Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 5 (4): 607-612, 2006
ISSN 1682-3974

Effect of Mechanical Inoculation of *Tomato spotted wilt tospovirus* Disease on Disease Severity and Yield of Greenhouse Raised Tomatoes

[1]R.C. Ramkat, [2]A.W. Wangai, [1]J.P. Ouma, [1]P.N. Rapando and [3]D.K. Lelgut
[1]Department of Crop and Soil Sciences, Egerton University P.O. Box 536 Njoro, 20115, Kenya
[2]Kenya Agriculture Research Institute Biotechnology Centre,
National Agriculture Research Laboratories, Kabete P.O. Box 14733, Nairobi, Kenya
[3]Kenya Agriculture Research Institute Njoro,
P.O. Box Private Bag Njoro, Kenya

Abstract: Tomato (*Lycopersicon esculentum)* is popular both in fresh market and processing industries. Tomato yields are limited by diseases, *Tomato spotted wilt tospovirus* (TSWV) disease being one of them. A study was conducted in a greenhouse to determine the effect of mechanical inoculation of tomato spotted wilt tospovirus on the severity of the disease on tomato varieties Cal J, Marglobe, Money maker, Roma and Riogrande and its impact on yield. The experiment was arranged in a split plot design replicated four times. Tomatoes variety Cal J was most severely affected by the disease whereas Marglobe and Riogrande were least affected. Mechanical inoculation reduced total yield of Cal J, Riogrande, Money maker, Marglobe and Roma by 60, 55.3, 45.1, 40.3 and 27%, respectively. Marketable yield was reduced significantly by inoculation for the varieties except for Roma with a slight reduction in marketable yield that was not statistically significant from healthy controls. Roma was shown to be a tolerant variety that can be given to resource poor farmers as a component of an Integrated Pest Management (IPM) system for control of TSWV in order to realize higher marketable yields.

Key words: Tomatoes *Lycopersicon esculentum, Tomato spotted wilt tospovirus*, yield mechanically inoculation

INTRODUCTION

The tomato (*Lycopersicon esculentum*) is an important source of vitamin A and C. Every 100 g of ripe tomato can supply about 20 and 40% of the recommended daily allowance of vitamin A and C, respectively for adults (Davis and Hobson, 1980). Tomato production under both field and greenhouse conditions is limited by pest and diseases, *Tomato spotted wilt tospovirus* (TSWV) being among these (Atherton and Rudich, 1986). *Tomato spotted wilt tospovirus* (TSWV) is causing severe yield losses on tomatoes in Kenya ranging from 20-100% (Wangai *et al*., 2001a). *Tomato spotted wilt tospovirus* is found in the *Genus* Tospovirus placed in the family *Bunyaviridae.* Tospovirus are the only members of this family that infect plants; other members of the virus family are arthropod-borne viruses that are serious pathogens of animals and humans (Pappu, 1997). The predominant vector of the disease in Nakuru region of Kenya is the western flower thrips (*Frankliniella occidentals* Pergande) (Lelgut *et al*., 2001).

Attempts to control TSWV by use of chemicals have been ineffective because some stains of *occidentals* have been found to be resistant to several pesticides (Martin *et al*., 1994). In the green house, biological control of *F. occidentalis* using phytoseiid mites *Amblyseius cucumeris*, *A. barkeri* and the *Orius* spp. has been successful in summer but during winter, they enter diapause and fail to control *F. occidentalis.* In addition the eggs of predatory mites are generally susceptible to low humidity conditions, which often arise in greenhouse when outside temperatures drop below zero (Hauten *et al*., 1995). The search for sources of resistance and development of resistant cultivars appears to be the only way to control TSWV disease (Adams *et al*., 1994). Mechanical inoculation is useful in identifying direct TSWV resistance such as virus replication and translocation which is useful in evaluating accessions for resistance/tolerance to the disease (Kumar and Ullman, 1993). An experiment was therefore carried out to determine the effect of mechanical inoculation of TSWV on the severity of the disease on tomato varieties and its impact on yield.

