ORIGINAL RESEARCH ARTICLE

Spontaneous vegetation in the horticultural belt of La Plata as host of Thripidae (Thysanoptera) vectors of Tospovirus: Relative risk as an epidemiological component

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ABSTRACT

Black Death is a virosis caused by the Tomato Spotted Wilt Virus (TSWV), transmitted by thrips, and represents a complex problem since weed hosts for thrips vectors and the virus is accentuated as virus reservoir and vector sustenance. The objective was to generate, from a list of weeds that act as hosts for the four vector thrips species in the horticultural belt of La Plata, a relative risk categorization as an epidemiological component. Between 2000 and 2003, three sites were selected within the horticultural belt of La Plata (Buenos Aires, Argentina) where flowers of 21 weed hosts of *Frankliniella occidentalis, Frankliniella schultzei, Frankliniella gemina* and *Thrips tabaci* were sampled monthly (60 in total). For analysis, the sampling results were grouped into three annual seasons, corresponding to the phenology of greenhouse crops in the region. For the four thrips vectors, the abundance of adult thrips and the presence of their larvae were considered using an unsupervised hierarchical cluster analysis and the DGC multivariate mean comparison test to obtain the number of significant groups. From this base grouping, three risk groups (RG) were defined as a source of inoculum for these vectors: high (H), medium (M) and low (L) according to the status of the reproductive host (RH). The groups that emerged were: (H): RH of *F occidentalis*, (M): RH of *F. schultzei* and *T. tabaci*, and (L): RH of *F. gemina* or non-vector thrips. Periodic survey and early flowering suppression of nine weed species categorized as high risk is proposed. This implies the continuous monitoring of three weed species, to which other companion weeds are added according to the growing season.

Keywords: Frankliniella Occidentalis; Frankliniella Schultzei; Frankliniella Gemina; Thrips Tabaci; TSWV

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1. Introduction

The Black Death (TSWV or Tomato Spotted Wilt Virus and other related serotypes) is a virosis transmitted to crops by thrips (Thripidae: Thysanoptera) that represents a complex problem throughout all horticultural areas of Argentina^[1–3]. TSWV is the type species of the genus Tospovirus, a Bunyaviridae characterized by being made up of phytopathogenic viruses, with a wide host range encompassing about 90 families and 1,000 plant species. The viral particles are spherical, with a lipid envelope (of the host) and glycoprotein projections encoded by the viral genome. Inside are three nucleoprotein particles, composed of circularized RNA segments and capsid protein. These three single-stranded RNA segments constitute the Tospovirus genome^[4].

Before the entry of Frankliniella occidentalis (Pergande) into the

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country^[5], although losses due to black plague in greenhouses had already been recorded, losses increased after its entry^[3]. Such epidemics show that at least one of the other three virus vectors recorded in Argentina (*Frankliniella schultzei* (Trybom), *Thrips tabaci* Lindemann or *Frankliniella gemina* Bagnall) would in itself imply a risk to crops.

Plants in the spontaneous vegetation surrounding greenhouses (considered as weeds) that are hosts (HR = reproductive, or "true", hosts) for thrips vectors can act as their reservoir and source of inoculum for the disease. However, eliminating all surrounding vegetation is irrational, both economically and environmentally. It is necessary to explore other options such as, for example, directing control only to those plant species preferred by vectors as breeding sites, which could act as the source of epiphytes^[6]. For this purpose, it is essential to determine the RH for the whole range of vector thrips, by identifying the species present in such hosts, both in the adult and larval stages^[7].

On the one hand, most research devoted to the search for HR for thrips vectors refers exclusively to F. occidentalis, the most efficient and globally dispersed vector^[8]. The second most cosmopolitan vector, Thrips tabaci, has a variable role in transmission depending on the area considered^[9]. Milne and Walter^[10] verified five HR among the plant species, cotton weeds, where this thrips is a pest. Similarly, Wilson^[11] found at least five species that can act as HR of this vector and virus reservoir in lettuce crops. Work on Frankliniella schultzei, mostly in tropical and subtropical areas of the southern hemisphere^[8], does not relate to its role as a vector. This is probably because this species is generally less important than F. occidentalis and T. *tabaci*^[8,12–14]. Finally, HRs for the South American endemic species, F. gemina, vector of GRSV (Groundnut Ring Spot Virus) in our country^[15] had not been considered until very recently^[7].

