequences: Two independent rotation sequence experiments were setup as one and two season rotations in the field plots of artificially inoculated fields as described in section 2.2. The one season rotation sequence was grown from September- December 2007 and the rotation effect was monitored during February-March, 2008. The two season rotation sequences were grown during September-December, 2007 and February-April, 2008. The two season rotations effect was monitored during May- June 2008. A field plot of 4.20 m x 4 m was demarcated and arranged in a randomized complete block design (RCBD) and replicated four times. A one-season rotation treatment consisted of: (1) tomato-maize- tomato, (2) Tomato – common bean - tomato (3) Tomato – tomato - tomato and the two season rotations treatments included **(**1) tomato - maize- common bean - tomato, (2) tomato –common beans – maize - tomato (3) tomato – tomato – tomato - tomato. Maize (Melkassa-1), common bean (Awash-1) and tomato moderately resistant genotype (King Kong 2) were used. The susceptible tomato cultivar (Marglobe) was planted to monitor bacterial wilt disease development at the end of each rotation sequences.

Assessment of disease development: In each treatment, bacterial wilt symptom development was evaluated daily until the fourth-day post inoculation and then at 7, 10, 14, 21, 30 and 45 days after transplanting. The two central rows of plants per plot were labelled and disease severity was assessed for each plant in all plots. A six-point rating scale (0-5) modified from Winstead and Kelman (1952) was used for wilt severity scoring, where 0 = no wilt symptoms, 1 = one leaf wilted, 2 = two or more leaves wilted, 3 = all leaves except the tip wilted, 4 = whole plant wilted and 5 = death (collapse) of the whole plant. Disease incidence was assessed as a percentage of wilted plants within each treatment and percent severity index (PSi) was calculated according to Cooke (2006).

$$PSi = \sum (scores*100) / NPR * MS$$

where, NPR = number of plants rated MS = maximum scale of the score.

The area under disease incidence progress curve (AUDiPC) and area under percent severity index progress curve (AUPSiPC) for each treatment and experiment were calculated on the basis of wilt incidence and percent severity index using the trapezoid integration of the disease progress curve over time with the following formula adopted from Shaner and Finney(1977) and Jeger and Vijanen-Rollinson (2001).

The AUDiPC (area under disease incidence progress curve) was estimated as follows:

$$AUDiPC = \sum_{i=1}^{n} [(I_{i+1} + I_i)] / 2 \times [t_{i+1} - t_i]$$

in which I_i = mean wilt incidence at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations. Similarly, the area under percent severity index progress curve (AUPSiPC) was calculated as:

$$AUPSiPC = \sum_{i=1}^{n} [(PSi_{i+1} + PSi_i)] / 2 \times [t_{i+1} - t_i]$$

in which PSi_i = mean of disease percent severity index at the i^{th} observation, t_i = time (days) at the i^{th} observation, n = total number of observations.

Survival of *R. solanacearum* under Rhizosphere Soils and Roots of crops

Growth of seedlings, bacterial strain and inoculation: Survival of *R. solanacearum* in the rhizosphere soils and roots of various crops was studied under glasshouse conditions using seedlings of presumable hosts and non-host crops of the pathogen. The same *R. solanacearum* strain, *TomNa3* used for field infestation in crop rotation experiment was used for survival study. The hosts of *R. solanacearum,* hot pepper (Marekofana, local cultivar) and tomato (King Kong 2) and the presumable not- host crops, maize (cultivar Melkassa-1) and common bean (Awash-1) were used in the study. Seeds of each crop species were sown on a plastic tray containing unsterilised sandy loam soil obtained from same field plot as used for crop rotation experiment but supplemented with commercial fertilizer [Urea and DAP (di-ammonium phosphate)]. Seedlings of each crop species were transplanted into an individual 16 cm diameter plastic pot containing same soil type as above 20-30 after days sowing. The seedlings were uprooted and roots were washed in tap water, then dipped into freshly prepared bacterial suspension with an optical density of OD_{620nm} = 0.06, corresponding to 10^8 colonies forming unit per ml water (CFU/ ml) prepared as described in section 2.2 in a big flask. From each crop species, 20 seedlings were inoculated in two repetitions. Five seedlings from each crop species were

included as a control. After inoculation pots were placed in a glasshouse with temperature ranging from 25-27 ^{0}C and relative humidity of 60 to 70% and watered daily.

