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Reduction of *Tomato spotted wilt virus* on Table Tomatoes in Greenhouses by Soil Fumigation

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***Tomato spotted wilt virus* (TSWV) has occurred on fields annually disease occurrence rates were 73.3% in 2005, 53.3% in 2006 and 41.6% in 2007 at Anyang area in Gyeonggi Province. Seasonal occurrence pattern of TSWV showed a dramatic increase from 8.7% in late May to 30.1% in early June in 2007 at Anyang area, coincided with the high population of a thrip, *Frankliniella occidentalis* at that time. The rate of viruliferous thrips with TSWV on lettuce and red pepper was 20.2% and 52.1%, respectively, in greenhouses. Dazomat, soil fumigation pesticide, reduced TSWV disease incidence drastically on table tomato as treatment the chemical into the soil with humidity in early spring in 2006 and 2007. Spraying insecticide periodically after treatment with Dazomat was more effective to control TSWV than spraying if on plants or applying into the soil of the insecticide during growing season. Control efficiency through treatments both of the soil fumigation and of spraying insecticide was significantly high with 85.3% in 2006 and 87.8% in 2007. Removing the potential vector from the soil of TSWV infested area can be an effective strategy for reducing TSWV disease.**

Keywords : *Frankliniella occidentalis*, soil fumigation, *Tomato spotted wilt virus*

Tomato spotted wilt virus (TSWV) has a wide host range, occurs great yield loss and distributes worldwide (Cho et al., 1989; Stobbs et al., 1992). *Frankliniella occidentalis*, one of TSWV vectors among 8 thrips species, is especially important carrier because of the ability to transmit easily the virus. In Korea, TSWV occurred on a paprika firstly at Yesan in 2004 (Kim et al., 2004). TSWV increased suddenly at Anyang area since 2005. Occurrence areas of TSWV have been spread especially on western part over the country year by year. Twenty nine plant species including tomato from 17 areas in Korea have been attacked seasonally (Cho et al., 2009; Kim et al., 2008). The best way to control of virus disease in cultural methods is prevention of infection. For prevention of virus disease, generally, it is important to remove the first inoculum thoroughly and to break the opportunity of transmission occurring by vary routes. TSWV is spread by thrips commonly and only, therefore, should be prevented by controlling the vectors. However, thrips are a fastidious pest with a unique life cycle through plant and soil and have changeable life cycle depends on the temperature. It makes hard to remove the pest effectively by chemicals, especially in growing season of crops. Generally, spraying pesticides and launching nets against the pest are used for control the thrips in the greenhouse. However, most greenhouses have been managed year-round, which can serve the viral inoculum continuously to crops cultivated in them. Espectally, a thrip, *Frankliniella occidentalis*, is small within 0.1 mm in length, and hard to be recognize a by naked eyes. In addition, the routine spraying of pesticide could bring resistance or tolerance problems of the chemicals (Park et al., 2002). Undoubtedly, resistance breeding science for crops against the TSWV has brought reducing yield loss, but the virus could make mutation easily by its genetic properties. Effective cultural methods, therefore, should be jointed such as spreading chemicals and blocking insect nets. For more effective solution to control of the thrips, fastidious vector for TSWV, the occurrence rate of the TSWV disease and vector population including viruliferous one during growing season had been conducted at Anyang area from 2005 to 2007. Effective elimination of the first inoculum by chemicals in a greenhouse in which TSWV occurred habitually for several years was investigated also for one of the methods to control the vector of TSWV in this research.

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Materials and Methods

TSWV survey. Crops infected with *Tomato spotted wilt virus* (TSWV) were investigated every week during growing season within greenhouses at Anyang area in Gyeonggi Province from 2005 to 2007. The collected samples were identified by transmission electron microscope, Carl Zeiss 906A, virion captured (VC)/RT-PCR and bioassay (Cho et al., 2005). Viruliferous thrips of *Frankliniella occidentalis* were also monitored in the collected flowers of table tomato and leaves of lettuce. Each thrips was tested by ELISA after grinding with 0.2 ml extraction buffer in 1.5 ml micro tube.

