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Article

Solanum elaeagnifolium and *S. rostratum* as Potential Hosts of the Tomato Brown Rugose Fruit Virus

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Abstract: Invasive weeds cause significant crop yield and economic losses in agriculture. The highest indirect impact may be attributed to the role of invasive weeds as virus reservoirs especially within the commercial growing areas. The new tobamovirus tomato brown rugose fruit virus (ToBRFV), recently identified in the Middle-East, overcomes the *Tm-2*^{2} resistance allele in the cultivated tomato varieties grown in greenhouses. In this study, we determined the role of invasive weed species as potential hosts for ToBRFV and pepino mosaic virus (PepMV). Out of all tested weed species, the invasive species Solanum elaeagnifolium and S. rostratum, mechanically inoculated with ToBRFV, were positive for ToBRFV in both enzyme linked immunosorbent assay (ELISA) and RT-PCR tests. S. rostratum was also positive for PepMV. No conspicuous phenotype was observed on ToBRFV infected S. elaeagnifolium plants suggesting a hostplant associated defense response. S. rostratum plants inoculated with either ToBRFV alone or a mixture of ToBRFV and PepMV-IL, contained high ToBRFV levels. In addition, when inoculated with ToBRFV or PepMV-IL disease symptom manifestations were observed in S. rostratum plants and the symptoms were exacerbated upon mixed infections with both viruses. The distribution and abundance of both Solanaceae species increase the risks of virus transmission between species.

Keywords: pepino mosaic virus (PepMV); *Solanum elaeagnifolium; S. rostratum;* tomato brown rugose fruit virus (ToBRFV); virus reservoir; weed management

1. Introduction

Weeds cause significant crop yield and economic losses in agriculture. Worldwide, the potential loss in overall yields of our major crops due to weeds (34%, on average) is higher compared to other crop pests, including insects, pathogens, viruses, and animal pests [1]. Among weeds, invasive species present major economic and ecological threats to agriculture and natural areas. In recent decades, we have experienced a rise in the reports of invasive weed species due to a significant manmade global change. Among the leading causes for this trend are import-export trades [2,3] and climate changes [4,5]. In the United States alone, annual losses caused only by crop-related invasive weeds were estimated to be more than $27 billion dollars [6]. In Israel, several invasive species such as *Parthenium hysterophorus*, *Solanaceae* spp., *Ambrosia confertiflora* and *Amaranthus* spp. have been documented. Apparently, the invasion route of these weeds is via imported animal feed shipments [7]. The damages of invasive weeds are not restricted to yield losses, but could be associated with increased spread of fire-fuel [8–10] and high allergenic effects [11–13]. However, although it may be underestimated, the highest indirect impact may be attributed to the role of invasive weeds as virus reservoirs especially within the commercial growing areas.

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The new tobamovirus tomato brown rugose fruit virus (ToBRFV), recently identified in the Middle East [14,15], overcomes the *Tm-2²* resistance allele in the cultivated tomato varieties grown trellised in greenhouses [14]. Subsequently, outbreaks of the ToBRFV disease were reported in North America [16,17], Germany [18], Turkey [19], Greece [20], Spain, Netherland and China [21]. ToBRFV causes a range of symptoms in tomato varieties [22]. Fruit yellowing and bleaching are the most commonly occurring symptoms, often accompanied by a necrotic peduncle [14,23]. In addition, a worldwide spread of the mechanically transmitted potexvirus, pepino mosaic virus (PepMV), has occurred as well [24–27]. PepMV could cause fruit necrosis and plant wilting [28,29]. These two viruses (ToBRFV and PepMV) most profoundly affect the yield and quality of tomato plants.

Plant manipulations such as planting and fruit picking, as well as pruning and trellising, which are essential for tomato plant cultivation, are the practices predisposing the plants for disease spread of mechanically transmitted viruses. Regarding PepMV, even direct contact between healthy and infected plants could spread the disease [30]. Interestingly, beneficial insects are also implicated in transmission of ToBRFV, which could occur through mechanical adsorption of the viruses to the insects [31,32]. Most importantly, the potexvirus PepMV and tobamoviruses in general are seed-borne viruses [24,33].

The emerging spread of ToBRFV in worldwide tomato production could have been accelerated by traded tomato fruits contributing to the disease spread across continents and countries [34].

Several weeds have been previously identified as potential reservoirs of plant viruses. In a study conducted on 98 weed species in the state of New York, 17 species were identified as hosts for iris yellow spot virus (IYSV), potato leafroll virus (PLRV) and potato virus Y (PVY) [35]. Various studies have shown that within identified host weed species, there was a high proportion of invasive weeds. *P. hysterophorus*, a prominent invasive wide spread weed species, was infected with viral genes with high nucleotide sequence identity with several tested viruses including cherry tomato leaf curl virus (CToLCV) and tobacco curly shoot alphasatellite (TbCSA) [36]. In Turkey, *A. retroflexus* appeared to be a common host of several viruses such as cucumber mosaic virus (CMV), PVY, tomato spotted wilt orthotospovirus (TSWV) and more [37]. *Ventenata dubia*, an invasive weed species infesting grasslands, rangelands and pastures throughout the USA was susceptible to barley or cereal yellow dwarf virus (B/CYDV) infection and served as a transient agent for crop infection [38]. Recently, Shargil et al [39] have documented new invasive weed species as optional hosts for the tobamovirus cucumber green mottle mosaic virus (CGMMV) in Israel. *A. graecizans* and *A. muricatus*, although a-symptomatic, tested positive for CGMMV, whereas the invasive weed *Datura stramonium* was CGMMV positive only in the inoculated leaves [39].

The main goal of the current study was to determine the potential of invasive weeds as virus hosts for ToBRFV and PepMV. Invasive weeds common within the commercial tomato growing areas (e.g. in greenhouses and open fields) were tested. Understanding ToBRFV and PepMV potential hosts in weeds throughout the production cycles of tomato crops during the sequential growing seasons provides additional information that may contribute to more efficient disease management via weed control approaches.

