Heat Treatment on the Bacterial Wilt Pathogen, *Ralstonia solanacearum*, in Soil

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Abstract: This study presents the experiments designed to test the efficacy of either continuous, 2-h cycle or short exposures to a variety of high temperatures and a range of time periods in reducing the viability of *Ralstonia* in wet or dry soils under laboratory conditions. One g of soil containing 10^8 *R. solanacearum* was prepared for inoculum in soil. For the wet soil treatments the soil was maintained at 100% MHC (Moisture Holding Capacity). In the dry soil treatments, air dry soil was maintained at 0-2% MHC. Each experimental unit kept for 10 days in an incubator adjusted to a temperature in the range between 30 and 60°C. To determine the minimum time required to inactivate *Ralstonia*, the units consisting of *Ralstonia* in soil were exposed to temperatures in the range of 45 to 60°C for 15, 30, 60, 120, 180 or 240 min. In wet soil viability was reduced to zero by a constant temperature of 45°C for 2 day or a minimum temperature of 60°C for 2 h. Viability of *Ralstonia* were destroyed by either a 4-day constant temperature of 40°C or a 2-h temperature cycle for 2 days at 50°C or 3 days at 45°C. Below 40°C *Ralstonia* were not affected. In dry soil at 55°C, a 2 h constant temperature or 45°C for 2 days 2-h temperature cycle decreased *Ralstonia* viability. The results indicate that *Ralstonia* in wet soil exposed to a constant temperature of 45°C and above had lost their viability after 24 h.

Keywords: *Ralstonia*, Soil, Temperature, Time, Viability

1. INTRODUCTION

Bacterial wilt is widely distributed in tropical, subtropical and some temperate regions of the world. The disease ranks as one of the most important if not the most important disease of bacterial origin in the world [1], causing sometimes total losses in tomato crops [2]. Causal agent is *Ralstonia solanacearum* (Smith) Yabuuchi et al. (1995) [3], a highly diverse and adaptive bacterium, that differs in host range, geographical distribution, pathogenicity, epidemiological interactions and physiological properties [4, 5]. *Ralstonia solanacearum* is the causal agent of potato brown rot and bacterial wilt in many crops [6], and it is responsible for losses of up to 75% of the potato crop in several countries [7]. Most of the outbreaks seem to be related to the irrigation of crops with *R. solanacearum*-contaminated water. However, little is known about its distribution and persistence in natural reservoirs such as water or the molecular or physiological bases of survival strategies for this bacterium [8].

On the basis of host range, *R. solanacearum* strains have been traditionally divided into races [9], while physiological and genetic characterization resulted in the formation of biovars and divisions [9,10]. The bacterium invades the plant vascular tissues through wounded roots or natural openings, which occur after the emergence of secondary roots. It progresses through intercellular spaces into the xylem. Colonization of stems results in browning of the xylem, foliar epinasty and lethal generalized wilt [11]. Temperature strongly influenced the survival of *R. solanacearum*, as optimal survival, in particular in water systems, was demonstrated at physiologically favorable temperatures [12] and reduction of *R. solanacearum* populations was observed at low temperatures[12, 13]. Evidence for the occurrence of viable-but-non-culturable (VBNC) cells of the pathogen was obtained in soil and water microcosms. The observation of these VBNC cells, but also the appearance of symptomless infections of the pathogen in, for instance, *Capsicum annuum* [14], indicate the limitations of cultivation-based procedures and observation of plant disease symptoms [15]. This study presents the experiments designed to test the efficacy of either continuous, 2-h cycle or short exposures to a variety of high temperatures and a range of time periods in reducing the viability of *Ralstonia* in wet or dry soils under laboratory conditions.

2. METHODOLOGY

2.1 Inoculations

*Inoculum of* *R. solanacearum* (from Bacteriology group, Plant Disease Research and Development Department of Agriculture) was prepared from overnight shaken cultures incubated at 30 °C. Cultures were centrifuged at 6000 g for 20 min at room temperature then the pellet was washed twice in distilled water. After the final wash the pellet was suspended in sterile distilled water (SDW) and the number of cells in the suspension was determined from optical density measurements at OD600. The suspension was diluted to give the required number of cells ml^-1 and samples were mixed into the soil.

2.2 Soil treatment

One milliliter bacterial suspension containing 1x10^9 cfu prepared from an overnight culture of *R. solanacearum* strains were mixed thoroughly with each gram of sterile soil. For wet soil treatment the containers were kept in glass dishes containing 100 ml water so that the soil was maintained at 100% MHC (Moisture Holding Capacity). In the dry soil treatments, air dry soil 2-3% MHC containing sclerotia was not watered.

2.3 Continual temperature

The unit consisting of soil mixed with 1x10^9 cfu/g culture of *R. solanacearum* strains and Petri dishes were covered in polyethylene bags, 40μm thick and kept for 10 days in an incubator adjusted to a range of temperature between 30 and 60°C.