Thrips population survey. The numbers of thrips were investigated by using blue sticky pest traps (20 × 25 cm) under greenhouse infested with TSWV at Anyang. Sticky pest traps were changed with new one every week from May 4 to July 12 and adjusted the position to the height of tomato plants. The thrips on the trap were counted and classified under the stereo-microscope (Park et al., 2002).

Experiment plots. Two greenhouses occurring TSWV disease habitually year-round since 2004 at Anyang area were served for control thrips vectored TSWV.

Plots in greenhouses were designed five partitions as follows: the first one is soil fumigation in early spring (F) before transplanting of tomato seedlings and spraying pesticide during cultivation (P), the second one is F+P and pesticide application for pupae stage into soil (PP), the third one is P+PP, the forth one is P for spraying pesticide alone and the final one is non-treatment (NC). Five treatments mentioned above had six replications and seedlings of table tomato commercial cultivar 'Superdoterang', which grown in the clean area, were planted on April 12 in 2006 and 2007.

Soil fumigation. Chemical used for soil fumigation was Dazomat powder (98%, Dongbu Co.). It was mixed with two folds of soil with 30 kg/10 a and spread evenly and mixed with rotary machine. Polyester sheets were covered entirely onto the soil surface after some irrigation keeping moisture for more effectiveness of fumigation. The polyester sheets were kept for one month from early March and the greenhouses were ventilated thoroughly for one week to prevent chemical damage on crops.

Pesticide application. Thiamethoxam (1.5%, Syngenta Co.) was treated as a granule pesticide with 3 kg/10 a amount into soil before planting tomato. Foliar application of spraying pesticide on all plots except the fifth plot (NC) was done on tomato plants with Spinosad (10%, Dongbang-agro Co.) and Emamectin benzoate (2.15%, Syngenta Co.) on May and June, respectively in the peak time of thrips population.

Detection of TSWV. Viral infection was judged by ELISA for thrips and VC/RT-PCR for plants. ELISA was done with the commercial kit (Agdia Co., TSWV reagent kit). For the diagnosis of thrips, each thrips was macerated in a 0.2 ml extraction buffer on a well of ELISA plate pre-coated with TSWV antiserum and incubated for 2 hrs at 37°C for binding with the specific antigen. After washing with washing buffer of 0.01 M potassium phosphate buffer, pH 7.0, the conjugated antiserum of TSWV was added 100 μl per well. The enzyme labeled immune-globulin was added and then visualized the positive reaction by the substrate of P-nitrophenyl phosphate for yellow color. VC/RT-PCR was also used for the detection of TSWV (Cho et al., 2006). The primer of TSWV for VC/RT-PCR was 777 bp product; TSWV-NCP, TSNCPR (5'-ATGTCT AAG GTT AAG CTC AC-3') and TSNCPS (5'-TCA AGC AAG TTC TGC GAG TT-3'). The condition of VC/RT-PCR was as follows, 48°C : 45 min → 94°C : 2 min → (94°C : 30 sec → 50°C : 30 sec → 72°C : 1.5 min) 35 cycles → 72°C : 7 min → 4°C. All procedures of VC/RT-PCR were carried out on ice surface. Plant specimen was macerated with same volume (W/V) of extraction buffer of 0.01 M potassium phosphate buffer, pH 7.0, containing 0.5% sodium sulfite (Na_2SO_3). The crude sap was treated for 30 min in a vial and then washing twice with 0.01 M potassium phosphate buffer, pH 7.0. Viral solution was made by adding 50 μl RNase-free water with ice treatment after heating 95°C for 1 min.