On the other hand, there are very few antecedents regarding the categorization of spontaneous vegetation species, not as HR of these thrips but for the relative risk they present when acting as the origin of migrating vectors to the crop. Research can be mentioned considering only *F. occidentalis* and using arbitrary scales according to its abundance in hosts and accompanying larvae belonging to Thripidae^[14,16]. In these arbitrary scales, the authors established, according to their own criteria, a scale without further substantiation, such as subjecting the abundance values found in the plant species to an objective analysis that validates their criteria in some way. It is precisely the valuation by means of an objective test to compare between species instead of using an arbitrary scale that is proposed in the present work.

The objective of the present work was to propose, for the species that act as Reproductive Hosts (RH) of Black Death vectors in the horticultural belt of La Plata, a categorization in Relative Risk Groups as a reservoir for these vectors as an epidemiological component.

2. Materials and methods

2.1 Study area

Three sampling sites were selected within the horticultural belt of Greater La Plata (Buenos Aires province, Argentina) that occupies a radius of about 40 km to the N, W and S of the City of La Plata (34°54′ S, 57°55′ W). Each of these sites was considered as a unit of experiment (UE):

Site 1: Gorina Experimental Farm, Ministry of Agrarian Affairs of the province of Buenos Aires; La Plata district; 34°54′36.10″ S, 58°02′13.05″ W.

Site 2: Commercial vegetable production establishment; located 1 km from Pereyra Station (Roca Railroad) in the district of Berazategui; 34°50′34.77″ S; 58°05′59.83″ W.

Site 3: Julio Hirschorn Experimental Station, Faculty of Agricultural and Forestry Sciences, National University of La Plata; in the Partido de los Hornos; 34°59′05.63″ S, 57°59′58.01″ W.

For the surveys of thrips host plants, areas without periodic weed control were considered: uncultivated areas, borders and roads, within the boundaries of each EU or in its most immediate area of influence.

The data correspond to samplings between 2000 and 2003; during that period, 1 to 3 samplings were performed per month, in different seasons of

the year, with the lowest intensity corresponding to autumn and winter and the highest to spring and summer. The sampling unit was 1 flower or inflorescence as appropriate (25 units/sp./date), in 21 plant species of spontaneous vegetation^[7].

2.2 Sample and data processing

The flowers were immersed and shaken in a container 15 cm high, 15 cm in diameter containing 60% ethanol, pouring the contents over a piece of muslin fitted to the mouth of the container, from where the individuals were extracted and placed in a Petri dish for subsequent counting. The material was preserved in 70% alcohol.

The thrips were conditioned in

semi-permanent microscope slides in Hoyer's liquid. They were identified under a Zeiss Axio Imager optical microscope equipped with Differential Interference Contrast (DIC) using keys for the adult stage and for the second larval stage.

The temporal abundance of thrips vectors in the 21 plant species sampled was analyzed, considering three seasonal stages: winter–spring (July to October), summer (November to February) and autumn (March to May), which correspond approximately with the management and phenology stages of bell pepper and tomato greenhouse crops in the La Plata horticultural belt, as well as with different activity for thrips (**Table 1**).

Autumn		
Postharvest		

Data were processed by plant species, at each site and sampling date. In the case of adults, the abundance per thrips species (number of individuals/corresponding plant species) was considered. For larvae, on the other hand, a binary variable (1, 0) (presence, absence) was considered, since this implies pointing to the plant species, at least as a reproductive host (RH) *sensu* lato for the vector thrips species^[7].

An unsupervised, hierarchical, cluster analysis^[17] was performed using InfoStat^[18] and representing the results in a dendrogram. Subsequently, the multivariate mean comparison DGC test^[19] was performed to determine the number of significant groups^[20].

A weed was considered to belong to a particular Risk Group (RG) as it acts as a Reproductive Host for the vector thrips species with decreasing efficiency in TSWV transmission^[8]: high (RH of *F. occidentalis*), medium (RH of *F. schultzei* and *T. tabaci*) or low (RH of *F. gemina* or non-vector thrips).