2. Results and discussion

2.1. Susceptibility of various weed species to ToBRFV and PepMV infections.

A wide variety of host plants for viruses detrimental to vegetable crops could have a major role in interference with the various measures pursued to control viral disease primary sources. Weeds growing adjacent to crops could serve as reservoirs of various disease-causing agents counteracting measures of crop rotations and quarantines that have been implemented to contain and overcome a disease. During the recent years, the tobamovirus ToBRFV has caused severe disease damages to elite tomato plants, harboring the durable Tm-2² resistance gene [14]. Partial host plant analyses of ToBRFV showed various weed species that belonged to the Solanaceae family such as *S. nigrum*, as well as the

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Amaranthaceae family members such as *Chenopodium murale*, which were serologically positive for the virus, with or without mottling symptoms [14,40].

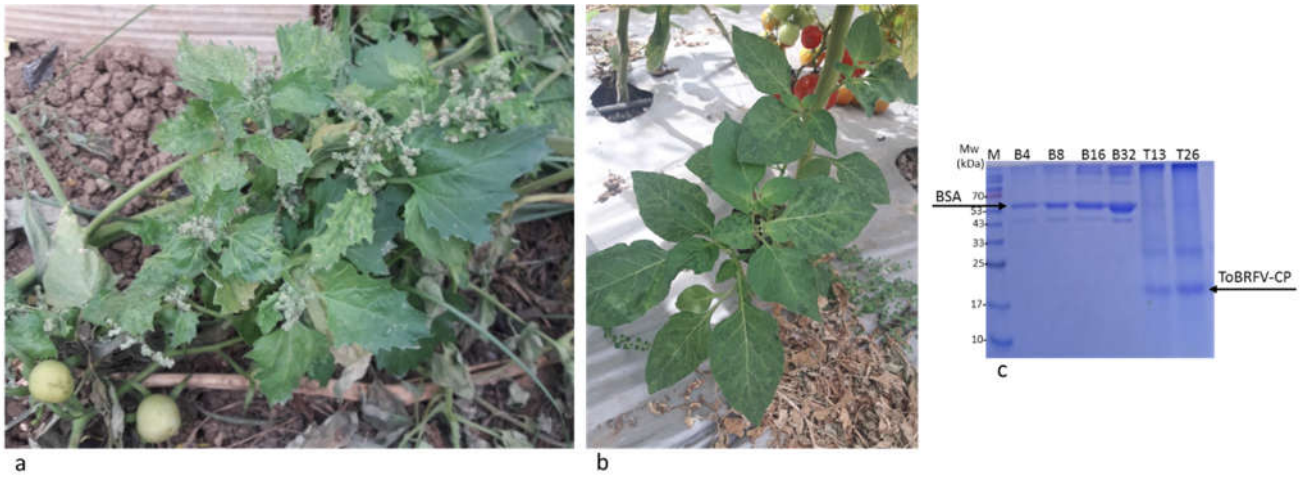

Figure 1. Natural occurrence of weeds adjacent to tomato plants in a commercial tomato greenhouse. (**a**) ToBRFV infected *Chenopodium murale*. (**b**) ToBRFV infected *Solanum nigrum*. (**c**) Quantification of ToBRFV inoculum by SDS-PAGE using a BSA reference followed by coomassie blue staining. M, molecular size marker; B, bovine serum albumin (BSA); T, ToBRFV; B4-B32 are μg of BSA; T13 and T26 are μl of 1.4 times diluted ToBRFV inoculum.

In our study, we have attempted to widen the scope of weed species susceptible to ToBRFV infection and included two endemic weeds and eight major invasive weeds of the Israeli flora in our tests. Out of all tested weed species, two invasive *Solanaceae* species were identified as possible hosts of ToBRFV. *S. elaeagnifolium* and *S. rostratum*, mechanically inoculated with ToBRFV, were positive for ToBRFV in both enzyme linked immunosorbent assay (ELISA) and RT-PCR tests (Table 1).

Table 1. Susceptibility of various invasive weed species to ToBRFV and PepMV infections. Diagnostics was carried out using enzyme linked immunosorbent assay (ELISA) followed by RT-PCR.

Species	Family	Invasive/ native	Infected plant part	ELISA		RT-PCR	
				ToBRFV	PepMV	ToBRFV	PepMV
Amaranthus blitoides	Amaranthaceae	invasive	leaves	-	-	-	-
Amaranthus retroflexus	Amaranthaceae	invasive	leaves	-	-	-	-
			inflorescence	-	-	x	x
Digitaria sanguinalis	Poaceaa	native	leaves	-	-	-	-
Solanum nigrum	Solanaceae		leaves	+	x	+	x
Conyza bonariensis	Asteraceae	invasive	leaves	-	-	-	-
Conyza canadensis	Asteraceae	invasive	leaves	-	-	-	-
Setaria adhaerens	Poaceaa	native	leaves	-	-	-	-
Solanum rostratum	Solanaceae	invasive	leaves	+	+	+	+
Solanum elaeagnifolium	Solanaceae	invasive	leaves	+	-	+	-
Sorghum halepense	Poaceaa	invasive	leaves	-	-	-	-
			inflorescence	-	-	x	x
Xanthium strumarium	Asteraceae	invasive	leaves	-	-	-	-
			fruits	-	-	-	-

+ = Positive results 2.5 fold compared to the negative control.

X = Not tested

- Negative results less than 2.5 fold the compared to the negative control.

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Following the first cycle of mechanical inoculations, the two weed species *S. elaeagnifolium* and *S. rostratum* were re-inoculated with ToBRFV using a larger plant number of 34 and 26 plants, respectively. Apparently, 35% of *S. elaeagnifolium* plants and 88% of *S. rostratum* plants were ToBRFV positive, as tested by ELISA.

Recently a synergism has been documented between the tobamovirus ToBRFV and the mild strain of PepMV-IL potexvirus, which were both found in mixed infections of commercially available tomato fruits and in elite tomato crops [34,41]. The new severe disease symptoms, conspicuously observed during the wintertime in Israel, were characteristic of an aggressive PepMV strain although only the mild PepMV-IL was present in the mixed infected plants [41]. The synergism between ToBRFV and PepMV-IL was manifested in accelerated levels of PepMV-IL in the presence of ToBRFV, when compared to PepMV-IL levels in PepMV-IL singly infected tomato plants. This synergism has been reproduced in a glass house grown crop, kept at 24±3°C growing temperature conditions [41]. We have therefore asked whether the tested weeds could host the mild PepMV-IL that could initiate synergism with the abundant ToBRFV. We have found that only S. rostratum, mechanically inoculated with either ToBRFV or PepMV-IL, tested positive for both ToBRFV and PepMV-IL using ELISA and RT-PCR tests (Table 1). None of the other analyzed weeds tested positive for PepMV-IL alone suggesting that those weeds could not be the source for PepMV-IL and the consequential synergism with ToBRFV in tomatoes in Israel [41].