Results and Discussion

TSWV occurrence. TSWV disease was occurred with the infection rate of 73.3% in average on infected field and ranged 7.6% to 100% at Anyang city of Gyeonggi province in 2005. The percentage of infected field was 53.3% and 41.6% in 2006 and 2007, respectively. However, there were no infected fields or plants at other cities of Gwangju, Icheon, Yangpyeong and Ansan in Gyeonggi province (Table 1). It made chase of the epidemiology for the TSWV infection, but couldn't find any clue without cultivating flowering annual, perennial, and woody plants imported from abroad in some greenhouses. There were some doubts about TSWV among them and it should be needed more an in-depth study in future.

Seasonal occurrence of TSWV. Seasonal occurrence of TSWV was investigated on table tomato cultivated in greenhouse at Anyang area during 3 years from 2005 to 2007. In 2005, TSWV infection was increased slowly and

Table 1. Occurrence rate *of Tomato spotted wilt virus* on tomato cultivated in Gyeonggi Province

Year	Areas	No. of fields		diseased fields (%)	diseased plants (%)
		investigated	diseased		
2005	Anyang	15	11	73.3	7.6~100
	Gwangju	50	0	0.0	0.0
	Icheon	5	0	0.0	0.0
	Yangpyeong	5	0	0.0	0.0
2006	Anyang	15	8	53.3	5.5~100
	Ansan	10	0	0.0	0.0
2007	Anyang	12	5	41.6	2.0~100
	Gwangju	10	0	0	0.0

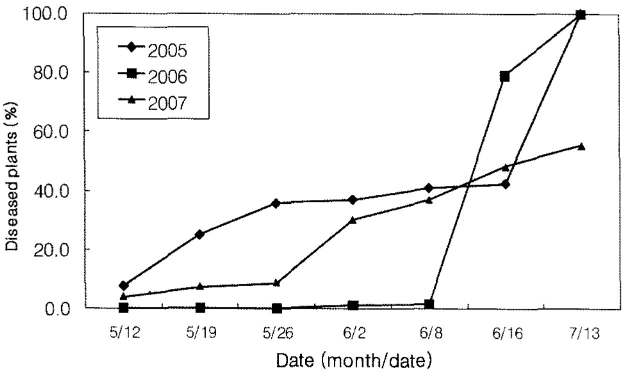

Fig. 1. Seasonal change of TSWV infection on the plants of table tomatoes cultivated in greenhouses for 3 years from 2005 at Anyang area.

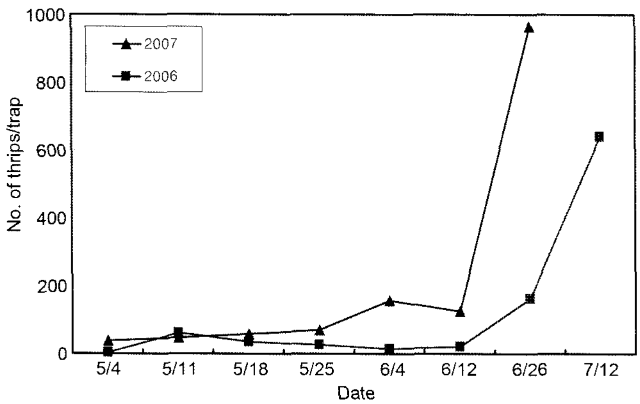

Fig. 2. The density of thrips collected from blue sticky pest traps hanged by tomato plants in spring season in 2006 and 2007.

continuously to 42.5% on mid June, however, almost all tomato plants were abruptly infected with the infection rate of 99.6% within a month after that. The infection rate of TSWV was also abruptly increased from 6.8% on early June to 78.9% on mid June in 2006 and slowly increased from 8.7% on late May to 55.3% on early July in 2007. There was not any special pattern of seasonal occurrences

Table 2. Occurrence rate by ELISA for the viruliferous thrips collected from fields in Anyang area

Year	No. of thrips [a]		Viruliferous thrips (%)
	tested	detected	
2005	94	14	15.0
2006	177	68	38.4
2007	188	68	36.2
Average	153	50	29.9