Corresponding Author: R.C. Ramkat, Department of Crops and Soil Sciences, Egerton University, P.O. Box 536 Njoro, 20115, Kenya Tel: 254-721-203873 Fax: +254-051-62145

MATERIALS AND METHODS

Study site: The experiment was conducted between January - September 2004 at National Plant Breeding Research Center (NPRRC)-Njoro, Nakuru district Kenya which is at an altitude of 2160 m above sea level and longitude 36°E and latitude 0°20″S. The soils at the experimental site are vintric mollic andosols. These are well-drained, dark reddish brown friable and silt clay soils with humic topsoil. The mean annual rainfall is 886 mm and minimum temperatures ranging from 7.8-10.5°C and maximum 11.5-23.3°C (Jaetzold and Schmidt, 1983).

Test plants: Healthy test plant seedlings were pre-germinated in wooden boxes measuring 100×30×10 cm and transplanted at 3-4 leaf stage to plastic pots measuring 20 cm diameter filled with sterilized mixture of soil and gravel chips at a ratio of 9:1. Di-ammonium Phosphate fertilizer (18: 46 N: P) was thoroughly mixed with the soil at a rate of 1 kg fertilizer to 250 kg of soil mixture. The greenhouse was sprayed on a biweekly basis with Mitigan® to control mites at a rate of 20 mL 30 L^{-1} of water, metasystoc® (Oxydemeton-methyl) at a rate of 20 mL 45 L^{-1} of water to control aphids, whiteflies and thrips and fungicide Ridomil® (75% Metaxyl and 56% Mancozeb) at a rate of 2.5 kg ha^{-1} to protect from most common fungal diseases.

Inoculum preparation and inoculation: The inoculum was prepared by grinding freshly harvested virus-infected leaves with phosphate buffer at pH 7.4 (1:5 weight/volume) under cooled conditions. A sterilized mortar and pestle was used for grinding. Disodium ethylenediaminetetraacetate (EDTA, 0.1M) was added as an antioxidant. Healthy tomato seedlings were pre-darkened for 24 h to increase susceptibility of the plants. The leaves were dusted with carborundum (silicon carbide, 400-600 mesh) to increases infection by providing minute wounds for the entry of virus particles. The inoculum was gently rubbed on to the leaves. Inoculated leaves were rinsed with water after the inoculation to remove natural toxins in the inoculum which interfere with infection and to reduce injury from chemicals which had been added to the inoculum. Rinsing also facilitates later observation of symptoms. The healthy controls were rubbed with buffer and carborandum only (Green, 1991).

Variable studied

Disease evaluation: *Tomato spotted wilt tospovirus* (TSWV) damage on tomatoes was rated beginning two weeks after inoculation on a subjective scale of 0-5 (Diez *et al.*, 1999) by visually examining for the disease symptoms on individual plants where; 0 - No observable symptoms of the disease, 1- Chlorotic spots on leaves, 2- Ring or linear pattern, 3- Chlorotic spots ring or linear patterns and necrotic lesions, 4- General yellowing, leaf defoliation and necrosis, 5- Dead plant. The symptoms were confirmed by serological test. Enzyme Linked Immuno Sorbent Assay (ELISA) was used for diagnosis and detection of TSWV in plant samples. The antibodies used were obtained from AgDia ELISA kit (Golnaraghi *et al.*, 2001).

Yield parameters: Yield data for tomatoes were taken on individual plants beginning when physiological maturity of fruits was attained until termination of the study on weekly basis. During each harvest, the parameters of interest were:

Fruit weights: During each data collection, fruits per plant were harvested and weighed. These readings were used to compute the total yields per plant in terms of fruit weight and fruit numbers. The fruit weights were converted to Mg ha^{-1}.

Marketable yields: After harvesting and getting the total yields, the fruits were graded into two lots those marketable and unmarketable. Unmarketable fruits were those with TSWV disease symptoms such as ripe fruits showing pale red or yellow areas on the skin, sunken finger like depressions and malformation. Marketable fruits were those without disease symptoms.