2.2. S. elaeagnifolium as a host for plant viruses.

S. elaeagnifolium is a deep-rooted perennial weed species native to the Western plains of the United States and Mexico [42]. According to a literature documentation by A. Dafni, the initial introduction of this species into Israel had occurred in 1956 through Egypt [43]. Today, this weed has spread intensely across the country and inhabited extensive areas and multiple habitats. Due to its high abundance across the country, we may assume that since the first introduction in 1950s multiple introductions have occurred. Propagation of this weed occurs via seeds, creeping rhizomes or root fragments [44]. Distribution into new habitats may include the transfer of commercial seed and plant material harvested from infested fields. Root fragments retain high sprouting ability and could be extended up to 2 m from the parent plant [45]. In addition, dried plants could break off and spread with the wind their attached berries similar to tumbleweed seed dispersal [43]. In Israel, *S. elaeagnifolium* infests agricultural and non-agricultural habitats including field crops, roadsides and waste grounds. We have therefore analyzed the risk of *S. elaeagnifolium* to serve as a potential host of ToBRFV.

In order to test the infectious potential of ToBRFV in infected *S. elaeagnifolium* plants we have first established the presence of the virus in the plants using RT-qPCR and western blot assays that quantitate the virus. We have found that indeed ToBRFV was detected in the inoculated plants but the virus titer was very low, compared to tomato plants (Fig. 2a, b). Importantly, in a bioassay performed on *N. glutinosa* plants with *S. elaeagnifolium*, inoculated either with ToBRFV alone or with a mixture of ToBRFV and PepMV, the test plants were infected in two out of three plants and one out of three plants, respectively. These results indeed confirm that although low in ToBRFV titer, *S. elaeagnifolium* plants infected with the virus could serve as a primary infection source. The low ToBRFV titer could be related to the genetic background of *S. elaeagnifolium* that has close genetic proximity to S. melongena (eggplants) [46], which were not susceptible to ToBRFV infections [14,40]. Interestingly, no conspicuous phenotype was observed on ToBRFV infected *S. elaeagnifolium* plants suggesting that hostplant associated defense response, specific to *S. elaeagnifolium*, has determined the phenotype preservation and the low ToBRFV levels (Fig. 1c-e). Solanaceae family members' characteristic defense response could be more effective under conditions of low ToBRFV systemic infections, determined by host susceptibility to ToBRFV replication or movement. Importantly, *S. elaeagnifolium* plant could not host the potexvirus PepMV-IL using both a single inoculum source of the virus and an

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inoculum source extracted from ToBRFV and PepMV-IL mixed infected tomato plants, in which a synergism between the co-infecting viruses accelerated PepMV-IL levels (Fig. 2a, b; [41]). The unique response of *S. elaeagnifolium* to inoculations with ToBRFV and/or PepMV-IL could indicate that the plant unique defense response determines the resistance towards ToBRFV induced disease manifestations and it is worth further studying as a tool for the development of virus-resistant crop varieties.