[a]Thrips collected from red peppers and lettuces growing outside of greenhouse were macerated in tubes singly with 0.2 ml extraction buffer per thrips.

of TSWV from the results during 3 years. However, the infection rate was increased dramatically under high temperature on June (Fig. 1). The population of thrips resulted from blue sticky pest traps was increased greatly from early June in 2006 and late May in 2007 (Fig. 2). These figures showed that the inclination of vector population was correspond to that of occurrence of TSWV on tomato plants. In spite of spraying pesticide against the thrips, increasing of TSWV disease and thrips population should be explained that TSWV viruliferous thrips are incoming into greenhouse from the outside weed hosts (Groves et al., 2003).

From red peppers and lettuces growing outside of tomato greenhouses, the rate of viruliferous thrips was 15.0% in 2005, 38.4% in 2006 and 36.2% in 2007 by ELISA test (Table 2). Therefore, increasing of TSWV in the greenhouse was highly related with viruliferous thrips from near fields infested with TSWV disease during growing seasons. It was supposed that the viruliferous thrips won't be eliminated in early stage of the TSWV disease and viral infection would be increased, and then healthy thrips were turned continuously to viruliferous thrips. Actually, Anyang area was already infested with TSWV and the average viruliferous thrips was 29.9% (Table 2). Plenty of viruliferous thrips should be inhabited in weeds alive, soil and under plant debris in greenhouse for the winter season and they would be influenced new crops for next season.

TSWV control. Research for prevention TSWV disease has been conducted with cultural methods besides genetic methods. Park et al. reported that treatment pesticide against thrips on chrysanthemum in greenhouse reduced leaf damage with 20% in the harvesting season compared with 100% damage on no chemical plots (2002). It is important to reduce the thrips density as early as possible by application of pesticide in the open fields (Nagata, 1999) and covering the UV reflective mulching films on tomatoes and peppers (Gitaitis et al., 1998; Momol et al., 2004).

For remove the first inoculums of plants and thrips as early as possible, soil fumigation is supposed the best way in cultural aspect. The five plots for study about effect of

Table 3. The control efficacy of soil fumigation and pesticide spray on TSWV infested greenhouse in 2006

Treatment[a]	Percentage of diseased plants on							Control efficiency (%)
	A[b]	B	C	D	E	F	Average (DMRT)	
F+P	13.0	27.3	8.6	24.1	11.7	3.3	14.7[a]	85.3
F+P+PP	52.4	25.0	0.0	18.2	0.0	4.5	16.7[a]	83.2
P+PP	100.0	63.6	45.5	77.3	27.3	22.7	56.1[b]	43.7
P	95.0	90.5	76.2	100.0	72.7	73.9	84.7[c]	14.9
NC	100.0	100.0	100.0	97.2	100.0	100.0	99.5[c]	–

[a]Soil fumigation(F) was done with Dazomat 30 kg/10 a before planting tomato from March 2 to March 28 for 27 days. Soil appliction of parvules pesticide (PP) were applied with thiamethoxam before planting. Pesticide spraying (P) was applied on tomato leaves with spinosad and emamectin benzoate. Tomato 'Superdoterang' was planted on April 12 and surveyed date was July 13.
[b]A~F of replications represented different rows of tomato plants in the greenhouse.

Table 4. The control efficacy of soil fumigation and pesticide spray on TSWV infested greenhouse in 2007

Treatment[a]	Percentage of diseased plants on							Control efficiency (%)
	A[b]	B	C	D	E	F	Average (DMRT)	
F+P	1.9	3.8	1.9	5.7	9.6	17.3	6.7[a]	87.8
F+P+PP	14.3	6.1	2.1	10.4	14.6	27.1	12.4[b]	77.3
P+PP	12.5	0.0	25.0	20.8	30.4	47.8	22.8[c]	58.3
P	29.2	16.7	20.8	20.8	32.0	28.0	24.6[c]	55.0
NC	51.4	47.1	38.2	55.9	55.9	79.4	54.7[d]	–