Sampling and quantification of *R. solanacearum:* Determination of *R. solanacearum* in rhizosphere soils was performed by the method of Granada and Sequeira (1983) and Pradhanang *et al*.(2000) on modified semi-selective media (SMSA) (Englebercht, 1994) consisting all ingredients of TTC and supplemented with antibiotics dissolved in 70% of ethanol. The volume of antibiotics added were 0.5 ml of 1% of crystal violet, 10.0 ml of 1% polymixin B sulfate, 2.5 ml 1% of bacitracin, 0.5 ml of 1% chloromycetin, 0.5 ml of 1% penicillin and 2.5 ml 1% cycloheximide. Determination of the bacterial population was performed at a regular interval of 30, 45, 60, 90 and 120 dpi. At each test interval, three seedlings from each crop species were used to determine the presence of *R. solanacearum* in the rhizosphere soil and roots. Seedlings were completely uprooted and the rhizosphere soil clenching to the root system was shaken into a clean beaker. About 10 g soil samples were suspended in 100 ml distilled sterile water in a clean polyethylene bag, shaken vigorously for 1-2 minutes, and allowed to stand for 1-2 minutes to settle. One milliliter of the supernatant was then removed and serially 10-fold diluted in distilled sterile water. One hundred micro-liter aliquots of least two dilutions were spread on modified SMSA in replicate per dilution. Plates were then incubated for 48-72 hours at 30^0 C. Typical characteristics features of *Ralstonia solanacearum* colonies were evaluated as bold, fluidal and creamy-white in colour on SMSA agar to distinguish from colonies of other bacteria and presumptive colonies were marked and counted and calculated as CFU/g soil. Similarly, determination of *R. solanacearum* from the root was done from the same uprooted seedlings for the rhizosphere soil quantification. The roots were washed thoroughly with tap water to remove adhering soil, and 1 cm sections were cut from upper, middle and lower parts of the underground tap and lateral roots. Approximately 0.5-1 g root tissues were weighed and surface sterilized by submerging the sample in 70% alcohol for about 5 minutes, rinsed two to three times in distilled sterile water and blotted dry with paper towel. The tissues were then macerated in 20 ml of distilled sterile water and allowed to stand for about 20 minutes. From each macerated suspension one milliliter suspension was removed and serially a ten-fold dilution was prepared. From the least two dilutions 100 µl were spread on TTC media in replicate and incubated at 30^0 C for 48 hours. Numbers of typical colonies as irregular with a characteristic red centre and whitish periphery on TTC medium (Kelman, 1954) were counted were counted per plate and the number of colonies forming units was calculated as CFU/g fresh root weight. All materials such as beakers, Eppendorf tubes and pipette tips were sterilized by autoclaving before use.

Calculation and statistical analysis: Data of bacterial colony counts from the rhizosphere soils and roots at the different test intervals were logarithmically transformed into $\log_{10}$ (CFU +1) before performing analysis of variance. The effect of rotation sequence on bacterial wilt incidence, percent severity index, area under disease incidence progress curve and area under percent severity index progress curve analyzed using SAS general linear model procedure (SAS,2003 for Windows, 1999-2003, SAS Institute, carry, USA) and means separation was based on the least significance difference at 5% using the Waller-Duncan K-ratio t -test.

RESULTS

Effect of one and two season crop rotation: The bacterial wilt incidence, the percent severity index, the area under disease incidence progress curve and the area under the percent severity index curve were generally significantly reduced ($P \leq 0.05$) in both one season and two season rotations after common beans and maize in comparison to continues tomato cultivation (Table 1). In the one season rotation treatments involving common beans and maize after tomato, a reduction of 6% and 16% final wilt incidence, respectively was observed. Similarly, in the two season rotations growing tomato after common bean-maize and maize-common bean, the final wilt incidence was reduced by 29%.

Table 1. Effect of one season (A) and two season rotations (B) on tomato bacterial wilt incidence (Wi), percent severity index (PSi) 45 days after planting (dap) and expressed as area under wilt incidence progress curve (AUDiPC) and area under percent severity index progress curve (AUPSiPC), Melkassa, Ethiopia.