3. Results

Results from 60 sampling dates were analyzed:

26 corresponded to site 1 with 224 samples; 18 to site 2 with 160 samples and 16 to site 3, with 127 samples. From 14,636 weed flowers, a total of 54,054 individuals were obtained, of which 40,356 were adults and 13,694 were 1st and 2nd instar larvae. For the analysis, only adults and larvae of *F. occidentalis*, *F. schultzei*, *F. gemina* and *T. tabaci* were considered, i.e., the vectors recorded in the horticultural belt of La Plata.

Figures 1, 2 and **3** show the dendrograms originated by the cluster grouping and the subsequent DGC test, with the cut-off line that determined the emergence of significantly different groups. The analysis generated five groups for the winter–spring and autumn periods and ten groups for the summer season (DGC test results in **Figures 1, 2, 3** and **Tables 1, 2, 3, 4**).

The grouping, in the case of adults, is based on the relative proportion of each vector thrips species in the corresponding season. On the other hand, since the presence of larvae for each species was entered into the analysis as a binary variable, it gives all of them the same weighting. To this grouping was added the classification of risk groups: high, medium and low. Consequently, the figures show the interpretation of the grouping of plant species according to their reproductive host trait (HR) for each of the vector thrips species.

The high-risk group included species acting as feeding hosts for three or four of the vectors and as HR of *F. occidentalis* or *F. occidentalis* and *F. schultzei*. For the summer season, adults of *F. occidentalis* were found in 18 of the 21 species, so it was not a useful character to separate the groups.

The high-risk species would be four for the winter-spring season (Brassica rapa, Raphistrum

rugosum, *Raphanus sativus* and *Echium plantagineum*), eight for the summer season (the same as for the previous season plus *Matricaria chamomilla*, *Carduus acanthoides* and *Galega officinalis*) and five for the autumn season (*B. rapa*, *R. rugosum*, *R. sativus*, *M. chamomilla*, and *C. acanthoides*). Most of them had also been previously qualified as HR *sensu stricto* in the same area^[7] which justifies their belonging to this group.



Figure 1. Winter-spring season. Dendrogram, significant groups of the DGC test.

		Larval species	Adult species			
Plant species	Group according to DGC test	Presence	Presence	Seasonal dominance	Risk groups	
Lotus tenuis	5	ST	GOTS	G	Medium	
Conium maculatum	3	G	GOTS	GT	Medium	
Picris echioides	5	GT	GOTS	GT	Medium	
Trifolium repens	5	GT	GOTS	GTO	Medium	
Brassica rapa	1	GOT	GOTS	GTOS	High	
Raphistrum rugosum	1	GOT	GOTS	GTO	High	
Echium plantagineum	4	GOST	GOT	GT	High	
Raphanus sativus	4	GOST	GOTS	GTO	High	
Convolvulus arvensis	2	S	GOTS	Т	Under	
Matricaria chamomilla	2	GOST	GOT	GT	Medium	
Taraxacum officinale	2	G	GOTS	GT	Low	
Cichorium intybus	2		GOTS	GO	Low	
Hypochaeris	2		GOTS	GTOS	Low	

 Table 2. Winter-spring season. Table of criteria and risk groups

References:

For vector thrips species:

G: Frankliniella gemina, O: Frankliniella occidentalis, S: Frankliniella schultzei, T: Thrips tabaci For column headers:

Larval species, presence: in alphabetical order, larval thrips species found in flowers

Adult species, presence: in alphabetical order, adult thrips species found in flowers.

Adult species, seasonal dominance: ordered by relative abundance for the season, adult thrips species found on flowers.



Figure 2. Summer season. Dendrogram, significant groups of the DGC test.

		Larval species	Adult spec	Adult species			
Plant species	Group according to DGC test	Presence	Presence	Seasonal dominance	Risk Groups		
Matricaria chamomilla	6	GOTS	GOTS	GTOS	High		
Echium plantagineum	6	GOTS	GOT	TGO	High		
Raphanus sativus	6	GOTS	GOTS	TGSO	High		
Carduus acanthoides	1	GOT	GOTS	GTOS	High		
Brassica rapa	1	GOT	GOTS	GTOS	High		
Raphistrum rugosum	1	GOT	GOTS	GTOS	High		
Galega officinalis	7	ОТ	GOT	GOT	High		
Lotus tenuis	8	ST	GOTS	GTOS	High		
Taraxacum officinale	2	G	GOTS	GO	Low		
Cichorium intybus	2		GOTS	GTOS	Low		
Hypochaeris	2		GOTS	GTSO	Low		
Conium maculatum	3	G	GOTS	GTSO	Low		
Cirsium vulgare	3	G	GOTS	GTOS	Low		
Dipsacum sativus	5	G	GOTS	GTOS	Low		
Pascalia glauca	5	G	GOTS	GTO	Low		
Convolvulus arvensis	4	S	GOTS	TGSO	Medium		
Solidago chilensis	10	GS	GT	GT	Medium		
Melilotus albus	9	GT	GOT	TGO	Medium		
Picris echioides	9	GT	GOTS	GTO	Medium		
Trifolium repens	9	GT	GOTS	GTOS	Medium		

