Survival of *Pseudomonas Syringae pv. tomato* in different soil types and moisture regimes

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Abstract

A study was carried out to determine the survival of *Pseudomonas syringae pv tomato* (*P. s. pv. tomato)* in the soil. Sandy, loam and clay soils were collected from Chinamhora communal area and sterilised before inoculation with an estimate of 5 x 10^{10} cfu/ml of *P.s.pv. tomato* bacterium. The inoculated soils were incubated at room temperature in the laboratory for four months. Two soil conditions wet and dry were maintained throughout the experiment for each of the soil types. Bacterial population in the different soils was measured by the plate dilution method on a monthly basis for 4 months. Soil type and soil moisture significantly ($P<0.01$) affected the survival of *P.s. pv. tomato* in the soil. The bacterium survived well in wet soils as was shown by higher number of colony forming units than in dry soils. Sandy soils had the highest number colony-forming units then loam and clay had the least number. It was concluded that as the texture becomes finer the bacterial growth decreases as air spaces decrease. Therefore sandy soils support more bacteria than loam and clay soils respectively. Wet soils had a higher population of the bacterium than dry soils. Therefore the combination, which had more bacteria surviving, was wet sand soil.

Introduction

The bacterium *Pseudomonas syringae pv. tomato* (Young dye and Wilkie, 1978) synonymously known as *Pseudomonas tomato* causes bacterial speck disease on tomatoes (*Lycopersicon lycopersicum*). In Zimbabwe bacterial speck is one of the four most significant diseases of tomatoes (Dillard, Cole, Hedges, Turner,Utete, Mvere, Gubba and Wilkinson, 1994).

Pseudomonas syringae pv. tomato causes lesions on leaflets, which are dark brown to black a yellow halo, may appear at later stages.

Superficial sunken black spots of 1-3 mm in diameter (Jones, 1991) may also occur. Speck lesions may be deep enough to reduce the quality of tomatoes after mechanical removal of skin. Fruits that develop after severe defoliation by the disease are sunscalded, small and of poor quality (Monroe. J.

Goode, Myron Sasser 1980). Infection at early stages may occur resulting in reduced photosynthetic ability defoliation and flower abscission (Yunis *et al* 1980 and McCarter 1982). Infection at an early stage can also result in 75% loss in yield whereas if it occurs later in the season, about 5% loss in yield can be experienced (Yunis *et al* 1980). *Pseudomonas syringae pv tomato* can behave as a root pathogen decreasing plant stem and root weights.

The disease was once considered to be of little economic importance (Bryan*.,* 1983) but is now recognized as a significant disease of tomatoes capable of causing both quality and yield reductions (Kim, 1979 and Smitely, 1982). In recent years the disease has become more widespread and damaging (Goode, Sasser, and Jones 1991). The disease has been of great concern to tomato growers in communal and smallholder farms in Zimbabwe. The disease is easily detected at grading. This reduces the quality for market as a result farmers are barred from entering the lucrative horticultural market.

The disease commonly occurs in the high rainfall areas of Zimbabwe. High humidity of over 60% and low temperatures between 18-20ºC are conducive for disease development. The disease was reported to be severe around April to May in Zimbabwe when conditions are cool and moist (Schneider and Grogan 1977). Temperatures up to 52º C can completely kill the pathogen (Devash *et al,* 1980).

Bacterial speck can be spread by splashing rain, irrigation water, wind blown rain, workers handling infected and healthy seedlings and contaminated farm implements. The bacterium can also be spread through contaminated seeds. This has been a major means of spread as some communal farmers prefer to use the seeds from tomatoes which were damaged from harvesting and packaging and will be unfit for the marketing in an effort to reduce the costs of production. *Pseudomonas syringae pv.tomato* has been reported to survive in soil and infected plant debris (Chamber and Merriman, 1975; Bosshard -heer 1977). Survival in the soil is an important means of spread from one season to another (Goto, 1972)

Bacterial speck is difficult to control once established in the field however in Zimbabwe copper ox chloride is the only registered control, which can be found in small packages in Zimbabwe. Surveys in Chinamhora West communal areas showed that most farmers depend on the use of chemicals for disease control (Manyangarirwa, 1996). Fungicides are commonly used regardless whether they are controlling a virus, bacterium or fungus. Forty seven percent of the farmers did not have proper spraying equipment whereas those who had the knapsack sprayers, they were not in proper condition. The farmers could not calibrate the knapsack

Properly and they did not have measuring cylinders to make accurate measurements. Most of the farmers wore no protective clothing during chemical applications and this is a health hazard to farmers.

The use of chemicals in controlling bacterial pathogens is not effective since there is no vulnerable stage in the life cycle of the pathogen that can be targeted, unlike in the control of fungal pathogen where one can target on the spores before they germinate or penetration stage or inhibit production of secondary inoculums.

