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RESISTANCE TO ONION THRIPS (THYSANOPTERA: THRIPIDAE) IN ONION CULTIVARS DOES NOT PREVENT INFECTION BY *IRIS YELLOW SPOT VIRUS* FOLLOWING VECTOR-MEDIATED TRANSMISSION

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ABSTRACT

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a global pest of onion, *Allium cepa* L., and the principal vector of *Iris yellow spot virus* (IYSV) that can cause 100% crop losses. The purpose of this study was to evaluate onion cultivars resistant to *T. tabaci* feeding damage for their reaction to IYSV following exposure to viruliferous *T. tabaci* in both laboratory and field experiments. In the laboratory experiment, virus-free onion cultivars grown in pots were infested with 32 *T. tabaci* second instars collected from onions in an IYSV-infected field. In the complementary field experiment, virus-free onion plants in pots were moved to an onion field where IYSV was present. In both laboratory and field trials, plants were tested for IYSV by DAS-ELISA after 2 and 3 wk, respectively. Although plants were exposed to *T. tabaci* for a short period, IYSV was detected in all onion cultivars with the percentage of infected plants varying from 3 to 25% and 37 to 70% in the laboratory and field experiments, respectively. IYSV infection levels did not differ statistically between thrips-susceptible and thrips-resistant onion cultivars in laboratory and field experiments.

Key Words: *Thrips tabaci, Allium cepa, onion resistance, virus*

RESUMEN

El trips de la cebolla, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), es una plaga global de la cebolla, *Allium cepa* L., y el principal vector de *Iris yellow spot virus* (IYSV) que puede causar 100% de pérdidas del cultivo. El objetivo de este trabajo fue evaluar genotipos (resistentes al daño ocasionado por *T. tabaci*) por su reacción a IYSV transmitido por *T. tabaci* en condiciones de campo y laboratorio. En el experimento en laboratorio, plantas libres del virus fueron infestadas con 32 larvas de segundo instar de *T. tabaci* colectadas de un campo de cebolla infectado con IYSV. En el experimento complementario en campo, plantas libres de IYSV fueron llevadas al campo donde IYSV estaba presente. En ambos experimentos, laboratorio y campo, las plantas fueron examinadas con la prueba de DAS-ELISA para detectar IYSV después de 2 y 3 semanas, respectivamente. Aunque las plantas fueron expuestas a *T. tabaci* por un periodo corto de tiempo, IYSV fue detectado en todos los genotipos con porcentajes de plantas infectadas que osciló entre 3 a 25% y 37 a 70% en los experimentos en el laboratorio y en el campo, respectivamente. Los niveles de infección de IYSV no fueron estadísticamente diferentes entre los genotipos susceptibles y resistentes a *T. tabaci* en los experimentos de campo y de laboratorio.

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a polyphagous pest with a host range of more than 100 plant species in more than 30 families (Ghabn 1948; Morison 1957; Ananthakrishnan 1973). *T. tabaci* is a widely distributed pest of onions wherever they are grown (Lewis 1997), including New York State where a total of 4,330 ha were planted in 2010 (NASS 2011). *T. tabaci* feeding on onion causes silvery leaf spots that turn into white blotches and silvery patches along the leaves (Bailey 1938). This damage reduces the photosynthetic ability of the plant (Parrella & Lewis 1997) and results in reduction of onion bulb weight (Kendall & Capinera 1987; Rueda et al. 2007) and yield loss from 50% (Fournier et al. 1995) to 60% (Waiganjo et al. 2008). In addition to the feeding injury, *T. tabaci* transmits *Iris yellow spot virus* (family *Bunyaviridae*, genus *Tospovirus*, IYSV) and is the principal vector of this pathogen (Pozzer et al. 1999; Kritzman et al. 2001). IYSV was first identified on onion in southern Brazil in 1981 (Pozzer et al. 1994), and was confirmed in the USA in 1989 in the Pacific Northwest (Hall et al. 1993). IYSV

has spread subsequently throughout other important onion producing states in the USA and worldwide (Gent et al. 2006). IYSV symptoms on leaves appear as straw-colored to white, dry, and sometimes elongate lesions along leaf edges (Gent et al. 2006). IYSV infection can reduce bulb size (Gent et al. 2004) and cause 100% crop loss (Pozzer et al. 1999). Tospoviruses are transmitted in a persistent propagative manner. Virus acquisition occurs only during the larval stages, and it is passed transstadially to the adult; and only adult thrips that acquired the virus during their larval stages can transmit tospoviruses (Whitfield et al. 2005).