	Season 1			
Treatment	Wi	PSi	AUDiPC	AUPSiPC
Tomato-common bean-tomato	70.92A	15.31B	1475.9B	298.00B
Tomato-maize-tomato	63.63B	16.32B	1404.5B	300.88B
Tomato-tomato-tomato	75.78A	23.89A	1860.4A	438.74A
Mean	70.54	18.92	1601.83	353.01
CV	5.97	17.55	10.10	14.03
LSD ($P < 0.05$)	6.33	4.99	241.34	73.19
	Season 2			
Treatment	Wi	PSi	AUDiPC	AUPSiPC
Tomato-common bean-maize-tomato	50.10B	23.03BA	1345.70B	419.09B
Tomato-maize-common bean-Tomato	50.46B	19.16B	1360.79B	434.49B
Tomato-tomato-tomato	71.45A	28.49A	2018.44A	691.08A
Mean	57.45	23.55	1574.98	514.88
CV	8.55	19.27	6.84	10.78
LSD ($P < 0.05$)	7.44	7.63	160.85	83.67

Means followed by a same letter(s) in same columns under the same heading are not significantly different at ($P \leq 0.05$) using the Waller-Duncan K-ratio t -test.

Comparison of disease progress in one season and two season rotations, the later involving common bean-maize-tomato and maize-common bean-tomato sequences resulted in delayed onset of wilt incidence up to 10 days after planting in comparison to tomato with out rotation (Figure.1). However, after the onset of wilt, the disease progress followed an increasing trend in all treatments but with significantly lower final wilt incidence for two season rotation in common bean-maize and maize-common bean rotation sequences (Figure1).

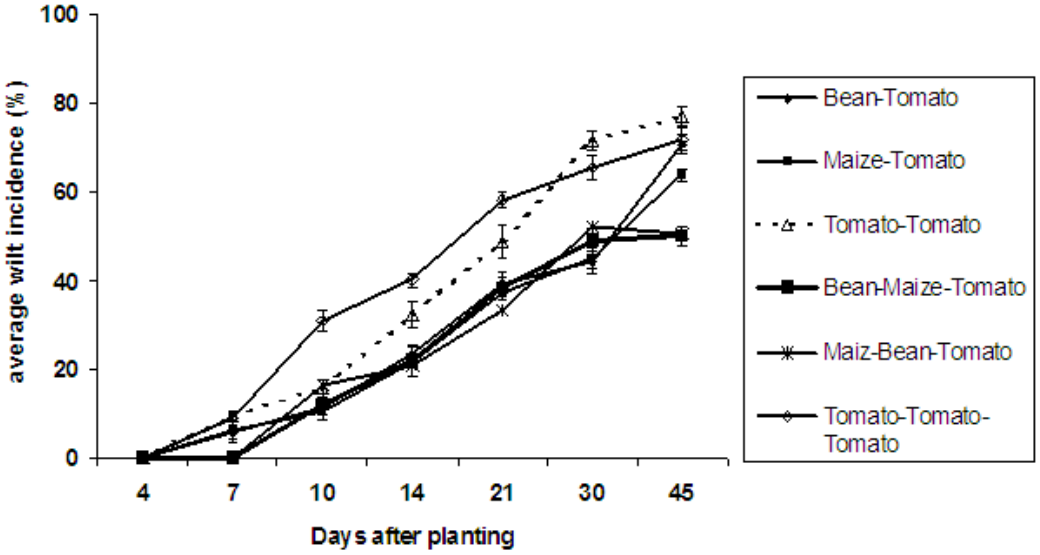

Figure 1. Development of wilt incidence under one and two season crop rotation and with out rotation on tomato at Melkassa, Ethiopia.

Survival of *R. solanacearum* Strain in Soil Rhizosphere and Crop Roots: *R. solanacearum* was detected in the rhizophere soils and roots of presumable non-host during all test intervals at 30, 45, 60, 90 and 120 days after inoculation. However, the population of *R. solanacearum* in the rhizosphere soils and roots of non-host crops was significantly lower ($P \leq 0.005$) as compared to presumable host crops at all sampling

dates. (Table 2). The overall bacterial population showed a declining trend but was detectable up to 120 days after inoculation both in the rhizosphere soils and roots of common bean and maize.

Table 2. Population of *R. solanacearum* [Log_{10} CFU+1/ g soil or fresh root weight] in rhizosphere soils and roots of tomato, common bean, maize and hot-pepper plants after 30, 45, 65, 90 and 120 days post-inoculation (dpi) under glasshouse conditions.

