International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 8, 2018, pp.-5845-5848.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

INSECT TRANSMISSION OF BUD NECROSIS VIRUS INFECTING TOMATO (*Lycopersicon esculentum* Mill.)

CHITRAPU RUTH*
Department of Plant Pathology, College of Horticulture, Anantharajupeta, 516105, Dr Y.S.R. Horticultural University, Venkataramannagudem, 534101, Andhra Pradesh
*Corresponding Author: Email - ruthbenerjee1@gmail.com

Received: April 16, 2018; Revised: April 22, 2018; Accepted: April 24, 2018; Published: April 30, 2018

Abstract- Insect transmission tests revealed that *Thrips palmi* is considered as the vector of the virus causing bud necrosis disease in tomato and cowpea. The larvae of *Thrips palmi* could acquire the virus with a minimum access period of 15 min. and the adults only transmit the virus with 1h inoculation access period (IAP). However, optimum virus transmission obtained with 48h/of AAP in the larval stage and 48h/of IAP in the adult stage, but beyond 48h of AAP and IAP resulted in decreased virus transmission. A single adult *Thrips palmi* could able to transmit the virus with a transmission rate of 24 to 32 percent and maximum transmission rate (100%) was achieved with 10 adults per seedling.

Keywords- *Thrips palmi*, Bud necrosis virus, insect transmission

Citation: Chitrapu Ruth (2018) Insect Transmission of Bud Necrosis Virus Infecting Tomato (*Lycopersicon esculentum* Mill.). International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 8, pp.-5845-5848.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop grown all over the world due to its varied climatic tolerance and high nutritive value. It is an important source of minerals and vitamin A and C. It is also known as 'Red Gold' and has high potential for developing value added products like soup, juice, ketchup, paste and powder through processing. It is also important for its edible fruits that can be consumed either directly as raw vegetable in sandwiches, drinks or cooked foods. About 36 viruses are reported to occur on tomato all over the world. Its occurrence in India was first reported by Todd, *et al*., (1975), from Nilgiris. Based on coat protein gene analysis TSWV is called as Groundnut bud necrosis virus in tomato (GBNV-T0) [1]. GBNV–To, a member of the genus Tospovirus of the family *Bunyaviridae*, is the most economically important virus of peanuts in the Indian subcontinent which is transmitted by melon thrips, *Thrips palmi* in a propagative manner [2]. The characteristic symptoms of TSWV are necrosis of the young growing bud, bronzing of the leaves with brown necrotic lesions, followed by wilting of the plants in severe cases [Plate-1]. The ripened fruits exhibit circular markings as concentric bands of starting from time of planting till harvest. According to the surveys revealed that the natural occurrence of thrips and PBNV were always high during *kharif* or rainy season than in *rabi* or post rainy season. Reddy, *et al*., (1983); Sreenivasulu, (1994); Krishnaveni, (1998); Kenchaiah and Porte, (1989) made similar observation [2-5]. Aswathi, *et al*., (2016), reported that Watermelon, *Citrullus lanatus* (Thumb.) is an annual trailing creeper belonging to the family *cucurbitaceae* [6]. Symptomatology and transmission studies are the important experimental tools to establish the etiology of viral diseases and plays vital role in disease spread and it is a serious threat in watermelon cultivation.

Materials & Methods

Insect Transmission Studies

Thrips collection, identification and rearing

Adult thrips from flowers and terminal buds of healthy tomato plants were collected at the Horticultural Research Station, Mahanandi and carried in a conical flask to the laboratory. A glass funnel was placed over it in an inverted position. The narrow end of the funnel was closed with a small glass vial as shown in [Plate-2].

Identification

The thrips collected in vials for identification were immobilized by placing them in a refrigerator at 4^0c for 15 min and then dislodged into an ice tray [7]. After immobilization by cold treatment, thrips to a level were identified using a stereo binocular microscope as per the key [Table-1] described by Amin and Plainer, (1985) [8]. Further confirmation was made at RARS, Nandyal [Plate-2].

Rearing

The suspected vectors *viz*., *S. dorsalis, F. schultzei* and *Thrips palmi* were reared on detached cowpea leaflets as described [8-11].