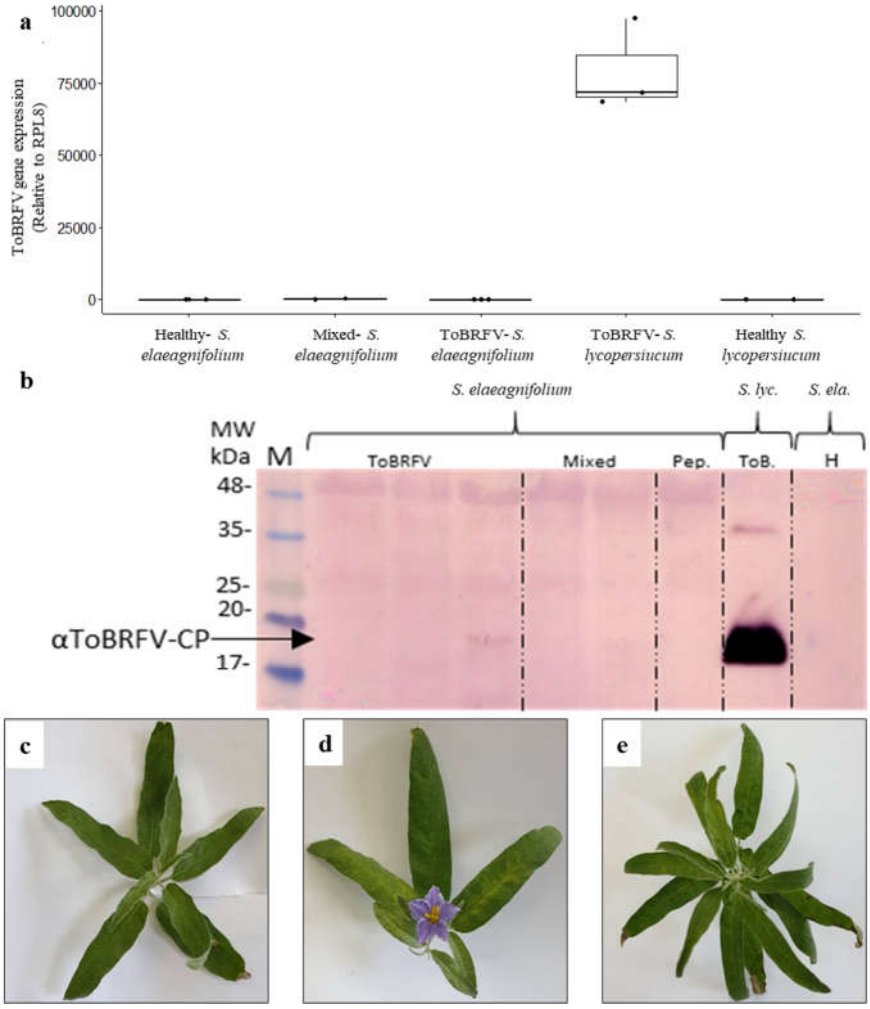

Figure 2. *S. elaeagnifolium* is a new host of ToBRFV, keeping a low virus titer. Graphical depiction of ToBRFV relative gene expression ratios in comparison to healthy controls (using the RPL8 endogenous gene). 2^-ΔΔCt was calculated from quantitative RT-PCR results (**a**). Western blot analyses showing high ToBRFV-CP levels in singly inoculated ToBRFV tomato plants compared to single and mixed-inoculated *S. elaeagnifolium* plants (ToBRFV n=5, PepMV n=2, Mixed n=5). M, molecular size marker; arrows indicate the ToBRFV-CP; H, healthy control (**b**). Depiction of *S. elaeagnifolium* plants (**c**, healthy controls) that were a-symptomatic at 30 dpi following inoculation either with ToBRFV alone (**d**) or a mixture of ToBRFV and PepMV (**e**).

Several studies have indicated *S. elaeagnifolium* potential as a host of crop plant pests. In a study aimed to identify potential sources of infection of both tomato plants and *S. elaeagnifolium* by tomato yellow leaf curl virus (TYLCV) and tomato yellow leaf curl Sardinia virus (TYLCSV), *S. elaeagnifolium* was identified as a natural host of the two viruses [47]. S. elaeagnifolium plants collected from pepper (*Capsicum annuum*) fields were infected and identified as hosts for pepper mottle virus (PepMoV) as well [48].

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2.3. S. rostratum response to plant viruses.

Unlike *S. elaeagnifolium*, *S. rostratum* plants inoculated with either ToBRFV alone or a mixture of ToBRFV and PepMV-IL, contained high ToBRFV levels when the inoculum source was *S. lycopersicum* plants (Fig. 3a). In a biological assay testing the infectious potential of ToBRFV in *S. rostratum* plants by inoculating either new un-infected *S. rostratum* plants (Fig. 2a) or un-infected *S. lycopersicum* plants (Fig. 3b), both test plants were infected. Nevertheless, the tomato inoculum source has constantly caused higher ToBRFV titer in either one of the inoculated plants (Fig. 3a, b). Importantly, similar to *S. lycopersicum* plants, *S. rostratum* plants were infected by PepMV-IL when either singly inoculated or in a mixture with ToBRFV and the plants showed synergism between the viruses manifested in increased PepMV-IL levels under mixed infection conditions (Fig. 3c, d). Manifestation of the synergism was also observed in symptom development showing yellowing and mottling on serrated leaves and leafroll (Fig. 3e-g). Severe disease symptom manifestations associated with ToBRFV and PepMV-IL mixed infections, resembling elite tomato plant response to the mixed infections, could serve as a warning sign designating a disease area detrimental for re-growing tomato crops. The similarities between *S. rostratum* and *S. lycopersicum* regarding ToBRFV and PepMV-IL infections could be an additional indication of genetic similarities between these two *Solanaceae* species with a possible common ancestor as previously assessed [49,50].

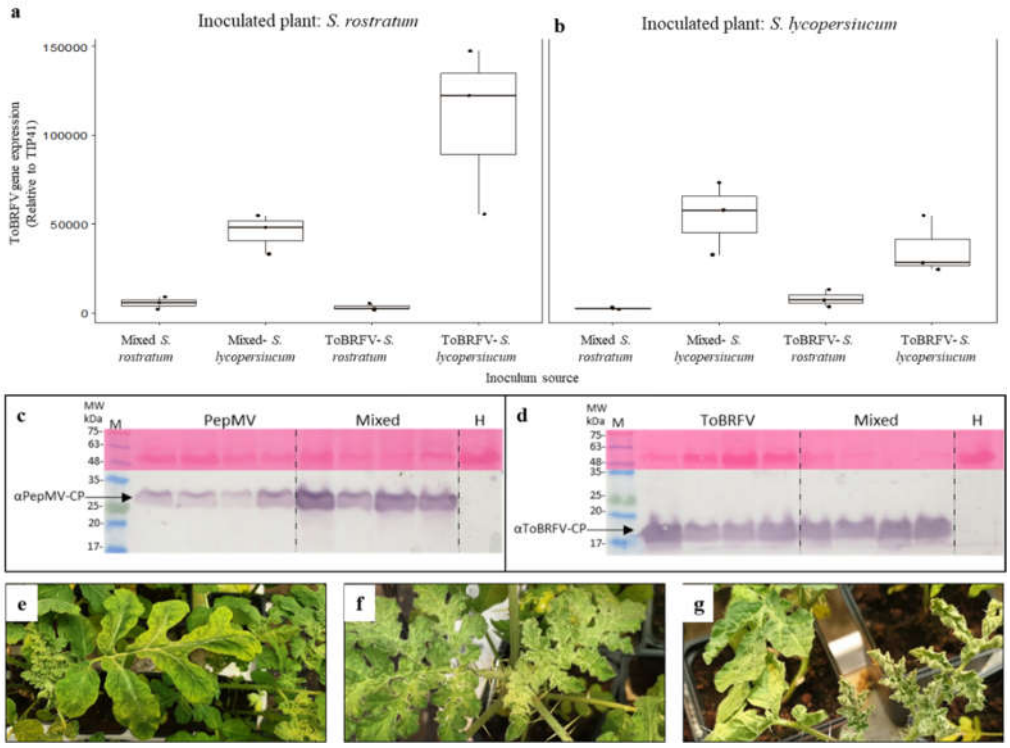

Figure 2. S. rostratum is a new host of ToBRFV and PepMV in a single and mixed infections. Graphical depictions of ToBRFV relative gene expression ratios in comparison to healthy controls (with the TIP41 endogenous gene). 2^-ΔΔCt was calculated from quantitative RT-PCR results showed that both inoculum sources, *S. rostratum* (**a**) and *S. lycopersicum* (**b**), were infectious. Western blot analyses showing CP levels of PepMV (**c**) ToBRFV (**d**) in singly and mixed-inoculated *S. rostratum* plants in comparison with (H), a healthy control. Symptomatic *S. rostratum* plants inoculated with PepMV alone (**e**), ToBRFV alone (**f**) or with a mixture of ToBRFV and PepMV showing leafroll and serrated leaves (**g**).

