THE SEASONAL OCCURRENCE OF VIRULIFEROUS *THRIPS TABACI* AND THE INCIDENCE OF IRIS YELLOW SPOT VIRUS DISEASE ON LISIANTHUS

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SUMMARY

The occurrence of viruliferous onion thrips (*Thrips tabaci*) outside lisianthus greenhouses was examined from 2003 to 2005. Adults were captured using blue sticky traps placed outside the greenhouses, and viruliferous individuals were identified using DAS-ELISA. While *T. tabaci* appeared from April to July, viruliferous individuals were mainly detected from the end of May to the middle of June. Leaf necrosis caused by Iris yellow spot virus (IYSV) developed soon after the massive appearance of viruliferous *T. tabaci* in the field. The disease appeared simultaneously in June on lisianthus plants at different growth stages. These results suggest that the infection source of IYSV is located outside the greenhouses. Surveys conducted in an onion field near the lisianthus greenhouses showed that viruliferous *T. tabaci* dispersed after harvesting of onions, suggesting that IYSV-infected onion plants are the inoculum sources in this area.

Keywords: *Eustoma grandiflorum*, Iris yellow spot virus, *Thrips tabaci*, epidemiology, DAS-ELISA.

INTRODUCTION

Lisianthus (*Eustoma grandiflorum*) is one of the most popular cut flowers in Japan. Although the acreage has doubled from 219 ha in 1990 to 466 ha in 2004, leaf necrosis disease (LND), caused by Iris yellow spot virus (IYSV), has hampered the Japanese lisianthus production since this disease was first recorded (Doi *et al.*, 2003).

IYSV was originally isolated from Dutch iris (*Iris hollandica*) and classified as a tentative species of the genus *Tospovirus*, family *Bunyaviridae* (Cortes, 1998). Subsequently, this virus was found in lisianthus (Kritzman *et al.*, 2000), onion (Hall *et al.*, 1993; Gera *et al.*, 1998; Pozzer *et al.*, 1999), and alstroemeria (Okuda and Hanada, 2001). In Japan, it affects primarily lisianthus and onion.

Two IYSV strains inducing a slightly different symptomatology have been reported from Japan (Doi *et al.*, 2003; Zen *et al.*, 2003). Onion thrips (*Thrips tabaci*) is the only known vector (Nagata *et al.*, 1999), transmission rates ranging from 33% to 50% (Kritzman *et al.*, 2001) or 33% to 80% (Doi *et al.*, 2003) under experimental conditions. Distributions and incidence of IYSV infection in onion fields suggested that infected volunteer onions serve as inoculum source for *T. tabaci* transmission to onion crops (Gent and Schwartz, 2004).

IYSV causes serious damage in Saga Prefecture (southern Japan) where lisianthus greenhouses are located near onion fields. Although the damage to onion is minor, IYSV-infected onion plants are suspected to serve as infection source for lisianthus.

In this study, we surveyed the occurrence of viruliferous *T. tabaci* and IYSV-infected lisianthus, to investigate the correlation between the thrips and LND.

MATERIALS AND METHODS

Preliminary tests. The number of *T. tabaci* present in the field was estimated following trapping on blue sticky traps (IT sheet, 30 × 10 cm, Sankei Chemicals, Japan). Single insects carrying IYSV were identified by DAS-ELISA according to Okazaki *et al.* (2007), but prior to field survey, we investigated whether IYSV could be detected in *T. tabaci* captured on sticky traps.

To this aim, thrips were made viruliferous by allowing first instar larvae to feed for two days on IYSV-infected lisianthus leaves. Larvae were then transferred to healthy broad bean seedlings until they became adults. Acquisition of IYSV was confirmed by the leaf disc assay (Wijkamp *et al.*, 1994). Some viruliferous adults were placed on a sticky trap and promptly removed (0 days), whereas others were kept on the trap for 7 o 14 days. The presence of IYSV in *T. tabaci* was individually assessed by DAS-ELISA using a commercial kit (Agdia, USA).

Incidence of viruliferous *T. tabaci* and leaf necrosis

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disease in lisianthus greenhouses. Surveys of lisianthus LND and viruliferous *T. tabaci* were conducted in greenhouses at Karatsu City (Saga Prefecture) from 2003 to 2005 (Table 1).

The first outbreak of LND in each greenhouse was identified by RT-PCR assays using the IYSV-specific primers IYSV-N5 and IYSV-N3T (Zen *et al.,* 2005). Subsequently, LND incidence was evaluated every 5 to 9 days by visual observation of each plant.