Therefore knowledge of the pathogen survival in the soil can help in disease control (Leben, 1981) by determining suitable crop rotations and rotation intervals, which are likely to kill off all resident populations of soil borne bacterium, and rotation crops, which can be sources of inoculum for the disease.

Therefore the objectives of the experiment are:

1. To assess pathogen survival in different soil types from tomato communal production areas.
2. To determine the effect of different moisture regimes on the survival of *Pseudomonas syringae. tomato*

The objectives were based on the hypothesis that:

1. Sandy soils support more bacteria than loamy soils and clay Soils (Leben, 1981)
2. *Pseudomonas syringae pv. tomato* bacterial population decreases with an increase in moisture level overtime.

Materials and methods

Soils were collected from Mutoko and Chinamhora areas. These areas are major smallholder tomato growing areas in Zimbabwe. Three different soil types were collected (1) red clay soil from Mwayera village in Chinamhora (2) sandy soil from Nyamukapa village in Mutoko and (3) loam soil from Chinamhora. Soils were randomly collected from 10 sites in the tomato fields and these were mixed to make one sample. The soils were collected from a depth of 0-15cm using a soil auger. A 2 X 3 X 4 Factorial experiment simple randomized design with three replications will be done. The factors will be as follows:

2 soil moisture regimes, Wet soil and Dry soil

3 soil conditions clay, loam and sand

Soil sterilization

The soil samples were sieved through a 400 um mesh to remove large particles, plant debris and stones. The soils were be sterilized in autoclavable plastic bags at 121^0 C for 1 hour at 24 hour interval as described by Devash *et al.,* 1980). One kilogram of each soil type was weighed and put in plastic pots.

Inoculum preparation

The *Pseudomonas syringae pv.tomato* was obtained from stored inoculum from Plant Protection Research lnstitute at the Department of Research and Specialists Services and this was kept at 0^0 C. The bacterium was isolated from infected tomato leaves collected from Chinamhora communal lands. Confirmation of *P.s.pv. tomato* isolate was done by the biolog test. Other tests, which were done, were growing on test and observation the colony characteristics. The bacterium was subcultured on King Medium B (KMB), which is selective for florescent bacteria. Plates were incubated at 25^0 C for 24-48 hours. Inoculum was prepared by suspending a two day old culture in 10ml sterile distilled water and this was an estimate of 2 x 10-^{8} cfu/ml (Conlin and McCarter 1983).

Soil inoculation and incubation

25ml of 2 x 10^{-8} cfu/ml bacterial suspensions from 24- 48 hour old cultures grown on KMB was sprayed uniformly onto 1kg soil sample using a fine spray laboratory atomizer. The soils were mixed thoroughly in plastic pots. Controls were sprayed with sterile distilled water and were kept at field capacity by watering once every week.

Determination of *Pseudomonas syringae pv. tomato* in the soil

10g of the soil were collected from of each pot after every 4 weeks and were thoroughly mixed. The soils were put in sterile flasks and 100ml of sterile distilled water (SDW) added. The flasks were be shaken for 20 minutes on the swirling table, and then left for 24 hours to allow the solution to settle .One milliliter of each supernatant was serially diluted ten fold by first transferring into a bottle holding 1ml SDW to obtain 1/10 dilution of the original supernatant 1ml of the 1/10 dilution will be transferred to another bottle containing 9ml of sterilized distilled water until the dilution is 10^{-9}. 0.1ml of dilutions 10^{-7} to- 10^{-9} was spread on fresh KMB. Plates were incubated at 25^0 C. After 24-48 hours colonies were viewed under UV light for fluorescence and then counted with the aid of Pakes Enumeration Disc. Colonies of 30-300 were considered for counting. (Kirally *et al.,*_1970). A representative suspected *P.s. pv tomato* was used to compare the characteristics of the colonies from the soil solution.

Data Analysis

Analysis of variance (ANOVA) was used to analyse the data. Standard error of difference (SED) was used to separate means where there are treatment differences. Regression analysis was carried out to determine relationship between soil type, soil moisture and time of sampling with the number of surviving bacterial cells.

Results

The soil type, soil moisture and time of sampling significantly ($P<0.05$) affected the survival of *P.s.pv.tomato* in the soil. The initial population of *P.s.pv.tomato* was $5x10^{10}$ cfu/g and the population decreased significantly with time from January to April in all the three soil types in either wet or dry soil as shown in Fig 1,2 and 3. There was a gradual change within the first three months and a drastic change in the fourth month with the population going to zero. The bacterial population decreased with the sampling time, with the highest population in January and least in April with no surviving bacteria.