Table 3. Summer season. Table of criteria and risk groups

For vector thrips species:

G: Frankliniella gemina, O: Frankliniella occidentalis, S: Frankliniella schultzei, T: Thrips tabaci

For column headers:

Larval species, presence: in alphabetical order, larval thrips species found in flowers

Adult species, presence: in alphabetical order, adult thrips species found on flowers.

Adult species, seasonal dominance: ordered by relative abundance for the season, adult thrips species found on flowers.



Figure 3. Autumn season. Dendrograms, significant groups of the DGC test.

Table 4.	Autumn season.	Table of	criteria	and risk groups
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		Larval species Adult species						
Plant species	Group according to DGC test	Presence	Presence	Seasonal dominance	Risk groups			
Convolvulus arvensis	3	S	GOST	GT	Medium			
Solidago chilensis	3	GS	GOST	GTOS	Medium			
Trifolium repens	5	GT	GOST	GSOT	Medium			
Carduus acanthoides	2	GOT	GOST	GTO	High			
Raphistrum rugosum	2	GOT	GOST	GTSO	High			
Picris echioides	1	GT	GOST	SGO	Under			
Brassica rapa	1	GOT	GOST	GTO	High			
Taraxacum officinale	1	G	GOST	GOST	Low			
Pascalia glauca	1	G	GOST	GTS	Low			
Cichorium intybus	1		GOST	GT	Low			
Cirsium vulgare	1	G	GOST	TGOS	Low			
Baccharis	1	G	GT	GT	Low			
Conium maculatum	1	G	GOST	G	Low			
Matricaria chamomilla	4	GOST	GOT	GT	High			
Raphanus sativus	4	GOST	GOST	GTSO	High			

References:

For vector thrips species:

G: Frankliniella gemina, O: Frankliniella occidentalis, S: Frankliniella schultzei, T: Thrips tabaci

For column headers:

Larval species, presence: in alphabetical order, larval thrips species found in flowers

Adult species, presence: in alphabetical order, adult thrips species found on flowers.

Adult species, seasonal dominance: ordered by relative abundance for the season, adult thrips species found on flowers.

The plant species considered medium risk presented adults for three or four of the vectors, acted as HR for one or two of them, but without including *F. occidentalis*. This category was related to the presence of larvae of *F. schultzei* and *T. tabaci*; the latter species does not behave as a vector in our country, where its reproduction is exclusive-

ly by thelytoky^[9]. It is not possible to predict whether this status can be modified in the future. Finally, plants grouped as low risk include those that act as HR of non-vector thrips species or only of *F. gemina*, among vector species.

4. Discussion and conclusions

On the one hand, it is not easy to categorize potential host plant species according to their risk. Although a first approximation generally refers to the abundance of individuals per flower, this variable can change by several orders of magnitude, from zero to 100 or more throughout the annual seasons, even for the same plant species and in a particular area. In addition, the plants that act as origin for vector migration vary not only between countries, but also across regions in Argentina. For example, Scotta^[21] in southern Santa Fe and de Breuil et al.^[22] in Córdoba identified F. schultzei as the prevalent species in various crops (along with F. gemina), both mainly vectors of GRSV. In the La Plata area, however, TSWV predominates^[2,3]. Therefore, as long as the latter remains the dominant Tospovirus in this area, the studies presented here and their application to the management of this vegetation would remain valid.