Three sticky traps were placed outside of each lisianthus greenhouses at a height of 150 cm and were replaced every 7-10 days. *T. tabaci* were recovered from the traps under a stereomicroscope. Insect batches were washed with chloroform and assayed by DAS-ELISA to determine the incidence of viruliferous individuals. Absorbance values (A_{405}) were measured for individual thrips, and a sample was considered positive if its A_{405} was 3 times higher than the mean absorbance value of a non viruliferous female adult of *T. tabaci*.

Incidence of viruliferous *T. tabaci* in an onion crop. To identify the source of IYSV infection, the incidence of viruliferous *T. tabaci* was investigated in 2004 and 2005 in an onion field, which was approximately 2 km away from the lisianthus greenhouses. Three sticky traps were placed at the edge of the field 10 m apart from one another and replaced every 6-10 days. The incidence of *T. tabaci* and the percentage of viruliferous individuals were assessed as described above. The incidence of IYSV-infected onion plants was evaluated by observing necrotic symptoms on the leaves.

Incidence of leaf necrosis disease in different cropping types. Incidence of LND in crops established at three different times was assessed in 2004 in lisianthus greenhouses at Karatsu City. To this aim, seedlings were transplanted in a greenhouse in September 2003 (plot 1), January 2004 (plot 2), and May 2004 (plot 3). The number of plants in plot 1, 2 and 3 were 4536, 11480, and 4172, respectively. The row width, furrow width, and intrarow spacing were 150, 12, and 12 cm, respectively. The incidence of LND was evaluated every 7-10 days as described previously.

RESULTS

Preliminary tests. ELISA values of viruliferous *T. tabaci* placed on sticky traps gradually decreased as the days passed (Table 2). Nonetheless, the average value for viruliferous *T. tabaci* trapped for 14 days was significantly higher than that of non viruliferous thrips (T test, $p < 0.01$).

Seasonal pattern of viruliferous *T. tabaci* incidence. Figure 1 shows the number of *T. tabaci* captured on the sticky traps outside lisianthus greenhouses from 2003 to 2005. In 2003, *T. tabaci* were first captured on April 17, and the average number of trapped insects ranged from approximately 1.00 to 8.56 (bodies/trap/week) from the middle of April to the end of May. The number of captured *T. tabaci* gradually increased reaching a maximum on June 14. The incidence subsequently decreased, so that no thrips was trapped after July 19. Although the insect number differed, their seasonal occurrence in 2004 and 2005 showed a similar pattern. In 2004 and 2005, *T. tabaci* was first captured on April 2 and April 22, the number of trapped *T. tabaci* had a peak on June 11 and June 17, and only few insects were trapped after July 1 and July 7, respectively.

The appearance of viruliferous *T. tabaci* did not correspond to the total incidence of *T. tabaci*. In 2003, viruliferous insects were detected on May 3, May 30, and June 20, but the peak was on May 30 (54.5%). In 2004,

Table 1. Some characteristcs of the greenhouses hosting lisianthus crops and timing of surveys for leaf necrosis and *T. tabaci.*

Year	Acres	Number of plants	Transplant	Survey periods	
				Lisianthus leaf necrosis [(a)]	*T. tabaci* [(b)]
2003	3.6	12192	May 5	May 10-August 16	March 29-August 16
2004	2.08	7920	April 23 –April 30	May 8-July 29	March 26-August 27
2005	3.6	12224	May 31	June 3-August 19	April 8-August 26

[(a)]Incidence of lisianthus leaf necrosis disease was determined by visual observation.
[(b)]Incidence of *T. tabaci* was determined using sticky traps

Table 2. Results of ELISA testing of viruliferous and non viruliferous *T. tabaci* captured on sticky traps for the presence of IYSV.

	Trapping period (days)		
	0	7	14
Viruliferous	0.159 ± 0.074 (n = 35)	0.087 ± 0.046 (n = 28)	0.068 ± 0.029 (n = 25)
Non viruliferous	0.005 ± 0.002 (n = 23)	0.003 ± 0.004 (n = 23)	0.002 ± 0.003 (n = 22)

Figures are mean absorbance values and standard deviations. Number of samples tested is in parenthesis.

viruliferous *T. tabaci* were detected on May 21 and from June 4 to 18, with the highest incidence on June 11 (33.3%). Likewise, in 2005, viruliferous thrips were detected from June 16 to 24, with the highest incidence on June 24 (10.7%).