S. rostratum is a noxious weed as it grows aggressively following habitat disturbance [51], livestock is discouraged from grazing on vegetation where it grows as thorns cover all the plant except the flowers [52]. This species is a native species of the Mexican highlands [53], and has invaded several different regions across the world including Canada,

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China, Russia, Australia, and Europe [51]. In contrast to *S. elaeagnifolium* this species reproduces only via seeds as dried berries open up and spread their seeds. However, similar to *S. elaeagnifolium*, plants may also break and move across the land as tumbleweeds improving seed distribution [53]. In Israel, *S. rostratum* was first documented at the Jezreel valley in 1953, since then several field populations have been located in the Jordan valley, the Hulla valley and at the Mediterranean Sea coast line [54]. S. rostratum can be found mainly within field and at field margins of crops, such as watermelon (*Citrullus lanatus* Thunb.), onion (*Allium cepa* L.), and tomato (*S. lycopersicum* L.).

Although the infectious potential of ToBRFV in *S. rostratum* was lower than that of ToBRFV in *S. lycopersicum* (Fig. 3a, b), an ongoing evolutionary process is possible when further viral host jumps may accelerate the spread and the damage of the virus in the future via *S. rostratum*. In order to confirm the preservation and infectious potential of ToBRFV in S. rostratum, we have planted in 100 L pots four ToBRFV infected plants for flowering and seed development under natural environmental conditions. New plants grown from the germinated seeds were subjected to virus identification. One out of the five mature plants was ToBRFV positive in ELISA test showing optical density (O.D.) values of 4.7 times the negative refrence with no visual symptomes. Further research is needed to understand the potential of transgenerational transmission of ToBRFV in S. rostratum.

3. Materials and Methods

3.1. Plant material and virus source for the inoculation experiments.

Seeds of various weed species collected during 2018-2019 in agricultural fields or field margins were tested for susceptibility to ToBRFV and PepMV infection. Tested species were; *A. blitoides, A. retroflexus, Digitaria sanguinalis, Conyza canadensis, C. bonariensis, Setaria adhaerens, S. rostratum, S. elaeagnifolium, Sorghum halepense, Xanthium strumarium*. Seeds were germinated in 500ml pots filled with commercial potting media (Tuff, Marom Golan, Israel) including Osmocote® slow release fertilizer. Seedlings were grown in a greenhouse under natural growing conditions for the spring season at Newe Yaar Research Center. At a three to four leaf stage, seedlings were transferred into plastic pots 0.3L containing one plant per pot.

ToBRFV and PepMV were extracted from infected tomato plants. To ensure single inoculations of either ToBRFV or PepMV ToBRFV was isolated on *Nicotiana tabacum* cv. Samsun, systemically infected by ToBRFV only (Oded Lachman, personal communication). PepMV was isolated on *D. stramonium* plants, systemically infected by PepMV and developed necrotic local lesions towards ToBRFV [14,55]. Cultures of each virus were propagated continuously on tomato plants cv. Ikram (heterozygote for the *Tm-2²* resistance allele), serving as a source of inoculum. The inoculum was prepared by grinding virus infected tomato leaves ~1 g/25 ml 0.01M sodium phosphate buffer pH=7.0. For estimating viral content viral proteins were extracted with urea-SDS-β-mercaptoethanol (USB) buffer using X1.4 dilution factor and proteins were run on SDS-PAGE adjacent to a bovine serum albumin (BSA) control (Figure 1c). Coomassie staining allowed estimation of viral CP in the inoculum, which was ~0.4 mg/ ml sap inoculum. The inoculation was performed mechanically by rubbing the ToBRFV or PepMV sap extract on the tested weeds 5-10 plants from each weed species. Plants were kept in a 24°C±3°C growth chamber. At the thirty days post inoculation (DPI) leaf samples were collected for virus diagnostics first by ELISA followed by RT-PCR.

3.2. Viral inoculations for quantitative RT-PCR and bioassays.

S. elaeagnifolium, S. rostratum and *S. lycopersicum* (tomato) plants were grown in growth chambers in a glass-house under controlled temperature conditions of 24°C±3°C. The tobamovirus tomato brown rugose fruit virus (ToBRFV) was mechanically inoculated onto the third leaf above the cotyledon. In parallel, a mixture of ToBRFV and the mild

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potexvirus pepino mosaic virus (PepMV-IL) that have shown synergism under mixed infection conditions, observed in tomato plants in Israel [34,41], were also inoculated onto the tested plants. PepMV-IL single inoculations were carried out for quantifying the increase in PepMV-IL under mixed infection conditions. At 30 days post inoculations bioassays of infected plants were carried out by using two inoculum sources: ToBRFV singly infected and ToBRFV and PepMV-IL mixed infected plants. For bioassays using *N. glutinosa*, equal ratios of inoculation buffer per leaf weight (6ml/g) of *S. elaegnifolium* served for inoculations. Systemic ToBRFV infection showing mild mottling were tested using ELISA [14]. Bioassays for *S. rostratum* singly infected with ToBRFV as well as mixed infected with ToBRFV and PepMV were also carried out by inoculating new wild *S. rostratum* plants and the cultivated tomato plants.

3.3. Serological tests for viral infections.

ELISA and western blot analyses were carried out as previously described [34]. For western blot analyses, the leaf samples were comparable by keeping constant ratios of USB lysis buffer and leaf weights. Accordingly, the increase in PepMV-IL in ToBRFV and PepMV-IL mixed infected plants was a quantitative comparison with PepMV-IL singly infected plants.

