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Susceptibility to virus infection of candidate plants used to enhance predatory dicyphine (Heteroptera: Miridae)

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Abstract: Selecting candidate plants in order to enhance predators for conservation biological control not only has to consider their contribution to enhancement of predators, but also avoid the risk of acting as reservoirs of potential pests or diseases. Mirid bugs are effective biological control agents of tomato pests and the introduction of insectary plants has been shown to be an effective means for the conservation of these important natural enemies. We studied the degree of susceptibility and the role of selected insectary plants as reservoirs of the most important viruses found in tomato crops in Spain. A virus survey conducted in the regions of Murcia and Barcelona, and a series of transmission experiments conducted under controlled conditions indicated that the species *Dittrichia viscosa* and *Marrubium vulgare* are susceptible and may play a significant role in *Potato virus Y* and *Cucumber Mosaic Virus* epidemics, whereas virus were never detected in *Carlina corymbosa* and *Ononis natrix* collected in the field and were immune to the transmission of both viruses by aphids. Our work suggests that there is a potential risk for virus epidemics to occur if *M. vulgare* and *D. viscosa* are introduced as insectary plants to attract and enhance biological control by predatory mirids. Further research is needed to confirm the role that *M. vulgare* and *D. viscosa* and *Verbascum thapsus* may play in the epidemics of PVY and CMV in tomato crops. *C. corymbosa* and *O. natrix* appear to be the species that show the lowest risk as reservoirs of viruses that commonly infect tomato crops.

Keywords: Heteroptera, Miridae, Dyciphinae, *Marrubium vulgare*, *Dittrichia viscosa*, *Ononis natrix*., *Carlina corymbosa, Verbascum thapsus,* insectary plants, PVY, CMV, TSWV, TYLCV, tomato

Introduction

The introduction of insectary plants has been shown to be effective for the conservation of natural enemies in the proximity of crops and to facilitate their establishment (Arnó *et al.*, 2000; Alomar *et al.*, 2002; Sanchez *et al.*, 2003a; Pascual-Villalobos *et al.*, 2006). However, the selection of plants also has to avoid or minimize the risk of plants enhancing pests or diseases that are common in the target cropping area (or avoid plants that augment the inoculum pressure). Vegetable production in Spain is threatened by a series of insect pests and insect-transmitted virus diseases that cause significant economic yield losses every year. Tomato crops are particularly susceptible to a wide range of virus diseases. *Tomato spotted wilt virus* (TSWV) has caused severe epidemics since the introduction of *Frankliniella occidentalis* (Pergrande) (Thysanoptera: Thripidae) in Spain in the late 80s (Lacasa, 1990). Important viruses are also *Potato virus Y* (PVY), *Cucumber Mosaic Virus* (CMV), and *Tomato yellow leaf curl virus* (TYLCV), this last one is transmitted by the whitefly *Bemisia tabaci* Gennadius, that causes the most destructive disease of tomato crops throughout the Mediterranean region (Gafni, 2003).

Several species of predatory mirids contribute to the control of important pests of vegetables in the mediterranean regions (Gabarra, 2002). *Macrolophus caliginosus* Wagner is an effective predator of *Trialeurodes vaporariorum* (Westwood) and *B. tabaci* in several crops, especially in tomato (Castañé *et al.*, 2000; Albajes & Alomar, 1999).) The proximity of refuge plants like *Dittrichia viscosa* (L.) Greuter, favoured the colonization of tomato crops by *M. caliginosus* (Alomar *et al.*, 2002). Mirid bugs have been also observed in other plant species like *Marrubium vulgare* L., *Ononis natrix* L. and *Carlina corymbosa* L. (Sanchez *et al.*, 2003b). However, natural vegetation may play a very important role in the epidemiology of plant viruses such as TSWV that cause severe virus epidemics in tomato crops (Kahn *et al.*, 2005). The objective of our work was to analyze the degree of susceptibility of selected candidate insectary plant species to the major viruses that infect tomato crops in Spain, and to study their likely role as virus reservoirs.

Materials and methods

Virus survey of insectary plants

A virus survey of selected insectary plant species was conducted in two separate vegetable producing regions of Spain (Murcia and Barcelona). A total of 104 samples of *M. vulgare, D. viscosa, O. natrix* and *C. corymbosa* were collected in spring of 2004 from Mazarrón, Murcia. Other 97 samples from *O. natrix* and *D. viscosa* were collected in autumn of 2004 and 2005 from various locations at Barcelona. All samples were analysed by ELISA to test for the presence of CMV, PVY, TSWV and TYLCV, which are the main viruses commonly found in the tomato crops in the regions under study. No obvious symptoms of virus infection were observed in the plants sampled, and therefore, samples were collected at random from the margins or across the field in the selected locations.

Transmission experiments

The viruses that were found present in our survey (CMV and PVY) were inoculated by aphids to healthy insectary test plants to assess the role of such plants as virus reservoirs. Both viruses were inoculated by aphids to several healthy test plants of *M vulgare, D. viscosa, C. corymbosa, O. natrix* and *V. thapsus*. CMV was inoculated by *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover, and PVY was inoculated by *M. persicae* only. Transmission experiments were conducted under laboratory conditions (21±2 ºC) using a procedure described by Fereres *et al.* (1993) with slight modifications. In summary, aphids were subjected to a 5 min acquisition access period on infected-pepper source plants, and then groups of 10-15 aphids were transferred to each test plant for a 2h inoculation access period. Test plants were sprayed with imidacloprid and tested by ELISA to assess virus infection.