No	Sample	Log_{10} [CFU+1]/g soil or fresh root weight]				
		30 dpi	45 dpi	60dpi	90dpi	120 dpi
1	Bean-rhizosphere	5.19B	5.35C	5.41D	4.73B	4.00B
2	Bean roots	5.04B	5.74B	5.46D	4.59B	4.26B
3	Maize-rhizosphere soils	5.11B	5.35C	5.41D	4.69B	4.00B
4	Maize –roots	5.18B	5.37C	5.43D	4.69B	4.32B
5	Tomato-rhizosphere soils	7.87A	8.04A	7.59BC	6.74A	6.51A
6	Tomato-roots	7.93A	8.00A	7.51C	6.86A	6.62A
7	Hot-pepper-rhizosphere soils	7.84A	7.95A	7.74BA	6.87A	6.47A
8	Hot-pepper-roots	7.90A	7.98A	7.85A	6.92A	6.53A
	Mean	6.51	6.72	6.55	5.76	5.34
	CV	1.95	3.02	1.97	1.88	5.06
	LSD	0.22	0.35	0.22	0.19	0.47

Means followed by a same letter(s) in same columns are not significantly different at (P≤0.01) using the Waller-Duncan K-ratio t -test. on LSD test.

DISCUSSION

A reduction in bacterial wilt incidence and associated wilt parameters measured in this study was achieved in crop rotation using non-host crops to *R. solanacearum*. Similar results were reported on the reduction of tomato bacterial wilt severity by rotation with corn, lady's finger and cowpea (Adhikari and Basnyat 1998). The study of Lemaga *et al.* (2001) on potato bacterial wilt indicate a reduction of wilt incidence with one and two season crop rotation under different infestation levels using potato- common bean- maize-potato rotation sequences. Furthermore, the result also is in good agreement with the available document on the beneficial effect of crop rotation with non-host crops for bacterial wilt of tobacco (Melton and Powell, 1991). In spite of the statistically significant reduction in bacterial wilt incidence and associated parameters assessed in one season and two-season rotation in this study, the practice could not be recommended as sufficient means for tomato bacterial wilt disease management when a susceptible tomato cultivar is used and the initial wilt incidence is very high. In one season rotation, the wilt incidence of 63 and 70% of bean and maize respectively indicate the inefficiency of the practice to minimise the disease completely or reduce it. Similarly, in the two season rotation, the final wilt incidence was 50% both after bean-maize and maize-bean rotation sequences. Therefore, crop rotation either as one season or two seasons sequence was not satisfactory to achieve bacterial wilt control. It requires an evaluation of more than one season rotations or other supplementary disease management options such as soil amendment with silicon (Diogo and Wydra, 2007) intercropping and more resistant cultivar (Michel *et al.,* 1997). Similarly, Abdullah & Sijam (1992) reported an increase in the bacterial population after the planting of tomato and decreased when all other non-host vegetable crops were grown in rotation sequence with tomato but bacterial populations persisted to detectable levels. However, a single or double sequence of non-host plants did not reduce wilt disease even though the population decreased significantly after each planting of the non-host crop. This indicates that more than two plantings of non-host crops in any combination are necessary to decrease the bacteria population to a low level and reduce bacterial wilt incidence.

R. solanacearum persisted for more than 120 days in the rhizosphere soils and roots both of under presumable non-host and hosts for the pathogen, however, with a declining trend in the case of non-host crops. One reason for this reduction in the population over time could be that association of the pathogen to roots of non-host plants remain localized without expression of symptoms and that the total population of bacteria multiplied from non-host plants is not as high as from susceptible ones

(Granada and Sequeira, 1983). Furthermore, the long-term survival of *R. solanacerum* strain for an extended period under host debris and live plants has been documented by Granada and Sequeira (1983). Hence, the ability of *R. solanacearum* to colonize the roots of non-hosts plants such as corn and other plants previously thought not to maintain a pathogen population accounted for the failure of crop rotation for the control of *R. solanacerum* in short-term condition (Granada and Sequeira, 1983).

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REFERENCES

Abdullah, H and K. Sijam.1992. Effect of selected vegetable crops on bacterial wilt pathogen population and their use in crop rotation programmes for bacterial wilt disease control. *Acta Horticulture*, 292: 161-165.

Adhikari, T.B and R.C.Basnyat. 1998. Effect of Crop Rotation and Cultivar Resistance on Bacterial wilt of tomato in Nepal. *Canadian Journal of Plant Pathology*. 20: (3) 283-287.