Experiment: Five tomato varieties were used; Cal J, Marglobe, Money maker, Roma and Riogrande. Cal J, Roma and Riogrande are indeterminate varieties (short) while Money maker and Marglobe are determinate varieties (tall). Two weeks after transplant, the plants were mechanically inoculated with a standard virus preparation. All plants were returned to the greenhouse and arranged in a split plot design replicated four times. The main plots treatments were those that were inoculated with virus and those not inoculated (control) while varieties made the sub plots.

RESULTS

Disease symptoms: *Tomato spotted wilt tospovirus* (TSWV) symptoms were observed two weeks after inoculation whereas none of the corresponding un-inoculated plants showed symptoms of the disease. The disease symptoms included leaf yellowing, leaf curl, necrotic lesions, chlorosis (Fig. 2) and severe stunting.

(a) (b)

Fig. 1: Comparison of healthy fruit (a) of variety Cal J and one showing symptoms that lower marketability of fruits (b)

Fig. 2: Tomato crop (Cal J) showing symptoms of inward curling, necrosis and yellowing of leaves

These symptoms however differed with variety. Variety Money maker and Roma did not show much necrotic spots with yellow halo, were stunted in growth but picked up and re-grew more rapidly until maturity. These show that the varieties showed some form of recovery hence lowering disease symptoms. Abscission of inoculated leaves was observed after leaves became necrotic on variety Roma. Variety Cal J had high necrotic lesion after mechanical inoculation, Leaf drying from down upwards and extensive chlorosis was a common symptom with the variety. Its fruits were reduced in size, had concentric rings with depressions and uneven ripening (Fig. 1). Similar symptoms have been reported by (Wangai *et al*., 2001a) in tomatoes and (Pappu *et al*., 1998) in squash (*Curcurbita eppo*) following infection with TSWV. Marglobe and Riogrande did not show local lesions on inoculated leaves and symptoms were mild and not easy to notice though increased at the time of fruit maturity and were clear on fruits.

Disease scores: Generally disease ratings were high in the inoculated plants that differed significantly from their health controls (Table 1). The disease ratings varied significantly with the varieties. Cal J was the most susceptible followed by Money maker, Roma and Riogrande and lastly Marglobe. The disease ratings continuously increased over the course of the study for variety Cal J while those of variety Roma and Money maker lowered towards plant maturity. The disease ratings were significantly related to the ELISA values.

Yield and yield components

Fruit weight: In absence of the disease, variety Marglobe had the heaviest fruits followed by Money maker, Roma, Riogrande and lastly Cal J (Table 1). Yield difference between inoculated and healthy plants was significant. TSWV inoculation on tomatoes caused reduction of varying magnitudes in total fruit weight when compared to their health controls. The disease caused a 60, 55.3, 45.1

Table 1: Effect of mechanical inoculation of TSWV on disease scores and ELISA values of five tomato varieties grown under greenhouse conditions

Tomato varieties	Treatment	Disease scores	ELISA	Fruit weights (Mg ha^{-1})	Marketable weight (Mg ha^{-1})
Cal J	+	2.83a*	0.182a	20.21c	3.7d
	-	0.83e	0.136cd	50.93abc	40.97abc
Marglobe	+	2.16d	0.155bcd	47.38abc	27.47bcd
	-	0.62f	0.132cd	79.39a	72.45a
Money maker	+	2.41b	0.167abc	40.01abc	29.37bcd
	-	0.62f	0.138bcd	72.92a	68.92a
Roma	+	2.26c	0.74ab	51.63a	44.11abc
	-	0.66f	0.130d	71.52abc	70.28a
Riogrande	+	2.19cd	0.163abcd	28.56ab	15.95cd
	-	0.61f	0.137cd	63.99bc	57.37ab

*Means within a column followed by the same letter are not significantly different at 0.05 level of probability., (+) denotes inoculated and (-) health controls

and 40.3% yield reduction in Cal J, Riogrande, Money maker and Marglobe, respectively compared to a 27% reduction in Roma.