3.4. Quantitative RT-PCR (RT-qPCR).

Leaves from ToBRFV-, PepMV- or mixed-infected *S. elaeagnifolium* and *S. rostratum* and tomato plants (50–100 mg) were subjected to total RNA extraction using a TRI Reagent kit (MRC, Inc., Cincinnati, OH, USA). RNA concentrations were measured by a spectrophotometer NanoDrop ND1000 (Thermo Scientific, Wilmington, DE, USA). cDNA synthesis was performed on 1 μg of total RNA using a Verso™ cDNA Kit (Thermo Fisher Scientific, Epsom, UK) with the oligo (dT) primer (10 pmol/μL). RT-qPCR was performed using the power SYBR Green PCR master MIX (Applied Biosystems, Thermo Fisher scientific, Vilnius, Lithuania) and running was performed using the StepOnePlusTM (Applied Biosystems, Fisher Scientific Company, Ottawa, Ontario). The endogenous gene TIP41 served as a reference gene for *S. elaeagnifolium* and *S. lycopersicum* [41] and RPL8 served as a reference gene for *S. elaeagnifolium* and *S. rostratum* [56] and were analyzed with each tested batch of viruses. Primers for the 2 reference genes TIP41 and RPL8, and the two target genes—ToBRFV-CP and PepMV-CP—were designed with Primer3 Plus software. The primer set for ToBRFV-CP was F 5′ CACAATCGCAACTCCATCGC 3′ and R 5′ CAGGTGCAGAGGACCATTGT 3′, amplicon size of 159 bp;;for TIP41 was F 5′ ATGGAGTTTTTGAGTCTTCTGC 3′ and R 5′ GCTGCGTTTCTGGCTTAGG 3′ ,amplicon size of 235 bp and for RPL8 was F 5' CAAATCCCACACCCACCACC 3' and R 5' GCAACACATTACCAACCATAAGACTAGC 3', amplicon size of 260 bp. The amplification of the tested viruses was performed in duplicates with the specific primers. Each sample was analyzed against the TIP41 endogenous gene (*S. elaeagnifolium* and *S. lycopersicum*) and RPL8 (*S. elaeagnifolium* and *S. rostratum*). Each reaction contained 100 ng cDNA (cDNA reverse transcribed from 100 ng RNA) in a 15 μL reaction mixture containing 4 μL of diluted cDNA, 3 pmols of each primer and 7.5 μL Absolute QPCR Sybr Green Mix (Thermo Fisher Scientific, Vilnius, Lithuania). Reaction conditions were: 10 min at 95 °C ("hot start") followed by 40 cycles of 3 sec at 94 °C, 15 sec at 60 °C, and 20 sec at 72 °C. The quantitative analysis was performed using the StepOnePlusTM bio system (Applied Biosystems, Fisher Scientific Company, Ottawa, Ontario). The percent amplification efficiency of each of the analyzed samples equaled: 1%. ΔCt, obtained by subtracting Ct of the endogenous gene from Ct of the tested virus, was calculated for each tested virus in all analyzed samples. ΔΔCt was calculated by subtracting mean ΔCt of each virus in the healthy control samples from each ΔCt of the respective infected samples. ΔΔCt of each infected sample served for calculation of $2^{-\Delta\Delta Ct}$ for estimation of relative gene expression in the infected samples relative to the respective healthy control samples. The mean

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$2^{-\Delta\Delta Ct}$ ± the standard deviation of the mean (s.d.) data for each gene in the various tested samples were analyzed and visualized using R software [57].

4. Conclusions

Several studies have indicated *S. elaeagnifolium* potential as a host of crop plant pests. However, to the best of our knowledge, this is the first report on *S. rostratum* as a host for tobamoviruses and potexviruses and most importantly the synergism between ToBRFV and PepMV-IL in weed plants. The distribution and abundance of both *Solanaceae* species within and in close proximity to agricultural fields in general, and specifically to tomato fields, increase the risks of virus transmission between species. Due to the fact that tomatoes constitute an important crop, developing weed management tools in order to prevent further buildup of *S. elaeagnifolium* and *S. rostratum* populations is highly important.

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References

1. Oerke, E.-C. Crop losses to pests. *J. Agric. Sci.* **2006**, *144*, 31–43.
2. Levine, J.M.; Vilà, M.; D'Antonio, C.M.; Dukes, J.S.; Grigulis, K.; Lavorel, S. Mechanisms underlying the impacts of exotic plant invasions. *Proc. R. Soc. B Biol. Sci.* **2003**, *270*, 775–781.
3. Shimono, Y.; Takiguchi, Y.; Konuma, A. Contamination of internationally traded wheat by herbicide-resistant *Lolium rigidum*. *Weed Biol. Manag.* **2010**, *10*, 219–228.
4. Hulme, P.E. Climate change and biological invasions: Evidence, expectations, and response options. *Biol. Rev.* **2016**, *1299*, 1297–1313.
5. Peters, K.; Breitsameter, L.; Gerowitt, B. Impact of climate change on weeds in agriculture: A review. *Agron. Sustain. Dev.* **2014**, *34*, 707–721.
6. Pimentel, D.; Zuniga, R.; Morrison, D. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* **2005**, *52*, 273–288.
7. Rubin, B.; Matzrafi, M. Weed Management in Israel-Challenges and Approaches. In *Weed Science in the Asian-Pacific Region*; **2015**; pp. 253–270.
8. Setterfield, S.A.; Rossiter-Rachor, N.A.; Douglas, M.M.; Wainger, L.; Petty, A.M.; Barrow, P.; Shepherd, I.J.; Ferdinands, K.B. Adding fuel to the fire: The impacts of non-native grass invasion on fire management at a regional scale. *PLoS One* **2013**, *8*, e59144.
9. Büyüktahtakin, İ.E.; Feng, Z.; Olsson, A.D.; Frisvold, G.; Szidarovszky, F. Invasive species control optimization as a dynamic spatial process: An application to buffelgrass (*Pennisetum ciliare*) in Arizona. *Invasive Plant Sci. Manag.* **2014**, *7*, 132–146.
10. Mound, L.A. *Octothrips lygodii* sp. n. (Thysanoptera:Thripidae) damaging weedy *Lygodium* ferns in south-eastern Asia, with notes on other Thripidae reported from ferns. *Aust. J. Entomol.* **2002**, *41*, 216–220.
11. Hamaoui-Laguel, L.; Vautard, R.; Liu, L.; Solmon, F.; Viovy, N.; Khvorostyanov, D.; Essl, F.; Chuine, I.; Colette, A.; Semenov, M.A.; et al. Effects of climate change and seed dispersal on airborne ragweed pollen loads in Europe. *Nat. Clim. Chang.* **2015**, *5*, 766–771.
12. Kaur, M.; Aggarwal, N.K.; Kumar, V.; Dhiman, R. Effects and management of *Parthenium hysterophorus* : A weed of global significance. *Int. Sch. Res. Not.* **2014**, 12.
13. Wopfner, N.; Gadermaier, G.; Egger, M.; Asero, R.; Ebner, C.; Jahn-Schmid, B.; Ferreira, F. The spectrum of allergens in ragweed and mugwort pollen. *Int. Arch. Allergy Immunol.* **2005**, *138*, 337–346.
14. Luria, N.; Smith, E.; Reingold, V.; Bekelman, I.; Lapidot, M.; Levin, I.; Elad, N.; Tam, Y.; Sela, N.; Abu-Ras, A.; et al. A new israeli *Tobamovirus* isolate infects tomato plants harboring *Tm-2²* resistance genes. *PLoS One* **2017**, *12*, e0170429.
15. Salem, N.; Mansour, A.; Ciuffo, M.; Falk, B.W.; Turina, M. A new tobamovirus infecting tomato crops in Jordan. *Arch. Virol.* **2016**, *161*, 503–506.
16. Ling, K.S., Tian, T., Gurung, S., Salati, R., and Gilliard, A. First report of *tomato brown rugose fruit virus* infecting greenhouse tomato in the United States. *Plant Dis.* **2019**, *103*, 1439.
17. Cambrón-Crisantos, J.M.; Johan Rodríguez-Mendoza; Jessica Berenice Valencia-Luna; Alcasio-Rangel, S.; García-Ávila, C.D.J.; López-Buenfil, J.A.; Ochoa-Martínez, D.L.; Cambrón-Crisantos, J.M.; Rodríguez-Mendoza, J.; Valencia-Luna, J.B.; et al. Primer reporte de *Tomato brown rugose fruit virus* (ToBRFV) en Michoacán, México. *Mex. J. Phytopathol.* **2018**, *37*, 185–192.