Seasonal patterns of lisianthus leaf necrosis disease. Figure 2 shows the incidences of LND in lisianthus greenhouses from 2003 to 2005. In 2003, LND was first observed on June 14, i.e. 42 days after the first capture of viruliferous *T. tabaci* by sticky traps. LND outbreak appeared to be consequent to the high incidence of viruliferous *T. tabaci* on May 30. Disease incidence increased rapidly until July 26, when it reached a plateau.

The survey conducted in the same greenhouse in 2004 gave a similar result. LND was first identified on June 18, i.e. 28 days after the first detection of viruliferous *T. tabaci*. The peak of viruliferous *T. tabaci* presence corresponded to the first detection and subsequent increase of LND. The disease incidence did not increase after June 26.

In 2005, leaf necrosis was first observed 14 days prior to the first detection of viruliferous *T. tabaci.* However, the viruliferous thrips population increased concomitantly with the subsequent increase of LND. Disease incidence attained the highest level on July 22.

Incidence of viruliferous *T. tabaci* in the onion crop. The developmental pattern of the *T. tabaci* population and the incidence of viruliferous individuals in the onion field resembled those registered outside the lisianthus greenhouses (Fig. 3). In 2004, thrips were first captured on April 23 in the field and their number increased after onion harvesting on June 6. The incidence subsequently decreased, and no *T. tabaci* was trapped after July 9. Likewise, thrips were first captured on May 2 in 2005, their number increased after harvesting on June 10, had a peak on June 14, and decreased thereafter.

Viruliferous *T. tabaci* were detected from May 14 to June 26 in 2004 (Fig. 3) whereas diseased onion plants were first identified on May 7. The infection rate gradually increased to reach a maximum of 1.27%. In 2005, one viruliferous *T. tabaci* was detected on May 2 but the highest number of viruliferous individuals was observed on May 31. Diseased onion plants were first observed on April 21, with a final incidence of 0.45%.

Incidence of leaf necrosis disease in different transplanting periods. LND appeared simultaneously in early June in all plots, regardless of the time at which they were established. However, the patterns of disease incidence differed depending on the time of transplanting (Fig. 4). In plot 2 (transplanted in January 2004), LND incidence was the highest and increased rapidly till the end of the cropping period. In plot 1 (transplanted in September 2003), LND spreading was rapid but apparently ceased at the beginning of July, whereas in plot 3 (transplanted in May 2004), there was a more gradual LND increase, which lasted till the end of the cropping period. Disease incidence was lowest in this plot.