[a]Soil fumigation (F) was done with Dazomat 30 kg/10 a before planting from March 2 to March 28 for 27 days. Soil application of parvules pesticide (PP) were applied with thiamethoxam before planting. Pesticide spraying (P) was applied on tomato leaves with spinosad and emamectin benzoate. Tomato 'Superdoterang' was planted on April 12 and surveyed date was July 10.
[b]A~F replications represented the different rows of tomato plants in greenhouse.

soil fumigation had been investigated for 2 years. The control efficiency of TSWV in the treatments of soil fumigation plus spraying pesticide (F+P) and soil fumigation, spraying pesticide plus application of parvules pesticide into soil (F+P+PP) were 85.3% and 83.2%, respectively in 2006. However, the treatments of spraying pesticides plus pupae pesticide into soil (P+PP) and spraying pesticide alone (P) were low as 43.7% and 14.9%, respectively (Table 3). In 2007, the control efficiency of TSWV in F+P and F+P+PP was 87.8% and 77.3%, respectively while that of P+PP and P were relatively low as 58.3% and 55.0%, orderly. Foliar spraying of pesticide (P) based on thrips density after planting of tomato showed 55.5% control effect, which was not big different with P+PP treatment (Table 4). These results from Table 3 and 4 suggested that the treatments of foliar spray only or soil treatment of pupae pesticide were not enough to reduce the incidence of TSWV in greenhouse (Todd et al., 1996). The control effects by F+P or F+P+PP were much higher with over 40% than that by P+PP in 2006. It is supposed that the control effect by single application of soil fumigation would be more over 70% than that by single spraying of pesticide (P).

Total 1,096 tomato plants planted on April 12 were numbered on each plant in a greenhouse and investigated individually on every week whether the plants were infected with TSWV or not from May 25 to July 10. Typical

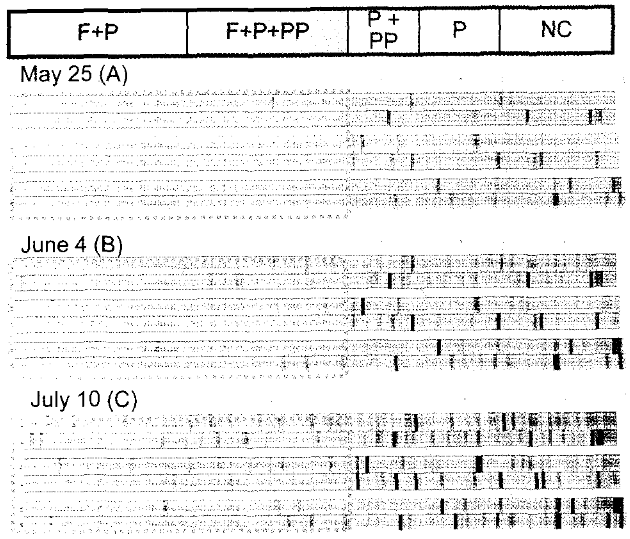

Fig. 3. Seasonal change of diseased tomato plants on Mat 25, June 4 and July 10 depending on chemical treatment. Tomato was planted on April 11. F+P : soil fumigation and spraying pesticide, F+P+PP : soil fumigation, spraying pesticide and parvules pesticide application into soil, P+PP : spraying pesticide and parvules pesticide application into soil, P : spraying pesticide alone, NC : no chemicals. The observed date of diseased plants was May 25(A), June 11(B) and July 10(C). Every tomato plant in greenhouse had its own designated number and checked symptoms caused by TSWV infection individually every week from May 25 to July 10. The different colors show the degree of symptomatology from light to dark on diseased tomato plants as time goes by during survey.