Transmission Tests

Three species of thrips *viz*., *Thrips palmi, Scirtothrips dorsalis* and *Frankeila schultzei* prevalent in tomato fields were tested in the laboratory for their ability in transmission of GBNV-To. GBNV-To infected leaves were collected and kept floating on water in Petri dishes Freshly emerged first instar nymphs were transferred over to the infected leaves in dishes with the help of a fine, moist camel hair brush and allowed to feed for 48 h (AFP) to acquire the virus. Then the nymphs from the infected leaves were transferred to individual glass vials each containing healthy leaflets. The vials were kept in an incubator at 22-26^0C for adult emergence. Freshly emerged ten adults each were transferred to individual healthy tomato seedlings covered with glass jars and allowed for 48 h (IFP) for inoculation. The seedlings were kept under observation for symptom expression. The transmission tests conducted with three species of thrips, *T. palmi* was found to transmit the GBNV-To while the two-other species *S. dorsalis* and *F. schultzei* failed in transmission of the virus. Hence, detailed transmission studies were conducted by *T. palmi*

Transmission of GBNV-To by Nymphs and Adults of *T. palmi*

In order to determine the ability of both nymphs and adults in transmission of GBNV-To, both nymphs and adults of *T. palmi* were allowed to feed on infected tomato leaves for an acquisition access period of 48 h. After acquisition, the nymphs were divided into two batches.

Plate-1 Monograph of Thrips palmi and necrosis and bronzing symptoms on tomato stem infected with GBNV-To

Table-1 *Identification characters of different thrips species*

Characteristics	*F. schultzei*	*T. palmi*	*S. dorsalis*
Adult female colour and length	Adult female pale in colour, 1 mm long	Straw yellow to pale brown 0.9 mm long	Relatively small, yellow in colour, 0.7 mm long
Antennae	8 segmented	7 segmented	8 segmented
Pronotum	Pronotum with 2 pairs of setae on the anteriolateral margin and 2 pair on the posteriolateral Margin	Pronotum having 2 pairs of setae on the posteriolateral margin no setae on the anteriolateral margin	No setae on the pronotum. Dark patches on the dorsal side of abdominal tergites
Wings	Fore wings with two complete rows of wing vein setae	Fore wings with broken rows of wing vein setae	Fore wings with few small setae on the veins, hind wings with 2 setae
Larvae	Larvae pale yellow move slowly and bend their abdomen while changing the direction	Larvae whitish	Larvae whitish. Both larvae and adults active, moving in a darted fashion

One batch of viruliferous nymphs after acquisition were immediately transferred to healthy tomato plants and allowed to feed for inoculation of the virus. After 48 h of inoculation feeding period (IFP), the test plants were sprayed with an insecticide to arrest the moulting of nymphs to become adults. The seedlings were kept under observation for symptom expression. The other batch nymphs were maintained on non-susceptible host till they become adults. The freshly emerged adults were transferred to healthy tomato plants for inoculation of the virus. After 48 h of inoculation feeding period (IFP), the test plants were sprayed with an insecticide. The seedlings were kept under observation for symptom expression. The third batch of adult insects after acquision, were transferred to healthy tomato plants for inoculation of the virus. After 48 h of inoculation (IFP) the test plants were sprayed with an insecticide. The seedlings were kept under observation for symptom expression. In all the cases, ten insects per plant were used for inoculation. In each case, a batch of 10 plants were included for testing.

Plate-2 Glass apparatus used for collection of thrips

Determination of Acquisition Access Period (AAP)

Transmission studies were conducted to determine the minimum acquisition access period for the transmission of GBNV-To in vector. Freshly emerged first instar nymphs of *T. palmi* were allowed to feed for different periods of 5 min, 15 min, 30 min, 1 h, 6h, 12h, 24hours, 48 hours and 72 h of acquisition. Each batch of these nymphs was transferred to healthy tomato plants for inoculation. Ten insects per plant were used for inoculation. The test plants were kept for symptom expression.