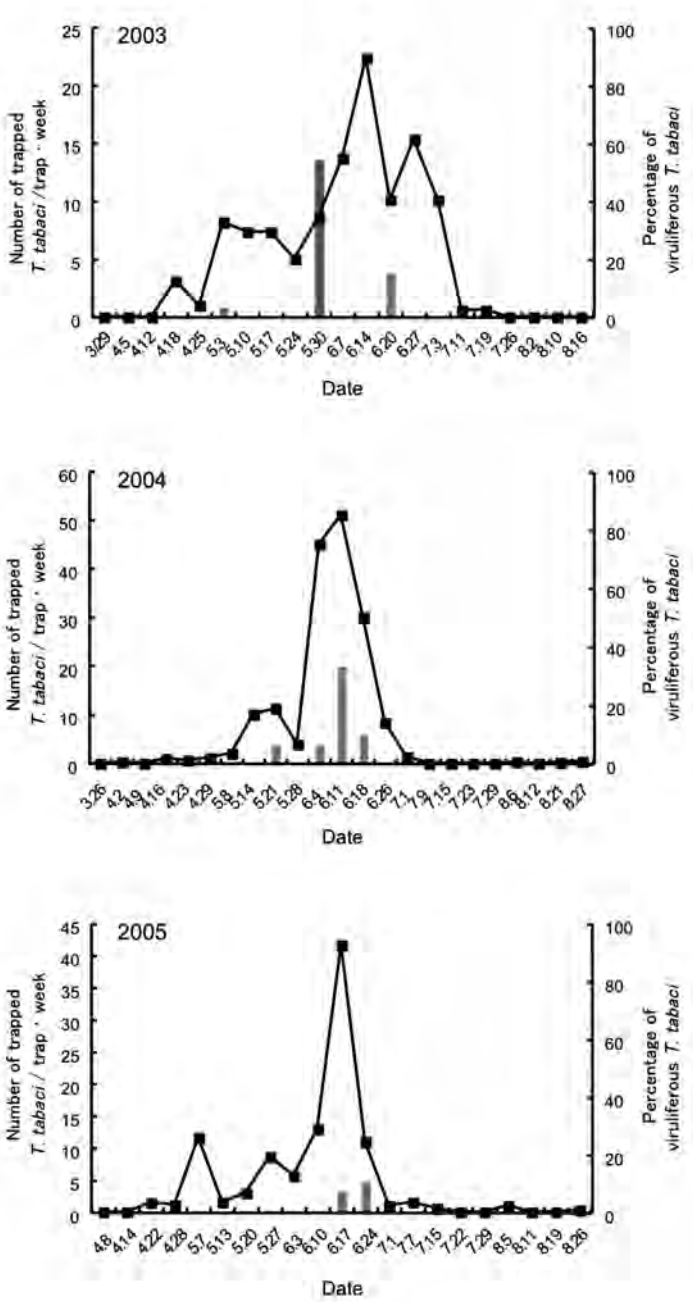

Fig. 1. Graphs of the number of *T. tabaci* and percentage of viruliferous individuals captured on the sticky traps outside the lisianthus greenhouses. Years are represented in each graph. Solid lines indicate the number of insects captured on the traps (left y-axis). Values are the average of 3 traps. Columns indicate the percentage of viruliferous *T. tabaci* (right y-axis).

DISCUSSION

T. tabaci is the only known vector of IYSV. In this

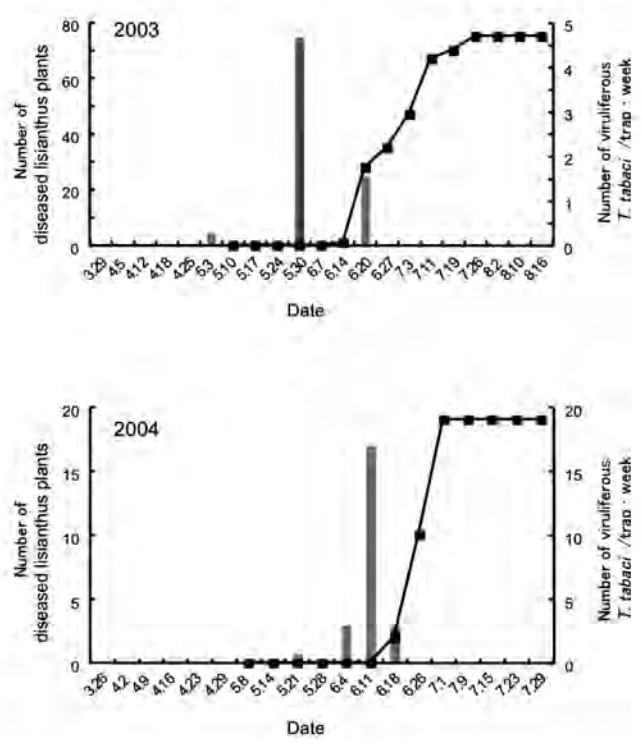

Fig. 2. Graphs of the number of diseased lisianthus plants and viruliferous *T. tabaci* captured on the sticky traps. Years are represented in each graph. Solid lines indicate the number of diseased lisianthus stocks (left y-axis). Columns indicate the average number of viruliferous *T. tabaci* captured on a sticky trap (right y-axis).

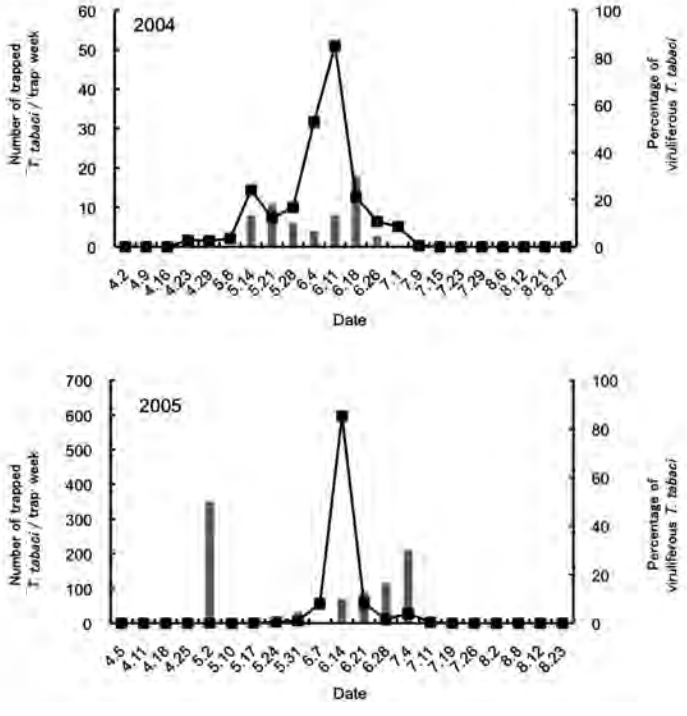

Fig. 3. Graphs of the number of *T. tabaci* and percentage of viruliferous individuals captured on the sticky traps in the onion field. Years are represented in each graph. Solid lines indicate the number of insects captured on the traps (left y-axis). Values are the average of 3 traps. Columns indicate the percentage of viruliferous *T. tabaci* (right y-axis).

