Transmission of Iris Yellow Spot Tospovirus

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Abstract: The biological transmission of *Iris yellow spot virus* (IYSV), a new tospovirus associated with a disease in onion (*Allium cepa*) that is known to growers in Israel as "straw bleaching", was studied. Virus distribution in the various onion plant parts, as well as, transmission during vegetative propagation was examined. IYSV distribution within the plant was uneven and the highest titres were in the inner leaves and near the bulb. The virus was not transmitted to onion seedlings from infected mother plants through the seed and was not located in bulbs of infected plants. Surveys of thrips populations in onion fields revealed that *Thrips tabaci* was the predominant species and that its incidence was strongly related to that of IYSV infection. Fifty percent of the thrips population collected from IYSV-infected onion fields transmitted the virus. In laboratory tests, IYSV was efficiently transmitted by T. tabaci, from infected to healthy onion seedlings. Two biotypes of *Frankliniella occidentalis*, failed to transmit the virus. The characterization of the virus in onion and the identification of its thrips vector provide valuable information to help growers control the disease.

Introduction

Of 12 tospovirus species described so far, three were reported in Israel: *Tomato spotted wilt virus* (TSWV) (Antignus *et al*., 1997), *Impatiens necrotic spot virus* (INSV) (Gera et al., 1999b) and *Iris yellow spot virus* (IYSV) (Gera et al., 1998a). The last was detected in *Hippeastrum hybridum*, onion (*Allium cepa*) and recently, in lisianthus (*Eustoma russellianum*) (Kritzman et al., 2000). In onion, the virus was associated with a disease known to growers in Israel as "straw bleaching". The incidence of the disease often reached 50-60%, resulting in heavy losses of onion-bulb production (Gera et al., 1998a).

Two species of tospovirus vectors are found in Israel, *Frankliniella occidentalis* and *Thrips tabaci,* of which the first is responsible for recent TSWV epidemic (Gera et al., 1998b). For effective integrated management of tospoviruses, information on virus occurrence and vector identity is necessary. The goals of the present study were to study the biological transmission of IYSV and to identify the thrips vector and its efficiency in transmitting the virus.

Materials and Methods

IYSV was isolated from onion (*A. cepa*) collected in the Bet-Shean Valley in Israel. The virus was maintained in *Nicotiana benthamiana*. Onion plants, naturally infected with IYSV, were used to determine virus distribution within the leaves. Five leaves of each plant were divided into four equal segments. Each segment was sampled and tested for IYSV by ELISA with a polyclonal antibody against the NC proteins (Gera *et al*., 1999a).

Mature seeds were harvested from naturally infected onion that showed severe symptoms. Non-infested seeds were collected from healthy plants and served as controls. Seedlings were grown for 5-8 weeks before being tested for IYSV.

To study thrips field populations, a randomized complete block design with four replications was used. Ten onion plants from each block were used for thrips counting. Both larvae and adults of *T. tabaci* were included. Field populations of *T. tabaci* were collected from naturally IYSV-infected onion. Onion seedlings grown from seeds and testing negative for IYSV by ELISA were used for thrips transmission experiments. A single adult thrips was placed on each healthy seedling leaf piece for 2 days. Virus presence was later ascertained by ELISA.

Adult *T. tabaci* and *F. occidentalis* were confined for up to 4 days on bean pods to lay eggs. Once the eggs had hatched, the larvae were collected for up to 12 h and used for transmission. Alternatively, first instar larvae were reared on naturally infected onions

and allowed to move freely to healthy onion seedlings in the same cage. Healthy plants exposed to thrips for inoculation access feeding were kept under greenhouse conditions and observed for symptom expression for 4 weeks. Virus presence was ascertained by ELISA.

Results

Symptoms of the virus in naturally infected *A. cepa* include straw-colored, chlorotic, and necrotic lesions on leaves. In order to develop a reliable test protocol for onion, we examined the virus distribution in the various plant parts as well as virus transmission during vegetative propagation. The virus was detected in all segments of the various leaves. The distribution of virus was not uniform within infected plants. Higher concentrations were consistently obtained in the internal leaves and in leaf segments close to the bulb (Table 1). Attempts to detect the virus in onion roots or bulbs of infected plants were unsuccessful.

No visible symptoms were observed in onion seedlings grown from seeds harvested from infected plants that were naturally infected with IYSV, and none of the seedlings were found to be infected with IYSV as determined by ELISA (0/535), up to 8 weeks after germination. Moreover, when onion bulbs taken from symptomatic plants that tested positive for IYSV by ELISA were planted, no leaf symptoms developed, and all leaf samples tested by ELISA were negative (n=25).

Surveys to relate the incidence of thrips populations to that of IYSV were conducted in onion fields. These surveys showed that *T. tabaci* was the predominant thrips species and that its incidence was strongly related to that of IYSV (Table 2). The mean numbers of *T. tabaci* per plant in an infected onion field, counted at 2-week intervals starting 2 months after sowing, were 13, 64, 48, 21 and 15 (Table 2). *T. tabaci* adults and larvae collected from infected onion fields in different locations in Israel were 33-50% efficient in transmitting IYSV to onion as confirmed by ELISA (Table 3).