Wet sandy soil in January had the highest population of 5.645×10^{10} loam soils had a population of 1.850×10^{9} and clay soils with the least population of 1.050×10^{8} (Table 1). Wet loam and sandy soils had significantly ($P<0.05$) higher populations than dry soils. In January for example, wet sand soils had a population of 5.645×10^{10} and a slight increase from the initial population of 5×10^{10} cfu/g. Dry sandy soil, had decreased to 1.778×10^{9}. The population decreased significantly at different rates as shown by figure Fig 1 and 2. Clay soils had a high rate of decrease compared to other soils. There was no significant ($P<0.05$) difference in the bacterial population in wet clay soil and dry clay soil from January to April.

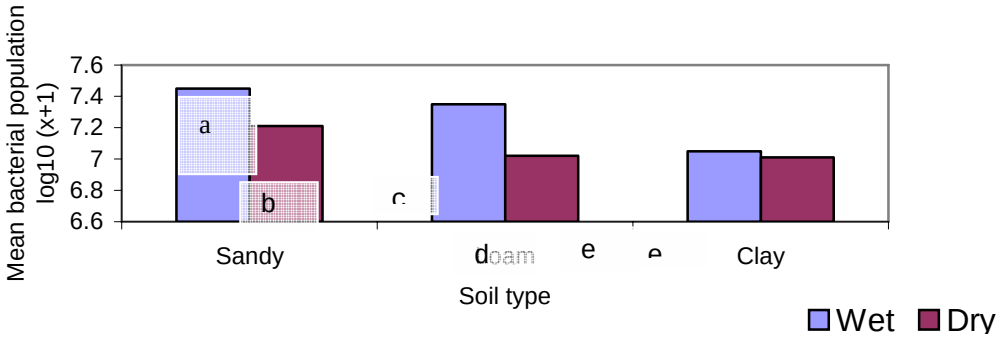

Fig 1. Bacterial cell survival in different soil types in January

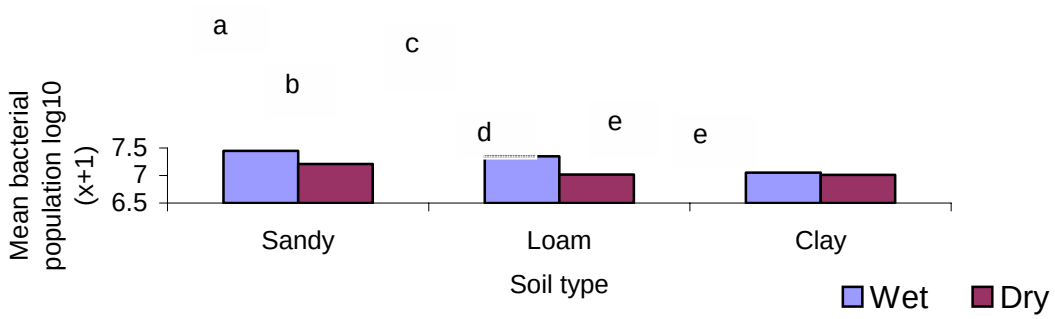

Fig 2. Bacterial cell survival in different soil types in February

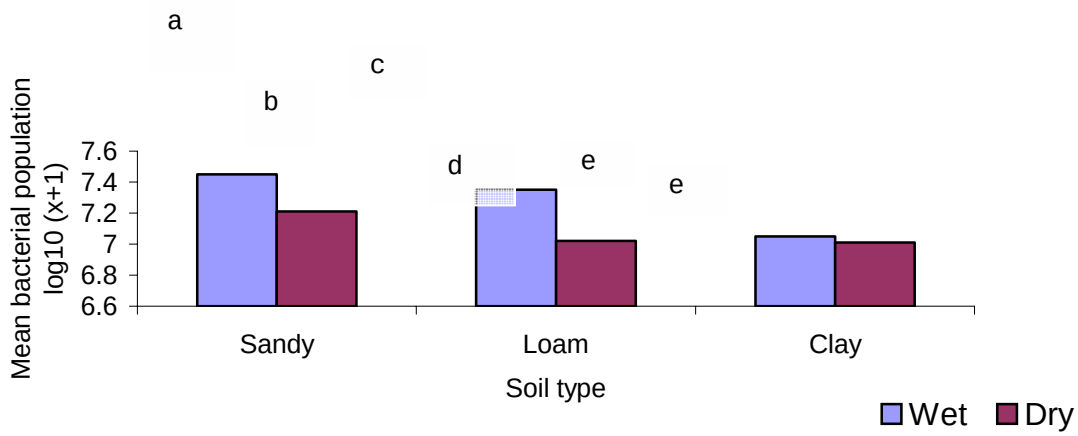

Fig 3 Bacterial cell survival in different soil types in March

Table 1. Mean Colony forming units (cfu) g^{-1} for surviving bacteria in different soil types from Chinamhora.