study, we examined its relationship with lisianthus LND. The results showed that LND outbreaks correlated with the appearance of viruliferous *T. tabaci* outside the greenhouses hosting lisianthus. Therefore, viruliferous *T. tabaci* that entered the greenhouses are likely responsible for the initial introduction of LND and for the subsequent spreading of the disease, following the successive entry of other viruliferous vectors.

Viruliferous individuals did not represent the totality of the *T. tabaci* population. Populations of this thrips species differ because of their reproduction system (Murai, 1990), morphological characteristics (Klein and Gafni, 1996), and habitats (Jenser *et al.*, 2001). Molecular characterizations of the cytochrome oxidase I (COI) and ribosomal RNA genes enabled categorization of intraspecific varieties and identification of genetic relationships (Toda and Komazaki, 2002; Frey and Frey, 2004; Brunner *et al.*, 2004). Nucleotide sequence analysis of the COI gene the *T. tabaci* population present in our surveyed fields, indicated that it was similar to the population commonly distributed in Japan (S. Toda, personal communication), suggesting that the IYSV-transmitting ability and feeding habit are consistent throughout the year.

Although *T. tabaci* were observed outdoors in April, viruliferous adults were not found in the greenhouses. While a few weed species are known as natural hosts of IYSV (Ghotbi *et al.*, 2005), alliaceous plants are suspected to be the major source of infection in the field. Gent and Schwartz (2004) reported that volunteer onions might serve as a source of IYSV inoculum, but wild onions have not been observed in Japan. *Allium grayi*, which is a species related to *Allium cepa*, commonly grows around onion fields. However, IYSV was never detected in *A. grayi* nor in any other weeds (unpublished information).

In Japan, lisianthus seedlings are transplanted at various time intervals to harvest flowers throughout the year. Our surveys showed that LND can develop simul-

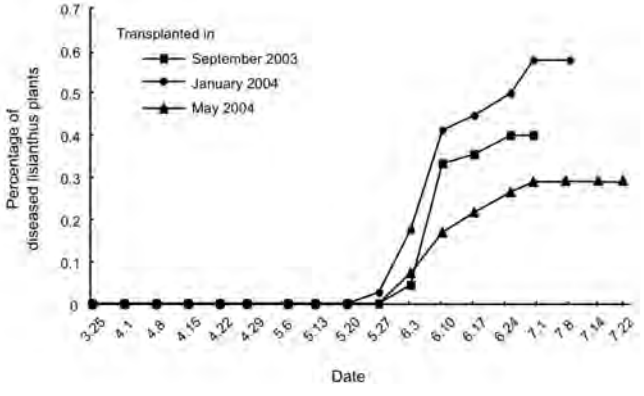

Fig. 4. Graph of the percentage of diseased lisianthus stocks in different cropping types. Solid lines indicate the percentage of diseased lisianthus stocks.

taneously on lisianthus plants at different growth stages, which supports the notion that the infection source of IYSV is located outside the greenhouses.

In the studied area, onion is grown near lisianthus greenhouses and harvested from the end of May to early June. We have shown that viruliferous *T. tabaci* thrive on onion plants and that IYSV infections to onion precede those to lisianthus. Hence, it seems plausible to conclude that onions are the main source of IYSV, which is spread by viruliferous *T. tabaci* when they abandon onion crops after harvesting. In the surveyed onion field, diseased plants were not identified until April. Since IYSV is not present in onion bulbs and roots (Kritzman, 2001), the potential of onion bulbs as IYSV reservoir is uncertain. Further studies are required to establish when IYSV infects onion plants.

The use of sticky traps was of assistance not only for estimating the number of thrips but also for monitoring the incidence of viruliferous thrips in the field. If insecticides are applied in accordance with the emergence of viruliferous *T. tabaci*, in principle, plants could be protected effectively from IYSV infection. Murai (2000) has determined the development and reproduction cycle of *T. tabaci*. Thus, a future forecasting model based on information on meteorological parameters, ecological characteristics, and cropping conditions would perhaps enable to predict the size of viruliferous *T. tabaci* populations.

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