Kasetsart J. (Nat. Sci.) 41 : 219 - 224 (2007)

Increased Tomato Yields by Heat Treatment for Controlling *Ralstonia solanacearum* in Soil

Jirasak Kongkiattikajorn[1]* and Sirichia Thepa[2]

ABSTRACT

Possibities of soil after heat treatment by incubation at high temperature were investigated for controlling *Ralstonia solanacearum*, the causal agent of bacterial wilt on tomato. A constant temperature of 45°C for 2 day or a minimum temperature of 60°C for 2 h was applied in soil, after which tomato seedlings were grown. The incidence of tomato drop was very low in the heated plots, but a 40-50% loss of tomato to the disease was observed in the control plots (no heat treatment). Reductions in biomass and in the number of tomato seedlings were observed in the non heated treatments, reduced the total bacterial population by 60-97%. The populations of native *Ralstonia* spp. were reduced from $2.4\text{-}7\times10^8$ colony forming units (cfu)g^{-1} to 0-115 cfu g^{-1}. Heat treatment reduced bacterial wilt incidence by 50-75%. This study presented the report on the efficiency of high temperature treatment on soil against tomato bacterial wilt pathogens.

Key words: *Ralstonia*, soil, temperature, tomato, seedling

INTRODUCTION

Bacterial wilt, caused by *Ralstonia solanacearum*, is responsible for severe losses to many important crops, mainly Solanaceous plants and bananas, in tropical and subtropical regions (Jenkins and Averre, 1983). This microorganism is the causal agent of potato brown rot and bacterial wilt in many crops and it is responsible for losses of up to 75% of the potato crop in several countries. 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 (Cook and Sequeira, 1994).

Sanitation is the process that reduces, excludes or eliminates the initial inoculum from which epidemics start. Reduction in primary inoculum found on the residue delays the onset of the epidemic and provids for an increase in spear production during harvest. Removal of primary inoculum to control monocyclic diseases is a very effective technique. However, Sumner *et al*. (1986) pointed out that secondary spread of foliar pathogens would not be affected by tillage practices. However, if tillage reduces the primary inoculum of the pathogen, it will reduce disease progress of both foliar and soilborne diseases. Burning of crop residue may also have other beneficial effects. These effects are attributed to the heat generated by burning of plant residue rather than to the ash. Obviously, burning has

1 School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand.

2 School of Energy and Materials, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand.

* Corresponding auther, e-mail: jirasak.kon@kmutt.ac.th

negative attributes including the generation of smoke and resultant air pollution, reduction in soil organic matter and visibility hazards to nearby motorists.

Knowledge has been accumulated to control soilborne diseases by heating of field soils (DeVay and Katan, 1991). Soil temperature extreme stress affects the competitive ability of microbial growth and are important in the mechanism of weakening of microorganisms in heat treated soil. Freeman and Katan (1988) reported that sublethal heating of conidia and chlamydospores of *Fusarium oxysporum* caused reduction in populations and loss of viability. Weakening of fungal propagules caused by sublethal heating has been reported for *Sclerotium rolfsii* (Lifshitz *et al*., 1983), *Rosellinia necatrix* (Sztejnberg *et al*., 1987) and *Armillaria melea* (Munnecke *et al*., 1976). This study presented the experiments designed to test the efficacy of short exposures to high temperatures and a range of time periods in reducing the viability of *Ralstonia* in soils and the effect on tomato sowing under laboratory conditions.

MATERIALS AND METHODS

Seeds

Commercial tomato seeds (*L. esculentum* Mill) were first washed in Tris buffer for 10 min in order to dissolve the fungicidal coating. Then, washed seeds were inoculated in the soil.

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 6000g 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 OD_{600}. The suspension was diluted to give the required number of cells ml^{-1} and samples were mixed into the soil.

Seeds and growth of seedlings

Seeds of tomato were sown in sterile flasks, each containing sterile soil used for growth of seedlings. Twenty seeds were sown in each flask at equal distances and watered as required to keep soil moist but not wet. All flasks were placed on a bench at room temperature.