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2.4.2 High temperature cycle
Since at least 3 h elapsed for soil to attain the high temperature of the incubator, the experimental units consisting of \textit{R. solanacearum} in soil was therefore incubated for 5 h which included 3 h gradual rise in temperature plus 2 h to obtain a constant temperature. The dishes were then transferred to 30°C for 19 h when at least 6 h elapsed to reach the ambient temperature. The effect of 2 h temperature cycle was tested every day for 10 days.

2.5 Short temperature exposure
To determine the minimum time required for the inactivation of \textit{R. solanacearum}, soils containing \textit{R. solanacearum} were exposed to temperatures in the range of 30-60°C for 0.5, 1, 2, 3, 4, 5 and 6 h.

3. RESULTS AND DISCUSSION
3.1 Effect of continuous temperature
Effects of continuous temperature on viability of \textit{R. solanacearum} in dry or wet soils are shown in Fig. 1-2. In wet soil (Fig. 2), continuous exposure for 48 h at temperatures of 45°C and above was sufficient to destroy \textit{Ralstonia} viability, but temperatures of 35°C and below had no effect. In dry soil held at 55°C, \textit{Ralstonia} viability was destroyed after 2 h with 45% of \textit{Ralstonia} surviving 1 h. Reduction of viability was less at lower temperatures.

3.2 Effect of a daily 2 h temperature cycle
Effect of daily 2 h high temperature exposure on the viability of \textit{R. solanacearum} in dry or wet soils are shown in Fig. 3-4. In the effect of a daily 2-h high temperature exposure of dry soil (Fig. 3), a minimum daily 2-h exposure of \textit{Ralstonia} to 60°C resulted incomplete loss of viability. Treatments lasting 1, 2, 3 and 4 days, to a daily 2-h exposure to 60, 55, 50 and 45°C, respectively, were necessary to destroy \textit{Ralstonia} viability. Daily exposure of \textit{Ralstonia} to 40°C for 2 h had no effect by 4 days. Daily exposure of wet soil to 45°C for 2 h had reduced the viability to 50% after 1 days and to zero after 3 days (Fig. 4). However, at 40°C and below 5 days of exposure to a high temperature for 2 h day$^{-1}$ caused no marked reduction in viability of \textit{Ralstonia} in dry soil (Fig. 3).
3.3 Effect of short temperature exposures

In the study of short temperature exposure, to determine the minimum time required to inactivate *Ralstonia*, the units consisting of *Ralstonia* in soil were exposed to temperatures in the range of 30 to 60°C for 30, 60, 120, 180, 240, 300 or 360 min as shown in Fig. 5-8.

For assessment of viability, three replicates from each treatment were removed at zero time and periodically thereafter. The *Ralstonia* culture from soil sample was determined by developing on PDA plate at 25°C for 3 days. For each treatment there were three replicates.
The results indicate that *Ralstonia* in wet soil exposed to a constant temperature of 45°C and above had lost their viability after 24 h. Such constant temperatures do not prevail when soil is tarped under field conditions. In studies of Usmani and Ghaffar [16], the maximum temperature attained in wet soil was 48°C for a duration of 2 h. At 60°C a 2-h exposure or a daily 2-h temperature exposure at 50°C for 3 days was necessary for complete loss in sclerotial viability. In a similar study 6-h high temperature exposures, the number of viable propagules of *Verticillium dahliae*, *Sclerotium cepivorum*, *Sclerotinia minor* were reduced by temperatures of 45°C and above after 2 weeks [17].

Simulation of elevated temperatures in a cyclic regime to study the survival of *Ralstonia* is, therefore, of prime importance to predict the efficacy of mulching treatments. Environmental factors such as soil moisture and temperature interact with each other in their effects upon soilborne pathogens. In experiments where dry soil was used, a uniform temperature for 2 h days at 55 °C or
exposure to 60°C for 2 h for wet soil was necessary to kill all the *Ralstonia*. Presumably microbial cells are more resistant to wet heat. According to the thermal death times *Ralstonia* is not among the temperature resistant bacteria. Therefore, it is possible that pathogen inoculum can be reduced with an increase soil temperature and therefore controlled with the low dose of fumigant treatment. It has been reported that *Sclerotium rolfsii* lost its viability in 4-6 h at 50°C, and it population is reduced at 15 cm death by means of solarization treatment.

4. CONCLUSION

*Ralstonia solanacearum*, is responsible for severe losses to many important crops, mainly Solanaceous plants in tropical and subtropical regions where several outbreaks of the disease were recently reported, the disease represents a serious threat. In this study, data on time and temperature relationships on loss in viability of *Ralstonia* in soil could thus be used to predict efficacy of soil solarization.

5. REFERENCES