Determination of Inoculation Access Period (IAP)

Another experiment was conducted to determine the minimum inoculation access/feeding period for the transmission of GBNV-To by *T. palmi*. Freshly emerged first instar nymphs of *T. palmi* were transferred to infected tomato leaves. The freshly emerged adults were transferred to healthy tomato plants and allowed to feed for different periods 15 min, 30 min, 45 min, 1 h, 6h, 12h, 24hours, 48 hours and 72 h of inoculation. The inoculated plants were sprayed with an insecticide to arrest for further feeding of the insect beyond its stipulated time. The test plants were kept for symptom expression.

Number of Thrips Required for Transmission of GBNV-TO

Freshly emerged nymphs of *T. palmi* were allowed to feed on infected tomato leaves. The freshly emerged viruliferous adults were transferred to healthy tomato plants in batches of 2, 3, 4, 5, 10 and 15 insects per plant. Both acquisition access period and inoculation access/feeding period were fixed as 48 h. The test plants were kept for symptom expression.

Results and discussion

Insect Transmission

Insect transmission tests were carried out in the laboratory with three species of thrips *viz., Thrips palmi, Scirtothrips dorsalis* and *Frankliniella schultzei*. Out of the three-species tested, only *T. palmi* could able to transmit the GBNV-To from diseased to healthy tomato and cowpea plants [Table-2]. The other two species S. *dorsalis* and *F. schultzei* failed to transmit the virus. Hence, the detailed studies of insect transmission were done with *T. palmi*.

Transmission Test with Nymphs and Adults of *T Palmi*

Transmission tests were carried out in the laboratory to determine the ability of

both nymphs and adults in transmission of GBNV-To. Freshly emerged nymphs and adults of *T. palmi* were allowed to feed on the infected tomato leaves and transferred to the healthy tomato plants in three batches as described in materials and methods. Results indicated that nyphs only could acquire and transmit the virus when it became adult. Nymphs immediately after transferred to heathy plants in the nymphal stage itself before it became adult, failed to transmit the virus. While adult thrips failed to acquire the virus [Table -3].

Table-2 *Insect transmission studies of GBNV-To with thrips species*

Thrips[a]	Test plant	No. of plants		Transmission %
		Tested[b]	Infected	
Thrips palmi	Tomato	22	32.3	32.35
	Cow pea	49	18	36.73
Frankliniella schultzei	Tomato	45	0	0
	Cow pea	45	0	0
Scirtothrips dorsalis	Tomato	50	0	0
	Cow pea	4	0	0

a: Larvae were allowed 2 days acquisition access period (AAP) and adults
b: ten adults were released per plant

Table-3 *Insect transmission studies of GBNV-To with larvae and adults of Thrips palmi*

Stage of insect	Test plant	No. of plants		Transmission%
		Tested[a]	Infected	
Larvae with 2 days of AAP and IAP	Tomato	56	0	0
Larvae with 2 days of AAP and adults with 2 days IAP	Tomato	60	40	66.4
Adults with 2 days of AAP and IAP	Tomato	59	0	0

a: Ten first instar larvae and adults were released per plant

Number of thrips required for transmission of GBNV-To

Transmission tests were carried out in the laboratory to determine the number of adult thrips required for transmission of GBNV-To. Results indicated that a single adult can capable to transmit the GBNV-To and the success was 32 percent. The percentage of success increased with the increase in number of adults. Hundred percent transmission was achieved with 10 adults [Table-4].

Table-4 *Determination of number of adults of Thrips palmi required for transmission of GBNV-To*

Thrips palmi a Adults plant -1	No. of plants		Transmission %
	Tested	Infected	
1	25	8	32
2	25	11	44
3	25	14	56
4	25	16	64
5	25	19	76
10	25	25	100
15	25	25	100

a: Larvae were given two days acquisition access period (AAP) and adults 48 hours inoculation access period (IAP)

Determination of Acquisition Access Period (AAP)

Results presented in [Table-5] indicated that a minimum of 15min is required for the transmission of GBNV-To and the success of transmission was 54.25 percent. The transmission efficiency was increased with the increase in acquisition time and reached to hundred percent at 48 h.