Management of *T. tabaci* through the use of insecticides and cultural practices has been challenging and additional methods of control are needed. The use of foliar insecticides is the most common tactic to control *T. tabaci* in onion, but this strategy has led to the development of populations resistant to pyrethroid and organophosphate insecticides in North America (Shelton et al. 2003, 2006; MacIntyre Allen et al. 2005) and other regions of the world (Martin et al. 2003; Herron et al. 2008; Morishita 2008). Other management practices have been investigated including host plant resistance. Different studies on onion resistance to *T. tabaci* have been conducted and resistance has been associated with bulb color (Verma 1966; Lall & Singh 1968; Brar et al. 1993) and leaf structure and color (Jones et al. 1934, 1935; Coudriet et al. 1979; Pawar et al. 1987; Patil et al. 1988; Hudák & Pénzes 2004; Loges et al. 2004a, 2004b; Diaz-Montano et al. 2010). Similarly, others have evaluated onion cultivars for incidence of or resistance to IYSV. For example, the response of 46 onion cultivars to IYSV infection was investigated by du Toit & Pelter (2005), and all cultivars were found susceptible to the virus with infection levels, as determined by visual symptoms, ranging from 58 to 97%. In 2007 and 2008, 47 onion cultivars were evaluated for resistance to IYSV, as determined by double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA), and the virus was present in all cultivars, but with highly variable levels of infection: 3 to 31% in 2007 and 15 to 79% in 2008 (Diaz-Montano et al. 2010). In that study, symptoms of IYSV were mild to absent and only appeared late in the season.

The present tests were conducted in order to study and compare the reactions of different onion cultivars resistant to *T. tabaci* feeding to infection by IYSV following *T. tabaci*-mediated transmission in field and in laboratory conditions.

Materials and Methods

Plant Material

A total of 17 onion cultivars were evaluated in this study, which was conducted in New York in 2009 (Table 1). Fifteen onion cultivars that were considered resistant to *T. tabaci*, based on reduced numbers of larvae and lower leaf damage ratings than susceptible cultivars (Diaz-Montano et al. 2010; Diaz-Montano 2011a), were tested. The cultivars 'Nebula' and 'Yankee' were used as the susceptible checks. Information on d to matu-

Table 1. Onion cultivars used to ascertain reactions of different onion cultivars resistant to *Thrips tabaci* feeding to infection by *iris yellow spot virus* in the laboratory versus the field.

Cultivar	Leaf color[a]	Response to *T. tabaci*	Days to maturity	Seed company
Yankee[1]	Blue-green	Susceptible[b]	108	Bejo
Nebula[1]	Blue-green	Susceptible[b]	100	Nunhems
OLYS05N5[1]	Yellow-green	Resistant[b]	120	Crookham
Tioga[1]	Yellow-green	Resistant[b]	118	Seminis
Peso[1]	Yellow-green	Resistant[b]	115	Bejo
Calibra[1]	Yellow-green	Resistant[b]	115	Bejo
Vaquero[1]	Yellow-green	Resistant[b]	118	Nunhems
Cometa[2]	Yellow-green	Resistant[b]	120	Nunhems
Medeo[1]	Yellow-green	Resistant[b]	106	Bejo
NMSU 03-52-1[1]	Yellow-green	Resistant[b]	120	d
Delgado[1]	Yellow-green	Resistant[b]	116	Bejo
T-433[1]	Yellow-green	Resistant[b]	117	Takii
Colorado 6[1]	Yellow-green	Resistant[b]	120	Crookham
Arcero[1]	Yellow-green	Resistant[c]	120	Nunhems
Mesquite[1]	Yellow-green	Resistant[c]	120	D. Palmer
White Wing[2]	Yellow-green	Resistant[c]	105	Bejo
Granero[1]	Yellow-green	Resistant[c]	118	Nunhems