Marketable yield: Variety Roma, Money maker and Marglobe, had the highest yield of marketable fruits followed by Riogrande and Cal J (Table 1). *Tomato spotted wilt tospovirus* (TSWV) caused a severe decrease in the marketable yield of the fruits that was significant across the varieties. A 90% reduction in marketable yield was observed in variety Cal J followed by Marglobe, Riogrande and Money maker, with 62, 41 and 40%. An exception however was observed in variety Roma which had a non-significant 37% reduction on the marketable yield between inoculated plants and health controls.

DISCUSSION

The objective of the study was to screen tomato varieties by mechanical inoculation in order to determine TSWV disease severity and its effect on yield. Results from the study should that disease severity based on symptoms varied with variety. The total fruit weight and marketability were lowered by the disease and that variety Roma demonstrated beneficiary of using tolerant varieties by producing more marketable yield. The positive correlation of R^2 = 0.6, between disease rating and serological (ELISA) test confirmed that most of the symptoms observed were due to the virus. Such an association between ELISA and symptoms has been observed by Luciane *et al.* (2000) following inoculation of tomatoes with TSWV. Symptoms observed on tomato plants in form of necrotic local lesions on the inoculated organs (leaves) are local symptoms. These lesions are circular and well delimited typical of a hypersensitive response without showing any negative effect on plant growth and development. Mosaic symptoms are systemic symptoms, which can evolve into stunting and general necrosis of the plant (Moury *et al.*, 1997). Thomas and Jones (2003) noted hypersensitivity reaction to be common on plants expressing Systemic Acquired Resistance (SAR) and vary with varieties. The high disease scores on variety Cal J could have been contributed by necrotic lesons that were very common. This has been observed following mechanical inoculation of tomatoes and pepper (Golnaraghi *et al.*, 2001). Leaf drying from down upwards observed with the variety is as a result of altered leaf senescence associated to changes in cytokinin levels (Fraser, 1987).Variety Marglobe and Riogrande had no local lesions and symptoms were light suggesting that the response was not mediated through hypersensitivity and such virus localization mechanism response can be symptom less in some cases or microscopic and confined to one or a small number of cells (Cebolla *et al.*, 2003). This varieties seems to allow low virus multiplication in plant a response that was observed by Stevens *et al.* (1991) when screening tomato varieties towards TSWV hence why the plants had the least disease scores. However the occurrence of delay in symptoms expression until plant maturity indicates that Marglobe and Riogrande lack mature plant resistance. Mature plant resistance is as a result of formation of tough tissues that lower virus multiplication (Fraser, 1987). Abscission of inoculated leaves after they became necrotic observed in variety Roma has been attributed by Torre *et al.* (2002) to low systemic movement of the virus in the plants hence leading to reduction of the amount the virus in the plant. This has been observed in pepper as a resistance strategy to TSWV (Boiteux *et al.*, 1995). The picking up and re-growth of Variety Roma and Money maker resulting to decreased symptoms could be a consequence of virus dilution when growth is prolonged resulting to a sort of “passive resistance” which is a defense mechanism. The virus tends to be silent and plants show recovery a mechanism that has been demonstrated in virus infecting herbaceous host plants (Ratcliff *et al.*, 1999).

There was a reduction in the yields both in fruit weights and marketability after inoculation with the disease. Losses of between 20-100% in tomatoes have been attributed to TSWV in Nakuru region of Kenya (Wangai *et al*., 2001a). Reduction in yields both in quantity and quality of tomato and pepper due to infection of plants is a common effect of TSWV (Gitaitis *et al*., 1998). Variety Cal J was very susceptible to the disease and this was reflected on the weight and marketability of the fruits. Inoculated plants of the variety produced fruits that were small in size leading to a reduction in fruit weights. Reductions in fruit sizes are as a result of the virus targeting reproductive cells of the plant affecting cell division, multiplication and subsequently yield (Bendahmane *et al*., 1999). Mavric and Ravnikar (2001) observed that when plants are affected by TSWV marketable yield is lowered due to systematic necrosis which is severe on the fruits. Marketable yield reduction out of viral infection has been observed in pineapple after infection with pineapple mealy bug wilt associated virus (PMBWAS) which caused a synchronous fruit ripening and fruit malformation (Sipes *et al*., 2002). TWSV infection markedly reduced marketable yield of all varieties except Roma, These observations are in agreement with reports of Costa *et al*. (1999) where some tomato varieties have been able to produce more marketable yields than others since they are tolerant and are able to localize virus with less reduction in yield quality.