Determination of Inoculation Access Period (IAP)

Results presented in [Table-6] indicated a minimum of 1h is required for the transmission of GBNV-To and the success of transmission was 40.95 percent. The transmission efficiency was increased with the increase in inoculation time and reached to hundred percent at 48 h. Further increase in inoculation time has no effect over the transmission. Three species of thrips *viz*., S. *dorsalis, F. Schultzei* and *T. palmi* were tested for the transmission of GBNV-To.

Table-5 *Determination of acquisition access period (AAP) of Thrips palmi in transmission of GBNV-To*

Acquisition Access Period [a]	No. of plants[b]		Transmission %
	Tested	Infected	
5min	37	0	0
10min	43	0	0
15min	46	2	4.34
30min	39	4	10.24
1h	47	2	4.42
6h	44	5	20.43
12h	30	7	23.31
24h	42	12	28.56
48h	46	25	54.25
72h	43	20	46.4

a: One day old (first instar) larvae were used
b: 10 adults with an inoculation access period of 2 days were released per plant

Table-6 *Determination of inoculation access period (IAP) of Thrips palmi in the transmission of GBNV-To*

Acquisition Access Period	No. of plants[b]		Transmission %
	Tested	Infected	
5min	37	0	0
10min	34	0	0
15min	46	0	0
30min	30	0	0
1h	47	2	4.24
6h	61	5	8.15
12h	54	8	14.8
24h	52	13	24.96
48h	53	30	56.4
72h	43	15	34.8

b: newly emerged 10 adults were released per plant

Out of the three species only *T. palmi* could transmit the virus. The rest of the two species have failed in transmitting the virus. *Thrips palmi* was identified as vector of groundnut bud necrosis virus. These findings are in accordance with the reports of Vijayalakshmi, (1994) and Sreekanth, (2002) where it was reported that peanut bud necrosis virus on groundnut, mungbean and urdbean was transmitted by *T. palmi* only. Nymphs acquired the virus and transmitted the disease when it became adult. However, the viruliferous nymphs could not transmit the virus at its nymphal stage itself. While the adults fed on the diseased samples failed to transmit the virus. These findings are in accordance with the observations made by Reddy, *et al*., (1983); Vijayalakshmi, (1994); Pappu, *et al*., (1998) and Sreekanth, (2002) [13]. Transmission tests of the present study revealed that a minimum of 15 min is required for the acquisition of GBNV-To and the success was 4.32 percent. The rate of transmission increased with the increase in acquisition access period up to 48 h. (54.25%) and further increase in the acquisition access period beyond 48 h resulted in decreasing transmission (46.40% at 72 h). Sakimura, (1963), also observed that a minimum acquisition access period of 15 min for the nymphs [7]. Reddy, *et al*., (1983), reported increased percentage of transmission with longer acquisition access period upto 48 h. Vijayalakshmi, (1994) found that *T. palmi* was able to acquire PBNV within 5 min with negligible increase in the transmission rate up to 12 h and maximum transmission rate at one day acquisition access period. The findings of Sreekanth, (2002), is in accordance with the present study who has reported that nymphs could acquire the virus within 15 min and the transmission rate increased with increase in acquisition access period up to 48 h and further exposure to longer period could not increase the rate of transmission. In the present investigation, the inoculation access period was one hour. There was considerable increase in transmission up to 24 h (24.96%). Beyond 24 h of exposure did not increase the transmission rate. In other studies, on inoculation access period by thrips for 5 to 30 min were found to be adequate on groundnut and Sreekanth 2002 on mungbean reported that the minimum inoculation access period for transmission of GBNV by *T. palmi* was 1 h.