[1,2]Bulb color. 1: yellow, 2: white.
[a]Leaf color obtained by visual observation.
[b]According to Diaz-Montano et al. (2010).
[c]Onion cultivars confirmed as resistant in other studies (Diaz-Montano 2011a) .
[d]Onion line developed by C.S. Cramer, Department of Plant and Environmental Science, New Mexico State University, Las Cruces, NM.

rity and bulb color was obtained from the respective companies or the breeder (Table 1). Seeds for each cultivar were planted into 200-cell 4.5 cm deep plug trays with one seed per cell filled with Cornell soil mix (Boodley & Sheldrake 1977), and then grown under greenhouse conditions at 20-30 °C and 20-40% RH with supplemental lights set for a photoperiod of 14:10 h L:D. After 8 wk in the greenhouse, 4 onion plants per cultivar were transplanted individually into plastic pots (15.0 cm diam × 15.0 cm high, with 4 plants per pot) filled with Cornell soil mix (Boodley & Sheldrake 1977). IYSV is not known to be transmitted through seed and there was no source of IYSV in the greenhouses. Therefore, experimental onions were presumed to be IYSV-free before plants were infested with *T. tabaci*.

Reactions of Onion Thrips-Resistant Onion Cultivars to *Iris yellow spot virus* following *T. tabaci*-Mediated Transmission

To characterize the reaction of onion thrips-resistant onion cultivars to IYSV, experiments were performed under laboratory and field conditions.

Laboratory Experiment

This trial was conducted at Cornell University's New York State Agricultural Experiment Station, Geneva, New York. Onion plants from the greenhouse were infested with *T. tabaci* collected from a commercial onion field near Elba, New York, from onion plants on which IYSV visual symptoms (straw-colored, dry diamond-shaped lesions on the leaves) were evident. A total of 32 *T. tabaci* second instars were placed on leaves and confined in each plastic pot with the 4 plants by using a 15-cm-diam by 30-cm-high acrylic tube inserted into the soil of each pot and the top of the tube was covered with a plastic lid. The tube had 2 holes (5 cm diam) in the middle and one additional hole (3 cm diam) on the center of the lid, and all holes were covered with an organdy cloth (thrips proof). There were 10 pots per cultivar for a total of 40 plants per cultivar.

The plastic pots were placed in racks arranged in a completely randomized design and the racks were put in a climatic chamber (25-30 ºC, 40% RH at 14:10 h L:D). Two weeks after confinement with the 32 *T. tabaci* larvae, all onion leaves were tested for IYSV by DAS-ELISA (Clark & Adams 1977) and commercially available antibodies, as well as positive and negative controls for IYSV (Agdia Inc., Elkhart, Indiana). ELISA was used to detect the virus because visual symptoms are less reliable and asymptomatic plants in New York have been found infected with the virus (Diaz-Montano et al. 2010).

Field Experiment

This study was conducted in a commercial onion field near Elba, New York. The plastic pots containing the plants, as described above, were placed adjacent to this field. Pots were left completely open so that *T. tabaci* could naturally infest the plants. After 3 wk of exposure, the number of *T. tabaci* larvae was counted in each pot, and onion leaves were taken to the lab and tested for IYSV by DAS-ELISA, as described above. There were 10 pots per cultivar for a total of 40 plants per cultivar.

Statistical Analyses

Analysis of variance (ANOVA) for *T. tabaci* larvae data among cultivars was conducted by using PROC GLM and controlled for blocks. Multiple comparisons were computed by using Tukey's studentized range test ($P < 0.05$) (SAS Institute 2003). A logistic regression model for IYSV infection levels was performed using PROC GENMOD and controlled for blocks (SAS Institute 2003). The correlation between laboratory and field IYSV infection rates and the correlation between *T. tabaci* larvae and IYSV rates in the field were compared using PROC CORR and the Spearman rank coefficient correlation (SAS Institute 2003).

Results

Reaction of Onion Thrips-Resistant Onion Cultivars to *Iris yellow spot virus* following *T. tabaci*-Mediated Transmission

Laboratory Experiment

A total of 472 plants were collected at the end of the experiment with 24 to 40 plants per cultivar, except for 'Medeo', 'Calibra', 'Mesquite' and 'Yankee' that had 18 to 23 plants. Across all cultivars, only 8.9% of the plants tested were infected with IYSV, as shown by DAS-ELISA. The percentage of plants infected ranged from 2.9 to 25% (Table 2) and there were no significant ($\chi^2 = 17.27$; df = 16; $P = 0.3685$) differences in the percentage of IYSV-infected plants among the *T. tabaci*-susceptible and resistant cultivars tested. These results indicated the absence of an association between resistance to onion thrips and their response to IYSV following *T. tabaci*-mediated transmission.