In the winter-pring season, the inclusion of *M*. chamomilla in the low-risk group was due to the fact that adults were recorded on its flowers on few sampling dates at that time and in no case corresponded to F. occidentalis. Due to the low relative abundance of thrips in the season and being a very short plant with small inflorescences and little development, it probably does not offer an interesting refuge for that season, compared to the greater height and vertical development of inflorescences in B. rapa, R. rugosum, R. sativus and E. plantagineum, also in bloom for the season. However, its position as a low-risk host for the early season and high for the remaining two implies some contradiction. It is a very frequent species at the edge of crops outside greenhouses and given that it is a Reproductive Host for F. schultzei^[7], its most accurate position would be in the intermediate group.

Similarly, when placed in the high-risk group of plants, *Lotus tenuis* seems to occupy a higher position for the summer season than it would correspond to in the risk classification, since no larvae of *F. occidentalis* were recorded on its flowers. However, the presence of *F. schultzei* larvae (a trait shared by only five other species) and *F. occidentalis* adults should not be underestimated. Although of small size, it has an extensive presence in the areas of low cut (weeding) where it stands out clearly and presents an abundant flowering from October and at least until March.

On the other hand, other plant species are included or not in the high-risk group depending on the season for other reasons. This is the case of *C*. *acanthoides*, *Melilotus albus* and *G. officinalis* due to their shorter flowering time.

Finally, with reference to high-risk plant species, it is worth noting that *E. plantagineum* is a Reproductive Host (RH) *sensu stricto* for a predatory thrips, a frequent *Aeolothripidae* in the -Platense horticultural belt^[7]. Therefore, selective elimination of this weed could affect the population of a potential biological control agent.

Considering the abundance of *F. occidentalis* only, Yudin *et al.*^[16] established categories based on the presence of adults and Ripa *et al.*^[23] did the same, although the latter authors took into account the specific identity for larvae. Similar to the proposal of Yudin *et al.*^[16], Carrizo^[14] combined this criterion with others of a qualitative nature, such as the season of the year in which the plants flower or the duration of flowering; however, in the latter case, the categories established cannot be generalized or their application is too complex.

In contrast, the significant groups obtained in this work (which intrinsically considers the relative duration of their flowering) are the result of an objective assessment of the reproductive hosts for the entire list of vectors. Therefore, it is a classification criterion or a form of analysis that can be extrapolated to other areas.

In the years following the detection of *F. occidentalis* in the region^[5] and prior to the surveys presented here, growers maintained the area around the greenhouses with tall plants. The dominance of this thrips in the greenhouse extended to the flowers of the spontaneous vegetation, both in the immediate area of the crop and in other more distant areas (local roads and routes)^[13,14]. Since the epidemics suffered in the region since 1995, the Buenos Aires producer generally adopted the control of plants or their deflowering, both in the vicinity of greenhouses and in outdoor crops^[3]. For this reason, currently and in commercial cultivation, the presence of tall plants or flowering specimens near greenhouses is no longer common.

Lathan and Jones^[24] demonstrated that Tospovirus infection in a new marigold crop from an inoculum source decreases to less than 5% at only 15 meters. Similarly, Wilson^[11] proved that TSWV infestation decreases from more than 20% to less than 5% in plants in lettuce crop, in only 100 m and downwind from the edge with weeds infested with vector thrips. According to these results, despite the high vagility of thrips, their dispersal, and therefore that of the virus they transmit, requires the contiguous and relatively close presence of favorable hosts. This could explain the low dominance of the main vector (*F. occidentalis*) in spontaneous vegetation plants during the present study, unlike previous studies in the horticultural belt of La Plata^[13,14].

Table 5. Proposal for the seasonal scheme of survey and management of high-risk weeds as a reservoir of Thripidae

Vectors of TSWV in CH Platense										
Season	ison Winter–Spring Summer			Autun	n					
Month	7	8	9	10	10 11 12 1 2			4	5	
Stages of crop management	Seedbed/Vegetative		Production: Harvest					Post-H	arvest/Senescence	
Continuous surveillance (CV)	(B.r. + R.s. + R.r.) = 3sp.			(B.r. + R.s. + R.r.) = 3 sp.					(B.r. +	R.s. + R.r.) = 3 sp.
Seasonal species (SS)	(E.p.) = 1 sp.			(E.p. + C.a. + M.c. + L.t. + G.o.) = 5 sp.					(C.a. +	M.a.) = 2 sp.
Seasonal management (SM) = (VC) + (SS)	4 sp.			8 sp.					5 sp.	