T. palmi failed to transmit PBNV in 30 min inoculation access period and the maximum transmission rate was observed after 2 h of inoculation access period. The present results were supported with those reported by Vijayalakshmi, (1994). So, the 48 h. of inoculation access period at which the maximum rate occurred may be considered optimum. A single adult of *T. palmi* was able to transmit the virus with a transmission success of 28 percent. The transmission rate increased with the increase in number of insects per seedling. Hundred percent transmission was recorded with 10 adults per seedling. These findings are in accordance with the observations of Vijayalakshmi, (1994), who reported that a single *T. palmi* adult was able to transmit PBNV on groundnut and the maximum (100%) was achieved with 10 adults. Guisuibou Daimei, *et al*., (2017), studies the effect of Groundnut bud necrosis virus (GBNV) infection on the life history traits of its vector Thrips palmi and its feeding preference on GBNV-infected plants were studied [12]. A significant difference was observed in the developmental period (1st instar to adult) between the GBNV-infected and healthy thrips, wherein the developmental period of GBNV-infected thrips was decreased. However, there was no effect on the other parameters such as pre-adult mortality, adult longevity and fecundity. Further investigation on settling and feeding choice assay of *T. palmi* to GBNV-infected and healthy plants, showed that *T. palmi* preferred GBNV-infected cowpea plants more than the healthy cowpea plants. This preference was also noticed for leaf disks from GBNV-infected cowpea, groundnut and tomato plants

Summary

Among the three species of thrips *T. palmi, S. dorsalis* and *F. Schultzei* tested, *Thrips palmi* has been proved to be vector of GBNV-To and the other two species failed to transmit the GBNV-To virus. A single nymph of *T. palmi* with an AAP of 15min on becoming adult with an IFP of one hour could able to transmit the virus. Only nymphs acquire the virus but on becoming adult only could able to transmit it.

Application of research

The insect transmission study is useful to know the population of thrips at different seasons, planting time and stage of the crop. So, it can be applicable to manage the vector (thrips) by suitable management practices under the control of bud necrosis Virus in tomato

Research Category: Plant pathology

Abbreviations:

GBNV: Groundnut bud necrosis virus

Acknowledgement / Funding: Author thankful to College of Horticulture, Anantharajupeta, 516105, Dr Y.S.R. Horticultural University, Venkataramannagudem, 534101, Andhra Pradesh

***Principle Investigator: Dr Chitrapu Ruth**

University: Dr Y.S.R. Horticultural University, Venkataramannagudem, 534101, Andhra Pradesh, India

Research project name or number: Nil

Author Contributions: Sole Author

Author statement: Sole Author read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Todd J.M., Ponnaiah S. and Subramanyam (1975) *Madras Agricultural Journal*, 521(3), 162-163.

[2] Reddy D.V.R., Amin P.P., Mc Donald D. and Ghanekar A.M. (1983) *Plant Virus Epidemiology*, 93-102.

[3] Sreenivasulu A. (1994) *M.Sc. Thesis, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad, Andhra Pradesh, India*, 90.

[4] Krishnaveni D. (1998) *Ph.D. Thesis, Acharya N.G. Ranga Agricultural University, Hyderabad*, 155.

[5] Kenchaiah R.P.M. and Porte B.S. (1989) *Plant protection bulletin, Fariabad*, 41, 7-10.

[6] Aswathi.K.K., Asha V. Pillai, Preethy T.T. and Mannambeth Renisha Jayarajan (2016) *International Journal of Recent Scientific Research Research,* 7(3), 9393-9395.

[7] Sakimura K. (1963) *Phytopathology*, 53, 412-415.

[8] Amin P.W. and Palmer J.M. (1985) *Tropical Pest Management*, 31, 286-291.

[9] Ghanekar A.M., Reddy D.V.R., Lizuka N., Amin P.W. and Gibbons R.W. (1979) *Annals of Applied Biology*, 93, 173-179.

[10] Vijayalakshmi K. (1994) *Ph.D. Thesis, Acharya N. G. Ranga Agricultural University, Rajendranagar, Hyderabad, Andhra Pradesh. India*, 99.

[11] Srikanth M. (2002) *Ph.D. thesis. Acharya N.G. Ranga Agricultural University, Ragendranagar, Hyderabad, Andhra Pradesh, India*

[12] Daimei G., Raina H.S., Devi P.P., Saurav G.K., Renukadevi P., Malathi V.G., Senthilraja C., Mandal B., Rajagopal R. (2017) *Phytopathology*, 107(11), 1440-1445.

[13] Pappu H.R., Todd J.W., Culbreth A.K., Bandla M.D. and Sherwood J.J. (1998) *Plant Disease*, 82,1282.