Field Experiment

In the field, 579 plants were tested with 28 to 40 plants per cultivar except for 'Medeo' that had 24 plants tested. Across all cultivars, the average percentage of infected plants was 52.3%. The per-

TABLE 2. IYSV INCIDENCE, AS SHOWN BY DAS-ELISA, ON 17 ONION CULTIVARS IN LABORATORY AND FIELD EXPERIMENTS AS WELL AS NUMBER OF *THRIPS TABACI* LARVAE IN THE FIELD.

Cultivar	Laboratory, % plants infected (mean ± SD)	Field, % plants infected (mean ± SD)	Field, No. larvae (mean ± SD)
NMSU 03-52-1	25.0 ± 33.9 a[a]	36.7 ± 36.7 a[a]	1.1 ± 3.4 c[b]
Medeo	16.7 ± 26.9 a	37.5 ± 41.4 a	4.1 ± 5.0 bc
Peso	3.7 ± 11.7 a	41.4 ± 21.8 a	5.9 ± 5.8 b
Cometa	8.3 ± 26.3 a	45.0 ± 40.5 a	5.8 ± 6.3 b
Granero	12.5 ± 20.1 a	46.4 ± 33.9 a	4.6 ± 5.3 bc
White Wing	3.3 ± 10.5 a	48.7 ± 18.9 a	7.5 ± 3.0 b
Calibra	16.7 ± 26.9 a	50.0 ± 41.9 a	7.2 ± 7.8 b
Colorado 6	8.8 ± 14.2 a	50.0 ± 31.1 a	3.8 ± 5.4 bc
Mesquite	4.5 ± 14.4 a	53.8 ± 25.5 a	4.7 ± 4.9 bc
Arcero	13.3 ± 23.3 a	54.3 ± 28.4 a	7.3 ± 6.3 b
OLYS05N5	2.9 ± 9.0 a	55.3 ± 31.5 a	4.7 ± 5.4 bc
Vaquero	4.2 ± 13.2 a	56.4 ± 23.6 a	4.0 ± 4.1 bc
Delgado	4.0 ± 12.6 a	56.7 ± 35.3 a	6.2 ± 6.9 b
Yankee	4.3 ± 13.8 a	56.8 ± 37.0 a	17.3 ± 6.1 a
Nebula	9.7 ± 15.6 a	63.2 ± 22.2 a	20.6 ± 9.1 a
T-433	7.5 ± 12.1 a	63.2 ± 30.9 a	6.3 ± 5.3 b
Tioga	8.8 ± 14.2 a	69.7 ± 37.9 a	5.6 ± 5.5 b
No. plants tested	472	579	
Avg. % infected	8.9	52.3	

[a]Within a column, means followed by different letters are significantly different ($\alpha = 0.05$, Logistic regression model using PROC GENMOD).

[b]Within a column, means followed by different letters are significantly different ($P < 0.05$, Tukey's test).

centage of plants infected with IYSV varied from 36.7 to 69.7% (Table 2) and there were no significant differences ($\chi^2 = 16.83$; df = 16; $P = 0.3967$) in infection levels among *T. tabaci*-susceptible and resistant cultivars. As expected, 2 susceptible checks 'Nebula' and 'Yankee' had significantly ($F = 29.73$; df = 16; $P < 0.001$) more *T. tabaci* larvae than the other cultivars (Table 2). These results confirmed the absence of an association between resistance to *T. tabaci* in onion cultivars and their response to IYSV following *T. tabaci*-mediated transmission; thus confirming previous field reports (Diaz-Montano et al. 2010; du Toit & Pelter 2005). In addition, there was a low correlation between IYSV infection levels and the number of larvae per cultivar ($r = 0.452$, $P < 0.0688$).