The insectary test plants that became infected by either PVY or CMV were used as source plants to run a second series of transmission tests to test their role as virus reservoirs. For such tests, the same transmission protocol was used and the viruses were transmitted by aphids from each infected insectary plant to healthy tomato test plants (28 replicates).

ELISA-assays

Viruses were identified by ELISA-assay using specific commercial antibodies against the following four virus species: CMV, TSWV, PVY and TYLCV. The procedure used was a DAS-ELISA (Clark & Adams, 1977). Plants were considered infected when absorbance values reached three times the mean value of the healthy controls. Healthy samples of *D. viscosa*, *M. vulgare*, *C. corymbosa* and *V. thapsus* were obtained from seeded plants grown in virus-free environmental chambers.

Results and discussion

Virus survey of insectary plants

The only two viruses detected in the plants sampled were CMV and PVY (Table 1). PVY was detected in a single *M. vulgare* from Murcia and in 3 *D. viscosa* plant samples collected in Barcelona. CMV was found in 3 *D. viscosa* plants in samples collected in Barcelona. The natural occurrence of CMV infecting *M. vulgare* has been reported by Fletcher (2001) who found asymptomatic infected plants in a field survey conducted in New Zealand.

These results show that both *M. vulgare* and *D. viscosa* appear to be infected in the field by PVY and CMV. The two other viruses (TYLCV and TWSV) included in our survey were never found in none of the insectary plants sampled. Also, none of the samples obtained from *C. corymbosa* and *O. natrix* appeared to be infected with any of the viruses included in our survey. However, TSWV was found in natural infections on *M. vulgare* in field surveys conducted in North America (Sclar *et al*., 2001) and Australia (Latham & Jones, 1997). Natural infections of TSWV were also found in *V. thapsus* in North America (Sclar *et al*., 2001).

Table 1. Virus detection in selected insectary plants sampled in different locations of Spain during 2004 and 2005

Locality	Host plant	N[b]	Viruses present[a]			
			CMV	PVY	TSWV	TYLCV
Murcia	*D. viscosa*	29	0	0	0	0
	M. vulgare	30	0	1	0	0
	C. corymbosa	25	0	0	0	0
	O. natrix	20	0	0	0	0
Barcelona	*D.viscosa*	94	3	3	0	0
	O. natrix	3	0	0	0	0
Total		201	3	4	0	0

[a]number of infected plants; [b]number of plants sampled.

Transmission experiments

The results obtained in the transmission experiments of the viruses CMV and PVY to insectary test plants are shown in Table 2. Transmission of CMV was positive in the case of *D. viscosa* and *M. vulgare* and PVY was readily infecting *V. thapsus* (20/28) but none of the other insectary plants tested became infected. However, *M. vulgare* and *D. viscosa* were found infected by PVY in our field survey although we failed to inoculate the virus when using *M. persicae* as a vector. It is possible that other aphid species are involved in the transmission of PVY to *M. vulgare* and *D. viscosa* in the field. We found that *C. corymbosa* and *O. natrix* never became infected with PVY or CMV after aphid inoculation although the number of test plants was low and further experiments are needed to confirm this result.

The results on the role of insectary plants as sources of inoculum of both CMV and PVY are shown in Table 3. Tomato test plants became infected by CMV when using *D. viscosa* or *M. vulgare* as infected source plants. However, tomato plants did not become infected with PVY when the virus was acquired from *V. thapsus* infected plants. This result suggests that *V. thapsus* might be susceptible to PVY inoculation but may not be a natural effective source

Table 2. Transmission of CMV and PVY to selected insectary plant species by *A. gossypii* and *M. persicae*.

Virus source	Test plant	Vector	
		A. gossypii	*M. persicae*
CMV-infected pepper	*D. viscosa*	1/14	0/14
	M. vulgare	1/13	1/13
	O. natrix	0/3	0/4
	C. corymbosa	0/4	0/4
	V. thapsus	0/14	0/14
PVY-infected pepper	*D. viscosa*	NT	0/47
	M. vulgare	NT	0/18
	O. natrix	NT	0/7
	C. corymbosa	NT	0/7
	V. thapsus	NT	20/28

NT: not tested

Table 3. Transmission of CMV and PVY from infected insectary plants to tomato test plants by *A. gossypii* and *M. persicae*

Virus-Source	Vector	
	A. gossypii	*M. persicae*
CMV-infected *D. viscosa*	1/15	0/15
CMV-infected *M. vulgare*	0/14	1/14
PVY-infected *V. thapsus*	NT	0/28

NT: not tested.

for virus acquisition by aphid vectors.

In summary, our results suggest that susceptibility of *M. vulgare* to virus transmission by aphid vectors is low, although it is likely to occur when a significant number of viruliferous aphids land on the plant. Therefore, there is a potential risk for virus epidemics to occur if *M. vulgare* and *D. viscosa* are introduced as insectary plants to attract and enhance biological control by mirid bugs such as *M. caliginosus.* However, more experiments are needed to confirm the role that *M. vulgare* and *D. viscosa* may play in the epidemics of PVY and CMV in tomato crops. We also found that *V. thapsus* was susceptible to the transmission of PVY by aphids although it was not a source of inoculum for acquisition of the virus. Therefore, this species needs to be investigated further before its release as an insectary plant because it has been reported as a susceptible host species of TSWV. Finally, we have shown that *O. natrix* and *C. corymbosa* were never found infected by none of the viruses tested in our field survey and were not susceptible to virus inoculation by aphids under laboratory conditions and therefore, they are good candidates to be used as insectary plants from the point of view of avoiding insect-transmitted virus diseases.

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