Using ratios of inoculated plants to that of healthy controls variety Cal J can be rendered highly susceptible. It had the highest disease scores and highest yield loss both in quantity and quality. Roma was shown to be the most tolerant variety since its inoculated plants produced the heaviest fruits and more marketable fruits and it had fairly high disease scores. These findings are in line with those of preliminary survey by Wangai *et al*. (2001b). Variety Marglobe though a heavy yielder in absence of the disease, it was rendered moderate susceptible since it had lowest disease ratings but its marketable yield was adversely affected. Variety Riogrande had low disease ratings after Marglobe but in terms of fruit weights loss it was ranked high after Cal J hence also termed moderate susceptible. Variety Money maker can be rendered moderate tolerant since they had high disease scores after Cal J and was able to produce higher marketable yields after Roma. These shows that disease severity based on symptoms after mechanical inoculation vary with variety and that TSWV disease cause yield loss in quantity and quality depending on varieties and that choice of variety could be an important strategy for integrated pest management.

ACKNOWLEDGMENTS

The authors wish to thank the Kenya Agriculture Research Institute (KARI) for providing the funds and facilities to carry out the research and all who participated towards the success of this project.

REFERENCES

Adams, G. and H. Kegler, 1994. *Tomato spotted wilt virus* and related tospoviruses. Arch. Phytopathol. Plant Prot., 6: 483-504.

Atherton, J.G. and J. Rudich, 1986. The Tomato Crop, Chapman and Hall Ltd. New York.

Bendahmane, M., M. Koo, E. Karrer and R.M. Beachy, 1999. Display of Epitopes on the surface of tobacco Mosaic Virus: Impact of charge and iso electric point of the epitope on virus Host. J. Mol. Biol., 290: 1-20.

Boiteux, L.S., 1995. Allellic relationships between *Genes* for resistance to *Tomato spotted wilt tospovirus* in capsicum chinese. Theor. Applied Gen., 90: 146-149.

Cebolla-Cornejo, J., S. Soler, B. Gomar, M.D. Soria and F. Nuez, 2003. Screening capsicum germplasm for resistance to *Tomato spotted wilt virus*. Ann. Appl. Biol., 143: 143-152.

Costa, J., A. Lacasa and M.S. Catala, 1999. Tomato production under mesh reduces crop loss to *Tomato spotted wilt virus* in some cultivars. Hortic. Sci., 34: 634-637.

Davis, J.N. and G.E. Hobson, 1980. The constituents of tomato fruit - The influence of environment, nutrition and genotype. Ceccrit. Rev. Food Sci. Nutr., 15: 205-80.

Diez, M.J., F. Nuez, B. Ricarte and S. Rosello, 1999. Resistance to tomato spotted wilt virus introgressed from *Lycopersicon peruvicurum* in line upv 1.m. Euphytica, 119: 357-367.

Fraser, R.S.S., 1987. Biochemistry of Virus Infected Plants. Institutes of Horticultural Research. England. Research Studies Press Limited, pp: 109-150.

Gitaitis, R.D., C.C. Dowler and R.B. Chalfant, 1998. Epidemiology of *Tomato spotted wilt* in pepper and tomato in Southern Georgia. Plant Dis., 82: 752-756.

Golnaraghi, A.R., N. Sharaeen, R. Pourrahim, S. Ghorbani and S. Farzadfar, 2001. First report of *Tomato spotted wilt virus* on soy bean in Iran. Plant Dis., 85: 1290.