Determination of fresh and dry weight

Seedlings of different treatments, after recording the symptom development and percentage of infection, were removed, washed with SDW, blotted with tissue paper, and fresh weight was determined. Seedlings were then dried at 60°C for 72 h and dry weight was recorded.

Soil treatment

One milliliter bacterial suspension containing 1×10^9 cfu prepared from an overnight culture of *R. solanacearum* strains was mixed thoroughly with each gram of soil. Four sets of flasks, each containing 4 flasks, were used in this experiment. The first set where the sterile distilled water was mixed with sterile soil. The second and third sets where the soil were mixed with bacterial suspension of *R. solanacearum* strain were incubated at 45°C and 60°C, respectively, before sowing. The forth set was where the soil was mixed with bacterial suspension of *R. solanacearum* before sowing. Twenty seeds were sown in each flask, then watered with tap water and maintained at room temperature. Two weeks after sowing, seedlings of each set were determined for the weight and germination.

Effect of the treatments on soil micro-organisms

The effect of the treatments was evaluated on the total population of soil *Ralstonia*

spp. The homologous samples from the same plot were mixed together and three sub-samples (1 g each) were analysed by the plate dilution method on potato synthesis agar (PSA). After 0-2 d and 0-2 h incubation at 45°C and 60°C the soil were counted for *Ralstonia* spp. growing on PSA. Bacteria growing on PSA were counted. The results were expressed as cfu g^{-1} of dry soil. *Ralstonia* spp. population was estimated by plate dilution in 1 g soil samples in triplicate. At the end of the heat treatments, samples were recovered and populations determined by plate dilution on PSA medium. The results were expressed as cfu g^{-1} of dry soil.

RESULTS AND DISCUSSION

Effect of heat treatment in soil on the infection of Tomato sowing by *Ralstonia solanacearum*

The experiments were carried out to investigate the heat treatment in reducing the deleterious effect of *Ralstonia solanacearum* on tomato germination. *Ralstonia solanacearum* strains were mixed with the soil before sowing of the seeds (soil application) In the experiments, *Ralstonia solanacearum* strains induced disease symptoms when inoculated into the soil of 2 week-old tomato seedlings (Table 1). In contrast to this result, the soil of the control resulted in disease symptoms of 60%. On the other hand, mixing the bacterial suspension of *Ralstonia solanacearum* strains with the soil and incubated at 45°C or 60°C for 2 d and 2 h, respectively, inhibited the infection by 100%. Results are presented in Table 1 and 2.

Fresh and dry weight of tomato seedlings

Highly significant reduction in fresh and dry weight was obtained when 2 weeks old tomato seedlings whose seeds were sown and grew in the soil mixed with *R. solanacearum*, treatment Control + *R. solanacearum*, relative to the control seedlings. On the other hand, the pretreatment of soil with incubation at 45°C for 2 d or 60°C for 2 h reduced the deleterious effect of *R. solanacearum* on tomato seedlings. This was revealed by the highly significant increase in fresh and dry weights of seedlings growing in the pretreated soil relative to that untreated soil mixed with *R. solanacearum* only (Table 3). The untreated soil mixed with *R. solanacearum* reduced fresh and dry weights of the seedlings relative to the control seedlings.

Effects on soil *R. solanacearum*

In the untreated control (non heat treated sterile soil + *R. solanacearum*) soil, the total bacterial population was 2.4-7 × 10^8 cfu.g^{-1}. The populations of *R. solanacearum* were strongly reduced in the treated soil by incubation at 45°C and 60°C for 0-48 h and 0-120 min, respectively. The treated soil at 45°C significantly reduced the

Table 1 Percentages of germination of 2 week-old tomato seedlings grown in the soil with *R. solanacearum* 10^8 cfu.g^{-1}.