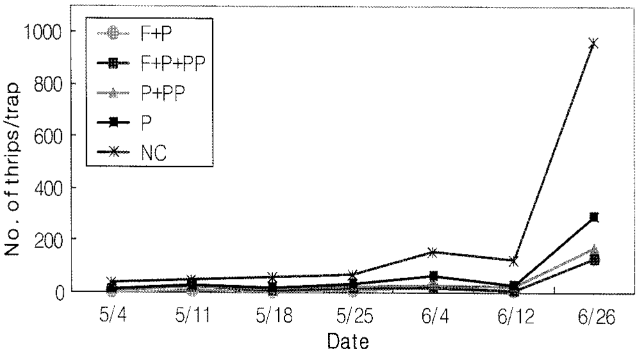

Fig. 4. Seasonal change of thrips population on tomatoes monitored by sticky blue pest traps in the experimented greenhouse at Anyang area in 2007. F+P : soil fumigation and spraying pesticide, F+P+PP : soil fumigation, spraying pesticide and parvules pesticide application into soil, P+PP : spraying pesticide and parvules pesticide application into soil, P : spraying pesticide alone, NC : no chemicals. Seedlings of tomato "Superdoterang" were transplanted on April 12.

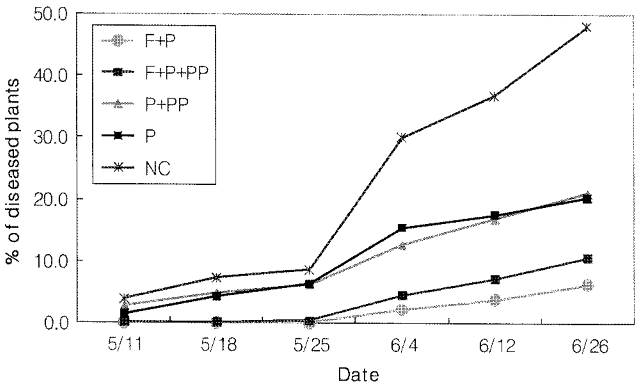

Fig. 5. Seasonal change of diseased plants infected with TSWV in the experimented greenhouse at Anyang area in 2007 depending on treatment. F+P : soil fumigation and spraying pesticide, F+P+PP : soil fumigation, spraying pesticide and parvules pesticide application into soil, P+PP : spraying pesticide and parvules pesticide application into soil, P : spraying pesticide alone, NC: no chemicals. Seedlings of tomato "Superdoterang" were transplanted on April 12.

symptoms of leaf necrosis and yellowing, ring spots on fruit were judged visually or VC/RT-PCR to viral infection. Symptoms of TSWV did not observed on plants in plots treated with soil fumigation, but those of other plots showed the symptoms of 8.7% from on May 25 (Fig. 3A). On June 4, plants showing the typical symptoms by TSWV were observed firstly on soil fumigation plots (Fig. 3B). On July 10, in three months of planting, the number of diseased plants were rapidly increased up to 55% in non-chemical treated plot and in plots of soil fumigation treatment, the numbers of diseased tomato plants increased slowly on plants planted near border of the greenhouse where the thrips could easily invade (Fig. 3C). Plots treated with soil fumigation had much less diseased plants showing typical symptoms of TSWV by naked eyes. In addition these results suggested that early reducing of thrips by soil fumigation was a very important factor to reduce TSWV in the greenhouse and launching the insect net could be helped to protect additive infection of TSWV from invading thrips from outside.

The population of thrips from the sticky trap in the greenhouse was increased slowly and most plots had almost same density of thrips to June 12 except NC plot. NC plot without treatment of chemical had the viruliferous thrips increased rapidly from the late May. The density of thrips in the NC plot increased up to 10 times compared to that of thrips in the soil fumigation plots (F+P and F+P+PP) on June 4 (Fig. 4). Increasing of diseased plants for growing season was corresponded with thrips population (Fig. 5). These results indicated that elimination of the first inoculum by soil fumigation and continual control of thrips during growing season would be greatly helpful to decrease yield losses.

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