Akiew,E and P.R.Trevorrow. 1994.Management of Bacterial Wilt in Tobacco. In: Hayward, A.C and Hartman, G.L, (eds.). Bacterial wilt: The Disease and its Causative Agent, *Pseudomonas solanacearum*. Wallingford, UK: CAB International, 179-198.

Cooke, B.M., 2006. Disease assessment and yield loss, (In B.M, Cooke, D. G. Jone and B, Kaye, (Eds.). The Epidemiology of Plant Diseases. Second edition.p.43-80.

Englebrecht, M.C. 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. P.3-5. In Hayward, A.C (ed.) Bacterial wilt Newsletter 10: Australian center for international Agricultural Research, Canberra, Australia.

Diogo, R.V.C and K.Wydra, 2007. Silicon–induced basal resistance in tomato against *Ralstonia solanacearum* is related to modification of pectic cell wall polysaccharide structure. *Physiology and Molecular Plant Pathology*, (2007), doi.10.10.1016/j.pmmpp.2007.07.008.

Granda, G.A and L.Sequeira. 1983. Survival of *Pseudomonas solanacearum* in the soil, rhizosphere, and plant roots. *Canadian Journal of Microbiology*: 29: 433 - 440

Hartman, G.L and J.G.Elphinstone. 1994 Advances in the control of *Pseudomonas solanacearum* race 1 in major food crops. P.157-177. In: A.C. Hayward and G.L.Hartman (eds. Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum*. Wallingford, UK: CAB International.

Hayward, A.C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annual Review of Phytopathology*.29: 65 -87.

Hayward, A.C. 1994. The hosts of *Pseudomonas solanacearum*. pp. 9 – 24: In: A.C. Hayward, and G.L.Hartman (eds.). Bacterial wilt: The Disease and its Causative Agent, *Pseudomonas solanacearum*. Wallingford, UK: CAB International.

Jeger, M.J and S.L.H. Viljanen-Rollinson. 2001. The use of the area under the disease progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars: *Theoretical and Applied Genetics*; 102:32–40.

Katan, J. 2000.Physical and cultural methods for the management of soil-borne pathogens. Crop Protection 19: 725 - 731.

Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a Tetrazolium medium, Phytopathology, 51:158–61.

Lemaga, B. R. Kanzikwera, R.Kakuhenzire, J.J.Hakiza and G.Manzi. 2001. Effect of crop rotation on bacterial wilt incidence and potato tuber yield. *African Crop Science Journal.* 9 (1): 257-266.

Melton, T.A and N.T.Powell. 1991. Effects of two-year crop rotations and cultivar resistance on bacterial wilt in flue-cured tobacco. *Plant Disease*, 75 (7): 695-698.

Michel, V. V and T.W.Mew. 1998. Effect of a soil amendment on the survival of *Ralstonia solanacearum* in different soils. Phytopathology, 88:300-305.

Michel, V.V., J.F.Wang, D.J.Midmore and G.LHartman. 1997. 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 Pathology*: 46: (4), 600-610.

Pradhanang, P.M, J.G.Elphinstone, and R.T.V.Fox. 2000. Sensitive detection of *Ralstonia solanacearum* in soil: a comparison of different detection techniques. *Plant Pathology,* 49: 414-418.

Sadler, G.S. 2005. Management of Bacterial Wilt Disease. P.121 – 132: In Allen, C Prior, P and Hayward, A.C (Eds). Bacterial Wilt Disease and *Ralstonia solanacearum* Species Complex, The American Phytopathological Society. St.Paul Minnesota, USA.

SAS Institute Inc., 2003. SAS/STAT Guide for Personal Computers, Version 9.0 edition. SAS Institute Inc., Cary, NC.

Shaner, G., and R.E.Finney. 1977. Effect of nitrogen fertilization on expression of slow-mildewing resistance in Knox wheat. Phytopathology; 67, 1051–1056.

van Elsas, J. D, P.Kastelein, P.van Bekkum, J.M.van der Wolf, P.M.de Vries and L.S.van Overbeek. 2000. Survival of *Ralstonia solanacearum* Biovar 2, the causative agent of potato brown rot, in field and microcosm soils in temperate climates. *Phytopathology* 90:1358-1366.

Wang, J and C. Lin. 2005. Integrated Management in tomato bacterial wilt: The world vegetable center brochure AVRDC. p. 1-14. Available at: http://www.avrdc.org/pdf/BW_brochure.pdf.

Winstead, N and A.Kelman. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. *Phytopathology*, 42: 628-634.