Treatment	Germination	% of Germination
Control[a]	15-18	75-90
45°C for 2 d[b]	14-17	70-85
60°C for 2 h[c]	15-17	80-90
Control + *R. solanacearum*[d]	0-8	20-60

Each treatment contained 20 seeds

[a]Tomato seeds sowed in the sterile soil.

[b]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1} after incubation at 45°C for 2 d.

[c]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1} after incubation at 60°C for 2 h.

[d]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1}.

population of *R. solanacearum* from 2.4-7 × 10^8 to 0-146 cfu.g^{-1} in the samples buried at 5 cm depth and to 0-115 cfu.g^{-1} in those treated at 60°C (Table 4).

Heat treatment of soil was proved to be able to protect tomato seedlings from infection by *R. solanacearum* mixed in the soil. This was revealed by the reduced percentage of infection of seedlings germinated in the soil pretreated by heating at 45°C or 60°C relative to the untreated soil.

The reductions in the number of bacteria were expected, since the soil temperatures during the heat treatment were high enough to cause the death of microorganisms.

The weakening effect of environmental stress on *Ralstonia* spp. depends on temperature, exposure times and soil moisture conditions to which bacteria are exposed. In this study, the bacterial viability and pathogenicity seemed to be due to enhanced loss according to exposure times. The effect of direct physical heat injury on bacteria during various heat treatments was, however, inevitable resulted from changes in morphology and biochemical activities of bacteria. Exposure of bacteria to near lethal heating can alter the permeability and fluidity of the cell membrane (Plesofsky-Vig and Bramble, 1985) and cause morphological deformities and cracks on the surface of bacterial cell walls (Lifshitz *et al*., 1983). Thermal inactivation or weakening of pathogenic bacteria of heated soil depends upon the sensitivity

Table 2 Percentages of infection of 2 week-old tomato seedlings sown in the soil with *R. solanacearum* 10^8 cfu.g^{-1}.

Treatment	Foliar		Root	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Control[a]	4.152	0.473	4.251	0.417
45°C for 2 d[b]	4.124	0.462	4.416	0.439
60°C for 2 h[c]	4.253	0.459	4.270	0.402
Control + *R. solanacearum*[d]	2.541	0.235	2.174	0.232

Each treatment contained 20 seeds

[a]Tomato seeds sowed in the sterile soil.

[b]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1} after incubation at 45°C for 2 d.

[c]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1} after incubation at 60°C for 2 h.

[d]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1}.

Table 3 Effect of *R. solanacearum* on fresh and dry weights of tomato seedlings sown in the soil with or without *R. solanacearum*.

Treatment	Foliar		Root	
	Infected seedlings	% of infection	Infected seedlings	% of infection
Control[a]	-	0	-	0
45°C for 2 d[b]	-	0	-	0
60°C for 2h[c]	-	0	-	0
Control + *R. solanacearum*[d]	8	40	10	50

Each treatment contained 20 seeds

[a]Tomato seeds sowed in the sterile soil.

[b]Tomato seeds sowed inthe sterile soil containing *R. solanacearum* 10^8cfu.g^{-1} after incubation at 45°C for 2 d.

[c]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1} after incubation at 60°C for 2 h.

[d]Tomato seeds sowed in the sterile soil containing *R. solanacearum* 10^8cfu.g^{-1}.

Table 4 Populations of *Ralstonia solanacearum* (cfu.g^{-1}) detected at the end of the treatments in colonised soil incubated at 45 and 60°C for 0-48 h and 0-120 min, respectively.

Treatment	Incubation time	cfu.g^{-1} of colonized soil
Control[a]	-	0
45°C [b]	0 h	2.4-7 × 10^8
	12 h	3.7-8.5 × 10^4
	24 h	3.7-5.2 × 10^2
	36 h	11-173
	48 h	0-146
60°C [c]	0 min	2.4-7 × 10^8
	30 min	2.5-9.3 × 10^4
	60 min	2.3-5.9 × 10^2
	90 min	20-215
	120 min	0-115
Control + *R. solanacearum* [d]	-	2.4-7 × 10^8

[a]The sterile soil.