Green, S.K., 1991. Guidelines for Diagnostic Work in Plant Virology. Asian Vegetable Research and Development Center, Technical bulletin No. 15, 2nd Edn., pp: 63.

Hauten, Y.M.V., P.C.J.V. Rijn, L.K. Tanigosho, P.V. Stratum and J. Bruin, 1995. Pre-selection of predatory mites to improve year round biological control of Western flower thrips in green house. Entomol. Exp. Appl., 74: 225-234.

Jaetzold, R. and H. Schmidt, 1983. Natural conditions and farm management handbook of Kenya. Central Kenya (Rift Valley and Central Provinces).

Kumar, N.K. and Ullman, 1993. Evaluation of *lycopersicon* germplasm for tomato spotted wilt tospovirus resistance by mechanical and thrips transmission. Plant Dis., 77: 38-941.

Lelgut, D.K., A.W. Wangai, E.W. Macharia, E. Alomba, S. Kilonzo and A.W. Gichangi, 2001. Preliminary survey of thrips species associated with potato (*Solanum tuberosum*) in potato growing zones of Nakuru district. Proceedings of NPBRC Workshop.

Luciane, C.J., R.M. Wilson, R.F. Antoria and T.V. Juliano, 2004. Correlation between symptoms of DAS-ELISA values in two sources of resistance against tomato spotted wilt virus. Brazilian J. Microbiol., 31: 204.

Martin, N.A., P.J. Workman and A.J. Popay 1994. Confirmation of a pesticide resistant strain of Western flower thrips in New Zealand. pp: 144-148. In Proceedings of the 4th New Zealand Plant Protection Conference. Waitiangi Note 1, New Zealand, pp: 9-11.

Mavric, I. and M. Ravnikar, 2001. First Report of *Tomato spotted wilt virus* and impatients necrotic spot virus in Slovenia. Plant Dis., 85: 1288.

Moury, B., A. Palloix, K.G. Selassie and X.G. Marchou, 1997. Hypersensitive resistance to *Tomato spotted wilt virus* in three capsicum Chinense accessions is controlled by a single gene. Euphytica, 94: 45-52.

Pappu, H.R., 1997. Managing Tospovirus through biotechnology. Progress and prospects. Biotechnology and Development Monitor, No. 32, pp: 14-17.

Pappu, H.R., J.W. Todd, A.V. Culbreath, M.D. Bandla and J.L. Sherwood, 1998. First report on the multiplication of *Tomato spotted wilt tospovirus* in tobacco thrips, ***Frankliniella fusca.*** Plant Dis., 82: 1282.

Ratcliff, F., B.D. Harrison and D.C. Baulcombe, 1997. A similarity between viral defense and gene silencing in Plant Science, 276: 1558-1560.

Sipes, B.S., D.M. Sether and J.S. Hu, 2002. Interaction between *Rotylenchulus reniformis* and pineapple mearly bug wilt associated virus in pineapple. Plant Dis., 86: 933-938.

Stevens, M., R. Scott and S.J. Gergeric, 1991. Inheritance of a gene for resistance to tomato spotted wilt virus from *Lycopersicon esculentum* Mill. Euphytica, 59: 9-17.

Torre, A., C. Dela, L. Diaz, H.A. Houston and R. Valverde, 2002. Phenotypic variation of some Mexican isolates of *Tomato spotted wilt virus* (TSWV). Agrociencia (Montecillo), 36: 211-221.

Thomas, C.M. and R.A.C. Jones, 2003. Selection, biological properties and fitness of resistance breaking strains of *Tomato spotted wilt virus* in pepper. Ann. Appl. Biol., 142: 235-243.

Wangai, A., B.J. Mandal, H.R. Pappu and S. Kilonzo, 2001a. First report of *Tomato spotted wilt virus* in Kenya. Plant Dis. J., 85: 1123.

Wangai, A.W., E.W. Macharia, D.A. Lelgut, S. Kilonzo and D. Onyango, 2001b. Host range studies of the *Tomato spotted wilt virus* in Kenya. Proceedings of NPBRC Workshop.