DISCUSSION

In this study, we tested the reaction of *T. tabaci*-resistant onion cultivars to IYSV in the field and in the laboratory by detecting the presence of IYSV by DAS-ELISA. Without a field component, the effects of multiple inoculation events, high vector pressure, inoculations across multiple plant stages, non-preference, etc. may not be considered. In the present study the correlation between laboratory and field IYSV infection levels was very low ($r = -0.279$, $P < 0.267$), indicating that there was not a strong pattern of IYSV infection using these 2 different experimental procedures. The lower infection rates in the laboratory suggest that initial screenings for detecting IYSV in a laboratory should not replace initial screening for IYSV detection in the field. However, differences of IYSV infection levels between our laboratory and field tests may also be due to the fixed number of *T. tabaci* used and confined in each pot in the laboratory compared with the field experiment, where unknown numbers and possibly more viruliferous *T. tabaci* may have landed on plants when pots were left uncovered.

In the present study, all cultivars became infected with IYSV in both lab and field experiments and this agrees with results from past studies, where more than 40 onion cultivars were tested for the presence of IYSV, and cultivars resistant to *T. tabaci* had high levels of IYSV incidence or vice versa from year to year or location to location (Diaz-Montano et al. 2010).

In the field experiment, the thrips-susceptible cultivars, 'Nebula' and 'Yankee', had 63.2 and 56.8% IYSV infection levels, respectively. However, there were no significant differences in IYSV levels between these thrips-susceptible and thrips-resistant cultivars, suggesting that cultivars resistant to *T. tabaci* are not necessarily free of the virus and vice versa; thus confirming previous studies in which several onion cultivars were tested for IYSV (Diaz-Montano et al. 2010; du Toit and Pelter 2005). This result is also explained by the low correlation ($r = 0.452$) found between IYSV infection levels and the number of larvae in the field.

Diaz-Montano et al. (2010) observed that visual symptoms of IYSV were mild to absent in onion fields in New York and usually appeared at the end of the season, which suggested that reductions in

plant and bulb size were due to *T. tabaci* feeding rather than IYSV. This agrees with studies by Hsu et al. (2010) that revealed a positive correlation between high populations of *T. tabaci* adults in onion fields at the end of the season and high levels of IYSV, but no evident yield reduction by IYSV. In New York, IYSV was first found in 2006 (Hoepting et al. 2007). Diaz-Montano et al. (2010) suggested that yield losses in New York might be devastating if IYSV infected onions earlier in the season, and in that study the average percentages of IYSV-infected plants in the field infected were 11 and 40% in 2007 and 2008, respectively. In this study (2009), younger onion plants were exposed to *T. tabaci* populations only for 3 wk in the field, and yet all the cultivars became infected with IYSV with the average percentage of plants infected being 52% (Table 2). This infection rate, or a higher one caused by a longer infection period on young plants, would likely have deleterious effects on yield.

Our results and those published in the literature have not found any onion cultivar to be resistant to IYSV, nor any onion cultivar to be free of the virus after exposing many cultivars to viruliferous *T. tabaci*. Because host resistance to the virus has not been found, efforts to control IYSV should focus on a combination of control or management strategies, including sanitation practices, such as elimination of volunteer onions and planting of transplants free of *T. tabaci* and the virus, selection of cultivars resistant to *T. tabaci,* crop rotation and *T. tabaci* control with insecticides.

There have been several studies on onion resistance to *T. tabaci* as summarized by Diaz-Montano et al. (2011b); however, the traits responsible for resistance have not been sufficiently well characterized for incorporation into a breeding program. However, our recent studies have identified some *T. tabaci*-resistant cultivars, and it appears that such resistance is associated with leaf color (Diaz-Montano et al. 2010). We have demonstrated that these cultivars possess strong antixenosis as a category of resistance to *T. tabaci*, and that leaf color may play an important role in that resistance (Diaz-Montano 2011a). These findings of cultivars with strong antixenosis to *T. tabaci* could have promise for IYSV management because plant traits may prevent the vector from colonizing these plants. However, as shown in this study and others (du Toit & Pelter 2005; Diaz-Montano et al. 2010) the situation is more complex because none of the cultivars examined showed any resistance to IYSV infection. Therefore, genetically engineering onions resistant to IYSV may be a more promising alternative for IYSV management. This strategy has proven extremely effective in controlling *Papaya ringspot virus* in papaya and several viruses in summer squash (Shelton et al. 2008).

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