18. Menzel, W.; Knierim, D.; Winter, S.; Hamacher, J.; Heupel, M. First report of *Tomato brown rugose fruit virus* infecting tomato in Germany . *New Dis. Reports* **2019**, *39*, 1.
19. Fidan, H.; Sarikaya, P.; Calis, O. First report of *Tomato brown rugose fruit virus* on tomato in Turkey . *New Dis. Reports* **2019**, *39*, 18.
20. Beris, D.; Malandraki, I.; Kektsidou, O.; Theologidis, I.; Vassilakos, N.; Varveri, C. First report of *tomato brown rugose fruit virus* infecting tomato in Greece. *Plant Dis.* **2020**, *104*, 2035.
21. Yan, Z.; Ma, H.; Han, S.; Geng, C.; Tian, Y.; Li, X. First report of *Tomato brown rugose fruit virus* infecting tomato in China. *Plant Dis.* **2019**, 2973.
22. EPPO, Pest Risk Analysis for Tomato Brown Rugose Fruit Virus. Available online: https://gd.eppo.int/taxon/TOBRFV/documents.
23. Smith, E., and Dombrovsky, A. Aspects in *Tobamovirus* management in intensive agriculture. In *Plant Diseases - Current Threats and Management Trends*. **2019**.
24. Hanssen, I.M.; Mumford, R.; Blystad, D.R.; Cortez, I.; Hasiów-Jaroszewska, B.; Hristova, D.; Pagán, I.; Pereira, A.M.; Peters, J.; Pospieszny, H.; et al. Seed transmission of *Pepino mosaic virus* in tomato. *Eur. J. Plant Pathol.* **2010**, *126*, 145–152.
25. van der Vlugt, R.A.A.; Stijger, C.C.M.M.; Verhoeven, J.T.J.; Lesemann, D.-E. First report of *Pepino mosaic virus* on tomato. *Plant Dis.* **2000**, *84*, 103.
26. Mumford, R.A.; Metcalfe, E.J. The partial sequencing of the genomic RNA of a UK isolate of *Pepino mosaic virus* and the comparison of the coat protein sequence with other isolates from Europe and Peru. *Arch. Virol.* **2001**, *146*, 2455–2460.
27. Verhoeven, J.T.J.; Van Der Vlugt, R.A.A.; Roenhorst, J.W. High similarity between tomato isolates of *Pepino mosaic virus* suggests a common origin. *Eur. J. Plant Pathol.* **2003**, *109*, 419–425.
28. Soler-Aleixandre, S.; López, C.; Díez, M.J.; Pérez De Castro, A.; Nuez, F. Association of *Pepino mosaic virus* with tomato collapse. *J. Phytopathol.* **2005**, *153*, 464–469.
29. Hasiów-Jaroszewska, B.; Borodynko, N. Characterization of the necrosis determinant of the European genotype of *pepino mosaic virus* by site-specific mutagenesis of an infectious cDNA clone. *Arch. Virol.* **2012**, *157*, 337–341.
30. Jones, R.A.C.; Koenig, R.; Lesemann, D.-E. *Pepino mosaic virus*, a new potexvirus from pepino (*Solanum muricatum*). *Ann. Appl. Biol.* **1980**, *94*, 61–68.
31. Levitzky, N.; Smith, E.; Lachman, O.; Luria, N.; Mizrahi, Y.; Bakelman, H.; Sela, N.; Laskar, O.; Milrot, E.; Dombrovsky, A. The bumblebee *Bombus terrestris* carries a primary inoculum of Tomato brown rugose fruit virus contributing to disease spread in tomatoes. *PLoS One* **2019**, *14*, e0210871.
32. Lacasa, A.; Guerrero, M.M.; Hita, I.; Martinez, M.A.; Jordá, C.; Bielza, P.; Contreras, J.; Alcazar, A.; Cano, A. Implication of bumble bees (*Bombus* spp.) on *Pepino mosaic virus* (PepMV) spread on tomato crops. *Plagas* **2003**, *29*, 393–403.
33. Dombrovsky, A.; Smith, E. Seed transmission of *Tobamoviruses*: Aspects of global disease distribution. In *Advances in Seed Biology*. **2017**.
34. Klap, C.; Luria, N.; Smith, E.; Bakelman, E.; Belausov, E.; Laskar, O.; Lachman, O.; Gal-On, A.; Dombrovsky, A. The potential risk of plant-virus disease initiation by infected tomatoes. *Plants* **2020**, *9*, 623.
35. Smith, E.A.; DiTommaso, A.; Fuchs, M.; Shelton, A.M.; Nault, B.A. Abundance of weed hosts as potential sources of onion and potato viruses in western New York. *Crop Prot.* **2012**, *37*, 91–96.
36. Qurashi, F.; Sattar, M.N.; Iqbal, Z.; Haider, M.S. First report of cherry tomato leaf curl virus and associated DNA satellites infesting an invasive weed in Pakistan. *J. Plant Pathol.* **2017**, *99*, 267–272.
37. Arli-Sokmen, M.; Mennan, H.; Sevik, M.A.; Ecevit, O. Occurrence of viruses in field-grown pepper crops and some of their reservoir weed hosts in Samsun, Turkey. *Phytoparasitica* **2005**, *33*, 347–358.
38. Ingwell, L.L.; Bosque-Pérez, N.A. The invasive weed *Ventenata dubia* is a host of *Barley yellow dwarf virus* with implications for an endangered grassland habitat. *Weed Res.* **2015**, *55*, 62–70.
39. Shargil, D.; Smith, E.; Lachman, O.; Reingold, V.; Darzi, E.; Tam, Y.; Dombrovsky, A. New weed hosts for *Cucumber green mottle mosaic virus* in wild Mediterranean vegetation. *Eur. J. Plant Pathol.* **2017**, *148*, 473–480.
40. Chanda, B., Gilliard, A., Jaiswal, N. & Ling, K. S. Comparative Analysis of Host Range, Ability to Infect Tomato Cultivars with Tm-22 Gene, and Real-Time Reverse Transcription PCR Detection of Tomato Brown Rugose Fruit Virus. *Plant Dis.* **105**, 3643–3652 (2021).
41. Klap, C.; Luria, N.; Smith, E.; Hadad, L.; Bakelman, E.; Sela, N.; Belausov, E.; Lachman, O.; Leibman, D.; Dombrovsky, A. *Tomato brown rugose fruit virus* contributes to enhanced pepino mosaic virus titers in tomato plants. *Viruses* **2020**, *12*, 879.
42. D'Arcy, W.. G.. *Solanum* and its close relatives in Florida. *Ann. Missouri Bot. Gard.* **1974**, *61*, 819–867.
43. Boyd, J.W.; Murray, D.S.; Tyrl, R.J. Silverleaf nightshade, *Solanum elaeagnifolium*, origin, distribution, and relation to man. *Econ. Bot.* **1984**, *38*, 210–217.
44. Heap, J.W.; Carter, R.J. The biology of Australian weeds. 35. (*Solanum elaeagnifolium* Cav.). *Plant Prot. Q.* **1999**, *14*, 2–12.
45. Molnar, V.M.; McKenzie., D.N. Progress report on silverleaf nightshade research. *Pamphlet No. 61. Vermin and Noxious Weeds Destruction Board, Dept. of Crown Lands and . Survey, Victoria, Australia*; **1976**;
46. Qasem, J.R.; Al Abdallat, A.M.; Hasan, S.M. Genetic diversity of *Solanum elaeagnifolium*, an invasive problematic weed in Jordan. *Weed Res.* **2019**, *59*, 222–234.
47. Zammouri, S.; Mnari-Hattab, M. First report of *Solanum elaeagnifolium* as natural host of tomato yellow leaf curl virus species (TYLCV and TYLCSV) in Tunisia. *J. Plant Pathol.* **2014**, *96*, 431–439.
48. Rodríguez-Alvarado, G.; Fernandez-Pavia, S.; Creamer, R.; Liddell, C. Pepper mottle virus causing disease in chile peppers