To confirm virus transmission by thrips, a colony of *T. tabaci* larvae grown in our laboratory was introduced onto naturally infected *A. cepa* plants and virus-free onion seedlings were exposed to adults that developed on those plants. *T. tabaci* transmitted the virus to 12 out of 20 plants, as confirmed by ELISA. When a population of *F. occidentalis* from mango in Bet Dagan was used, none of twenty tested onions became infected, although this thrips is a vector of TSWV.

Two *F. occidentalis* populations, collected from mango (n=65) and from sunflowers (n= 78), an efficient and a poor transmitter population of TSWV, failed to transmit the virus from *Emilia sonchifolia* to onion leaf pieces.

ELISA values (E_{405})[b]					Slice position[a]
Leaf # V	Leaf # IV	Leaf # III	Leaf # II	Leaf [c] # I	
0.518±0.635	0.593±0.676	0.914±0.833	0.412±0.429	0.299±0.298	A
0.656±0.835	0.730±0.435	1.218±0.987	0.689±0.649	0.228±0.227	B
0.861±0.707	1.703±0.624	1.836±0.902	1.038±0.749	0.566±0.494	C
1.895±0.772	2.326±0.556	2.706±0.156	2.069±0.902	1.860±0.673	D

[a]Slice A is the tip of the leaf, and slice D is closest to the bulb.
[b]Mean values of eight different onion leaves.
[c]Leaves I and V are the external and leaf III is the internal one.

Table 1. Distribution of *Iris yellow spot virus* (IYSV) in naturally infected onion leaves as determined by ELISA.

Date of count	Mean no. thrips per 10 plants (±SD)	Virus	
		incidence[a]	(%)
17.4.00	130±53	nt	nt
1.5.00	642±195	20/35	57
15.5.00	476±51	nt	nt
29.5.00	212±26	nt	nt
12.6. 00	162±30	12/20	60

[a]Virus incidence is the number of infected plants out of total plants tested.
nt=not tested.

Table 2. Mean numbers of thrips, and *Iris yellow spot virus* (IYSV) incidence in the field

Transmission rates by thrips collected as[a]				Location
Adults		larvae		
(%)	No./total[b]	(%)	No./total[b]	
42.1%	8/19	49.2%	16/37	Ein Harod
44.4%	8/18	47.9%	10/24	Mitzpeh
50.0%	2/4	33.3%	4/12	Yagor

[a]In all tests, only adults were used for transmission. Inoculation access feeding was 48 h.
[b]Denominator, number of onion leaf pieces used: numerator, number of onion leaf pieces infected, with one thrips per leaf piece.

TABLE 3. Transmission rates of *Iris yellow spot virus* (IYSV) by adult *Thrips tabaci* collected as larvae or adults from infected onion fields grown in different location in Israel.

Discussion

We provide here further information about the biological transmission of IYSV in onion. This newly emerged virus has been reported from the New and Old Worlds. IYSV was isolated from iris in the Netherlands (Cortes *et al*. 1998), from onion in Brazil (Pozzer *et al*., 1999) and from lisianthus in Israel (Kritzman *et al*., 2000).

The virus distribution within the onion plant was uneven. The highest titres were in the inner leaves and near the bulb. That area is also most preferred by the thrips as a feeding site, and it offers the best opportunity for virus acquisition and/or infection by larvae and adults. The failure to detect the virus in bulbs or roots of infected onions and the lack of virus transmission to the subsequent generation by bulbs and seeds is similar to the results obtained in Iris (Derks and Lemmers, 1996). Both, TSWV and INSV were detected mainly in the inner symptomatic leaves of infected iris, and no virus was detected in the subsequent generation of TSWV or IYSV infected and symptomatic plants (Derks and Lemmers, 1996). The virus distribution observed within onion leaves in this study may reflect local infection by feeding thrips. The factors which restrict the movement of the virus from leaves to bulb are not yet known.

The outbreak of IYSV in Israel was associated with large populations of *T. tabaci*. We demonstrated that the virus is transmitted by *T. tabaci*, and not by *F. occidentalis* populations. *T. tabaci* acquired and transmitted IYSV from infected onion plants. High rates of transmission by the field population of *T. tabaci* reflect the high proportion of viruliferous insects in the field. The failure of *F. occidentalis* to transmit IYSV may be attributed to a barrier preventing the infection of the salivary glands (Ullman *et al*., 1992). Salivary glands must contain large amounts of virions for thrips to transmit the virus (Nagata *et al*., 1999).

The data showing that neither seeds nor bulbs from infected onion plants serve as sources of IYSV for the subsequent generation indicates that thrips transmission is the way in which this virus is transmitted. However, despite the wide distribution of *T. tabaci* in natural vegetation and various crops in Israel, outbreaks of the disease are limited to certain areas. The identification of the virus and its thrips vector provides valuable information to help growers control the disease.

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