Soil Condition	Soil type		
	Sand (pH 5.6)	Loam (pH 5.8)	Sand (pH .6.8)
WET			
January	5.645×10^{10}	1.850×10^{9}	1.050×10^{8}
February	9.333×10^{8}	2.673×10^{9}	1.542×10^{7}
March	2.812×10^{7}	2.265×10^{7}	1.119×10^{7}
April	1.000	1.000	1.000
Mean	6.194×10^{6}	1.828×10^{6}	3.673×10^{5}
DRY			
January	1.778×10^{9}	2.168×10^{8}	2.793×10^{7}
February	1.064×10^{8}	3.412×10^{7}	1.271×10^{7}
March	1.622×10^{7}	1.047×10^{7}	1.023×10^{7}
April	1.000	1.000	1.000
Mean	1.324	5.272×10^{5}	2.45×10^{5}

Discussion

The study showed that soil moisture, soil type and time of sampling affect the survival of *P.s pv. tomato* in the soil. Sandy soil supported more bacteria than loam and clay soils respectively. This tallies with Leben's conclusion (1981) that sandy soils support more bacteria because of free air spaces access. Low bacterial populations in clay soils can also explain that *P.s pv. tomato* is strictly aerobic bacterium, which does not prefer conditions with limited air (Jones, 1991). There was no significant difference in wet or dry clay soil suggesting that the texture of clay is so fine and air is limited that wetting would not make much difference.

The results showed that moisture affects the survival of the bacterium. Wet conditions are more conducive for survival of the bacterium than dry soils. A significant higher bacterial population has showed this in wet loam and sand soils. This is possibly due to the presence of water solutes that flow easily in and outside the bacterial cells. This explains why *P.s.pv.tomato* multiply rapidly under moist conditions (Scheinder and Grogan, 1977). These findings also tally with a study by Wattterson, (1986) that for initiation of disease moist conditions are required and for initiation of disease in leaf, one-day leaf wetness is required

There was a decrease in the population from January to April. This bacterial cell decrease and ultimate death can be due to depletion of essential nutrients and accumulation of toxic wastes products as described by Tate, (1985).

P.s.pv. tomato survived in autoclaved soils for 3 months in this study. Devash *et al.,*(1980) reported survival of the bacterium in autoclaved soils for 6 months. In Switzerland the bacterium survived for 120 days approximately 4 months (Bosshard Heer and Vogelsanger, 1977).

The study of the bacteria survival in the soil has been reported to be a poor measure under natural conditions because autoclaving kills all microorganisms that live in association with the bacterium (Devash, 1980). The conditions in this experiment apply, for example in transplant production where steam-sterilized soils are used in an effort to reduce soil borne pathogens. Studying the survival under natural conditions would be difficult in pathogen identification and isolation because the presence of many different bacteria in the soil. This was shown by the soil borne pathogen diagnosis before autoclaving where many bacteria were observed in the soil and some could not be identified. This justified the need to sterilize the soils so as to start with an initial known population of *P.s.pv tomato* Wet and dry conditions can help the conditions which are conducive for disease establishment in either wet or dry conditions.

The results show that *P. s. pv tomato* is soil borne as the bacterium was isolated from the soils from Chinhamora and Mutoko. Jones, (1981) reported that *P.s.pv tomato* survives in the soil. The bacterium can survive in the soil for considerable periods of time. Since the pathogen is difficult to control once established in the field (Dillard *et al.,* 1995). It is quite important to reduce initial inocula in the soils when the propagules are few. This can be achieved by cultural control practices, e.g. rotations.

The combination, which had more surviving bacteria, was wet sandy soil suggesting that if the soil has enough nutrients and temperature for growth is optimum, bacteria would multiply more and survive longer in the wet sandy soils.

Conclusions and recommendations

The study has shown that *Pseudomonas syringae pv.tomato* can survive in the soil for considerable period of time most preferably in wet sandy soils. In this study *P.s pv.tomato* survived in autoclaved soils for three months and the population was decreasing with time.

Recommendations

Wet sandy soils had higher surviving populations of *Pseudomonas syringe pv tomato* and the populations decreased with time. From these findings the following are recommended.

1. The fields for transplants should be treated with steam to kill all resident populations of the bacterium, temperatures up to 55ºC can completely kill the pathogen.

2. Farmers with sandy soils should be aware that these soils could support more bacteria. Hence should avoid contamination of soil by planting treated seeds. Rotations can also help in reducing the inocula in the field. If possible one should not plant in same field for at least 2 years to be sure that no resident populations of the bacterium are present in the soils

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