[b]The sterile soil mixed with *R. solanacearum* 2.4-7 × 10^8cfu.g^{-1} and incubated at 45°C for 0-48 h.

[c]The sterile soil mixed with *R. solanacearum* 2.4-7 × 10^8cfu.g^{-1} and incubated at 60°C for 0-120 min.

[d]The sterile soil mixed with *R. solanacearum* 2.4-7 × 10^8cfu.g^{-1}.

of the pathogen to sublethal temperatures (35-40°C) (Katan, 1987). The bacteria subjected to the sublethal intermittent heating in this experiment were more likely to occur in solarized soil because in solar-heated soil a diurnal time (Katan, 1981). Therefore, heat treated soil pathogens were probably killed or severely weakened by direct heat injury. However, it was logical to assume that the lower temperature to soil weakening of bacteria occurred by the synergistic effect of heat-stress combined with microbial nutrient stress. The involvement of other abiotic factors such as pH, soil moisture and texture, and interaction among them in inducing greater competitive stress on bacteria in such soil cannot be ruled out and merits further investigation. Considering also its effectiveness in controlling *Rhizoctonia* spp, *V. dahliae* and weeds (Tamietti and Valentino, 2001), it should be practiced on a larger scale in biological and traditional farms.

CONCLUSIONS

In conclusion, heat treated soil should be regarded as an ecologically friendly alternative to traditional-soil disinfestation methods effective against bacterial wilt of tomato.

LITERATURE CITED

Cook, D. and L. Sequeira. 1994. *Bacterial Wilt:* **The Disease and Its Causative Agent, Pseudomonas solanacearum**. CAB International Mycological Institute, pp. 137–142 *In* A. C. Hayward and G.L.Hartman (eds.). Wallingford. UK.

DeVay J. E. and J. Katan. 1991. Mechanisms of pathogen control in solarized soil, pp. 87–101 *In* J. Katan and J. E. DeVay (eds.). **Soil Solarization**. CRC Press. Boca Raton. FL.

Freeman, S. and J. Katan. 1988. Weakening effect on propagules of *Fusarium* by sublethal heating, **Phytopathology** 78: 1656–1661.

Jenkins, S. F. and C. W. Averre. 1983. Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. **Plant Dis.** 67: 968–970.

Katan, J. 1981. Solar heating (solarization) of soil for control of soilborne pests. **Annu. Rev. Phytopathol.**19: 211–236.

Katan, J. 1987. Soil solarization. pp. 78–105. *In* I. Chet (ed.). **Innovative Approaches to Plant Disease Control**. Wiley. London.

Lifshitz, R., M. Tabacbink, J. Katan and I. Chet. 1983. The effect of sublethal heating on sclerotia of *Sclerotium rolfsii*. **Can. J. Microbiol.** 29: 1607–1610.

Munnecke, D. E., W. Wilbur and E. F. Darley. 1976. Effect of heating and drying on *Armillaria mellea* or *Trichoderma uiride* and the relation to survival of *A. mellea* in soil. **Phytopathology** 66: 1363–1368.

Plesofsky-Vig, N. and R. Bramble. 1985. Heat shock responses in fungi. **Exp. Mycol.** 9: 187–194.

Sumner, D. R. , E. D. Threadgill, D. A. Smittle, S. C. Phatak and A. W. Johnston. 1986. Conservation tillage and vegetable diseases. **Plant Dis.** 70: 906–911.

Sztejnberg, A., S. Freeman, I. Chet and J. Katan. 1987. Control of *Rosellinia necatrix* in soil and in apple orchards by soil solarization and *Trichoderma hanzianum*. **Plant Dis.** 71: 365–369.

Tamietti, G. and D. Valentino. 2001. Soil solarization: a useful tool for control of Verticillium wilt and weeds in eggplant crops under plastic in the Po valley. **J. Plant Pathol.** 83: 173–180.