in southern New Mexico. *Plant Dis.* **2002**, *86*, 603–605.
49. Bryson, C.T.; Reddy, K.N.; Byrd, J.D. Growth, development, and morphological differences among native and nonnative prickly nightshades (*Solanum* spp.) of the Southeastern United States. *Invasive Plant Sci. Manag.* **2012**, *5*, 341–352.
50. Shi, X.; Qiu, J. The complete chloroplast genome sequence of an invasive plant *Solanum rostratum* (So--lanaceae). *Mitochondrial DNA Part B Resour.* **2020**, *5*, 626–628.
51. Zhao, J.; Solís-Montero, L.; Lou, A.; Vallejo-Marín, M. Population structure and genetic diversity of native and invasive populations of *Solanum rostratum* (Solanaceae). *PLoS One* **2013**, *8*, e79807.
52. Abu-Nassar, J.; Gal, S.; Shtein, I.; Distelfeld, A.; Matzrafi, M. Functional leaf anatomy of the invasive weed *Solanum rostratum* Dunal. *Weed Res.* **2022**, *62*, 172–180.
53. Bassett, I.J.; Munro, D.B. The biology of Canadian weeds. 78. *Solunum carolinense* L. and *Solanum rostratum* Dunal. *Can. J. Plant Sci.* **1986**, *66*, 977–991.
54. Abu-Nassar, J.; Matzrafi, M. Effect of herbicides on the management of the invasive weed *Solanum rostratum* Dunal (Solanaceae). *Plants* **2021**, *10*, 284, doi:10.3390/plants10020284.
55. Blystad, D.; Vlugt, R. Van Der; Alfaro-fernández, A. Host range and symptomatology of pepino mosaic virus strains occurring in host range and symptomatology of pepino mosaic virus strains occurring in Europe. *Eur. J. Plant Pathol.* **2015**, *143*, 43–56, doi:10.1007/s10658-015-0664-1.
56. Zhao, D.; Wang, X.; Chen, J.; Huang, Z.; Huo, H.; Jiang, C.; Huang, H.; Zhang, C.; Wei, S. Selection of Reference Genes for QPCR Normalization in Buffalobur (Solanum Rostratum Dunal). *Sci. Rep.* **2019**, *9*, 1–10, doi:10.1038/s41598-019-43438-6.
57. R Core Team, G. *R : A Language and Environment for Statistical Computing*; 2020; ISBN 3900051070.