References for plant species:

Br: Brassica rapa; Ca: Carduus acanthoides; Ep: Echium plantagineum; Go: Galega officinalis; Lt: Lotus tenuis; Mc: Matricaria chamomilla; Ma: Melilotus albus; Rs: Raphanus sativus; Rr: Raphistrum rugosum

The usual strategy for the management of this and other persistent viruses is disease resistance^[3]. Cultural management is usually neglected when the producer has varieties with this quality; consequently, this strategy as an isolated practice is risky. In this regard, surveys in the Platense area during 2008-2009 showed that changes in TSWV were generated, since the resistance of peppers grown in the area was broken^[3]. The epidemics recorded from 2006 to 2009 in the horticultural belt of La Plata were not only the result of these changes in the virus, but also due to the drought that favored the increase in thrips populations, even in other crops in which it is a sporadic pest, such as soybean^[25]. Therefore, the management of this disease requires the implementation of strategies related to vector management, both in the crop and in the spontaneous vegetation.

Mitidieri mentioned a series of sanitary management practices for the horticultural area of San Pedro that involve the crop, stubble and weeds, in order to reduce the impact produced by these viruses and by the populations of thrips migrating from the outdoor vegetation to the greenhouse crop of the cultivated plots. This author agrees with Pearsall^[26] that such control on spontaneous vegetation should be carried out before flowering, since the removal of plants that have already reached this stage produces the opposite effect, stimulating a massive migration of adults into the crop.

In the area of the horticultural belt of La Plata, the management of spontaneous vegetation is currently based on the elimination of spontaneous vegetation without discrimination between species. This implies periodic removal by cutting at a low height (landscaping) in a strip of a few meters around the greenhouses; beyond this limit, the vegetation is left to grow freely within the property^[3]. Although removal by mowing is effective and it would be advisable to maintain it, it is possible to add the control of reproductive hosts for the uncultivated area within the boundaries of the property, using the categorization into risk groups proposed here.

The rational management of a sanitary problem requires, in the first place, an objective and reasonable way in economic terms to assess and survey the pest population in the plant species subject to such management; in this case, the non-cultivated hosts. It would not be possible to express in a simple number the necessary simultaneous survey of the 4 vector species on several plant species. However, it would be possible to monitor, as an alarm system, the feeding and reproductive activity on flowers of species that were rated as high risk and express it cumulatively, in a single measurement.

The sampling of thrips in flowers of weed species with barely detectable densities for these insects implies processing dozens of samples with almost null results, without being able to extract valuable information. Therefore, the proposed management is focused on a small group of species: nine in total (eight at most during the summer season, with greater specific richness of weeds in flower). In addition to being quicker and simpler than considering the monitoring of all flowering vegetation, it implies less expenditure of inputs (in herbicides, weeding or other means for subsequent measures). Finally, this means surveying the density of adults and larvae in four plant species in winterspring, or in five species in autumn and in eight species in summer (Table 5: last row, Seasonal Management).

The suggested consist of measures pre-flowering suppression (in pre-flowering or bud stage) of the selected weed species by season and in the vegetation patches where they predominate. Such measures would be complementary to those currently carried out, of low cut in the vegetated surface adjacent to the cultivated areas. Regarding the technical possibility of its application under cultivated conditions, it could be carried out in patches, in patches of high infestation for these plant species. Regarding the possibility of its implementation, the horticultural producer is accustomed to an artisanal management of crops since they require almost individual monitoring, very different from extensive crops, so there would be no inconvenience for its application.

For this purpose, it would be necessary to train those responsible for sanitary management in the farms regarding which plant species and at what time of the year to take the necessary measures. One of the fundamental aspects to consider in such training is that the frequency of application cannot be determined in advance, since it depends on the results obtained from the monitoring of the phenology of the plants.

Finally, this way of categorizing Reproductive Hosts (RH) in relative risk groups presents the advantage of being plausible for future modifications, since their membership could change if the consideration as vectors of certain thrips species changes (as mentioned for *T. tabaci*) as well as by the eventual entry of other Tospoviruses or even other vectors in the area. One aspect that could be considered in the future could be, for example, the estimation of the infestation (coverage) of high-risk plant species, in order to economically value the application of measures for their control.

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Conflict of interest

The authors declare that they have no conflict of interest.

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