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Article

Evaluation of Weeds as Virus Reservoirs in Watermelon Crops

Avaliação de Plantas Daninhas como Reservatórios de Vírus na Cultura da Melancia

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ABSTRACT - Watermelon is one of the most important vegetable crops in Brazil, which is grouped among the greatest producers worldwide. Viruses stand out among the most damaging disease agents, which can drastically reduce fruit production. In this context, weeds present in the field can also interfere in crop production, acting as reservoirs for viruses. Thus, this study aimed to investigate virus occurrence in weeds at the main watermelon-growing regions in the State of Tocantins. Viruses identification (e.g. potyviruses: *Watermelon mosaic virus* - WMV; *Papaya ring spot virus* – type watermelon -PRSV-W; *Zucchini yellow mosaic virus*- ZYMV; the cucumovirus *Cucumber mosaic virus* - CMV, and the orthotospovirus *Zucchini lethal chlorosis virus* - ZLCV) infecting weeds was performed by serology and confirmed by RT-PCR tests. Serological and molecular test results indicate that *Amaranthus spinosus*, *Nicandra physaloides*, *Physalis angulata* and *Heliotropium indicum* were infected by at least one virus species. The highest infection rate was represented by ZYMV (52.7%), followed by PRSV-W (22.2%); CMV, WMV, and ZLCV that showed the same infection rate (8.3%) each. Plants of *P. angulata* were infected by all five viruses, singly or in mixed infection, and represented 50% of the total number of infected samples. The highest virus infection rates, 50% and 44.4%, occurred in weeds collected at Lagoa da Confusão and Formoso do Araguaia, respectively. The results on occurrence and distribution of viruses infecting weeds in watermelon commercial plantations in the State of Tocantins provide important information about the role of weeds as virus reservoirs contribute to the knowledge of the epidemiology of these diseases, and enable a proper weed management aiming at reducing the secondary spreading control of viruses by insect vectors.

Keywords: *Citrullus lanatus*, alternative hosts, Potyvirus, Cucumovirus, Orthotospovirus.

*RESUMO - A melancia é uma das olerícolas mais importantes do Brasil, situando o país entre os maiores produtores mundiais. Os vírus se destacam como agentes de doenças prejudiciais, que podem reduzir drasticamente a produção de frutos. Plantas daninhas presentes em campos cultivados também podem interferir na produção de hortaliças, atuando como reservatórios de vírus. O presente estudo teve como objetivo determinar a ocorrência de vírus em plantas daninhas nas principais regiões produtoras de melancia do estado de Tocantins. A identificação de vírus (em geral: os potyvírus **Watermelon mosaic** virus - WMV, Papaya ringspot virus – type watermelon - PRSV-W, e **Zucchini yellow mosaic** virus - ZYMV; o cucumovírus **Cucumber mosaic** virus - CMV e o orthotospovírus **Zucchini lethal chlorosis** virus - ZLCV) foi realizada por meio de testes serológicos e confirmada por RT-PCR. Os resultados sorológicos e moleculares indicaram que as espécies*

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Amaranthus spinosus, ***Nicandra physaloides***, ***Physalis angulata*** *e* ***Heliotropium indicum*** *estavam infectadas com pelo menos uma espécie viral. A maior taxa de infecção foi representada por ZYMV (52,7%), seguido por PRSV-W (22,2%), CMV, WMV e ZLCV com a mesma taxa de infecção (8,3%). A espécie* ***Physalis angulata****, na qual foram detectadas todas as cinco espécies virais, representou 50% do total de plantas infectadas. As maiores porcentagens de infecção viral em plantas daninhas, 50% e 44,4% foram encontradas em amostras coletadas em Lagoa da Confusão e Formoso do Araguaia, respectivamente. Os resultados de ocorrência e distribuição de vírus infectando plantas daninhas em áreas cultivadas com melancia no estado de Tocantins geram informações importantes sobre o papel destas plantas como reservatórios de vírus em campos cultivados com melancia, bem como contribui para o conhecimento da epidemiologia dessas doenças de modo a propiciar o manejo adequado de plantas daninhas e a disseminação secundária desses vírus por meio de insetos vetores.*

Palavras-chave: *Citrullus lanatus*, hospedeiras, potyvírus, cucumovírus, orthotospovírus.

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is one of the most important vegetable crops in Brazil. Production of this fruit is estimated at 2, 079, 547 tons on a total planted area of 94, 690 ha. The northern region of Brazil produces approximately 18.60 ton ha^{-1} of watermelon, 41% of which comes from the Tocantins State (SIDRA, 2014; Furlaneto and Bertani, 2015).

Among the problems associated with watermelon worldwide, viruses stand out as the main pathogens that can drastically reduce production and fruit quality. At least ten virus species are known to infect watermelon crop in Brazil from which five are the most important: *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* – type watermelon (PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV), genus *Potyvirus*, in the family *Potyviridae*, and *Cucumber mosaic virus* (CMV), genus *Cucumovirus*, family *Bromoviridae* transmitted by aphids; and, *Zucchini lethal chlorosis virus* (ZLCV), genus *Orthotospovirus*, family *Tospoviridae* transmitted by thrips. The majority of these viruses are widely distributed in the main Brazilian cucurbit growing areas (Lima; Alves, 2011; Soares et al., 2016; Lima et al., 2017), including the State of Tocantins (Aguiar et al., 2015). Viral infection affects plant development and physical and chemical fruit characteristics, reducing production and yield (Valle et al., 2006; Aguiar et al., 2013).

Furthermore, species of spontaneous vegetation, such as the weeds, can affect cultivated crops, in many ways, for example, when they are infected with viruses serving as virus reservoirs for secondary spreding by insect vectors to infect field crops. Insect vectors can feed on infected weed plants acquiring virus particles and transmitting afterwards to healthy crops (Goyal et al., 2012). Studies have been conducted to investigate the role of weeds as alternative hosts of potyviruses and begomoviruses in vegetable crops favoring virus perpetuation and dissemination in producing regions of *Solanum* species (Silva et al., 2010; Sierra et al., 2012).

In the Tocantins State the occurrence, the distribution and the damage caused by PRSV-W, WMV, ZYMV, CMV and ZLCV in the main watermelon-producing regions have been recently reported (Aguiar et al., 2013; Aguiar et al., 2015). However, no information is available on alternative host plants of these viruses in commercial areas of the state. Considering the diversity of weed species found in producing regions of watermelon in the State of Tocantins and the frequent viral infection detected in watermelon fields, this study aimed to identify weed species as potential hosts of viruses and determine their role in the epidemiology of these diseases.

MATERIAL AND METHODS

Characterization of the experimental fields

All the analyses were performed in the Laboratory of Pest Integrated Management, at the Federal University of Tocantins - Campus Gurupi, TO. Weed sampling was carried out within and in the vicinity of watermelon fields in the following municipalities of the Tocantins State:

Figueirópolis (12°7'35'' S; 49°9'53'' W), Formoso do Araguaia (11°47'45'' S; 49°31'52'' W), Gurupi (11°43'45" S; 49°04'07" W), and Lagoa da Confusão (10°47'22'' S; 49°37'50'' W) (Figure 1).

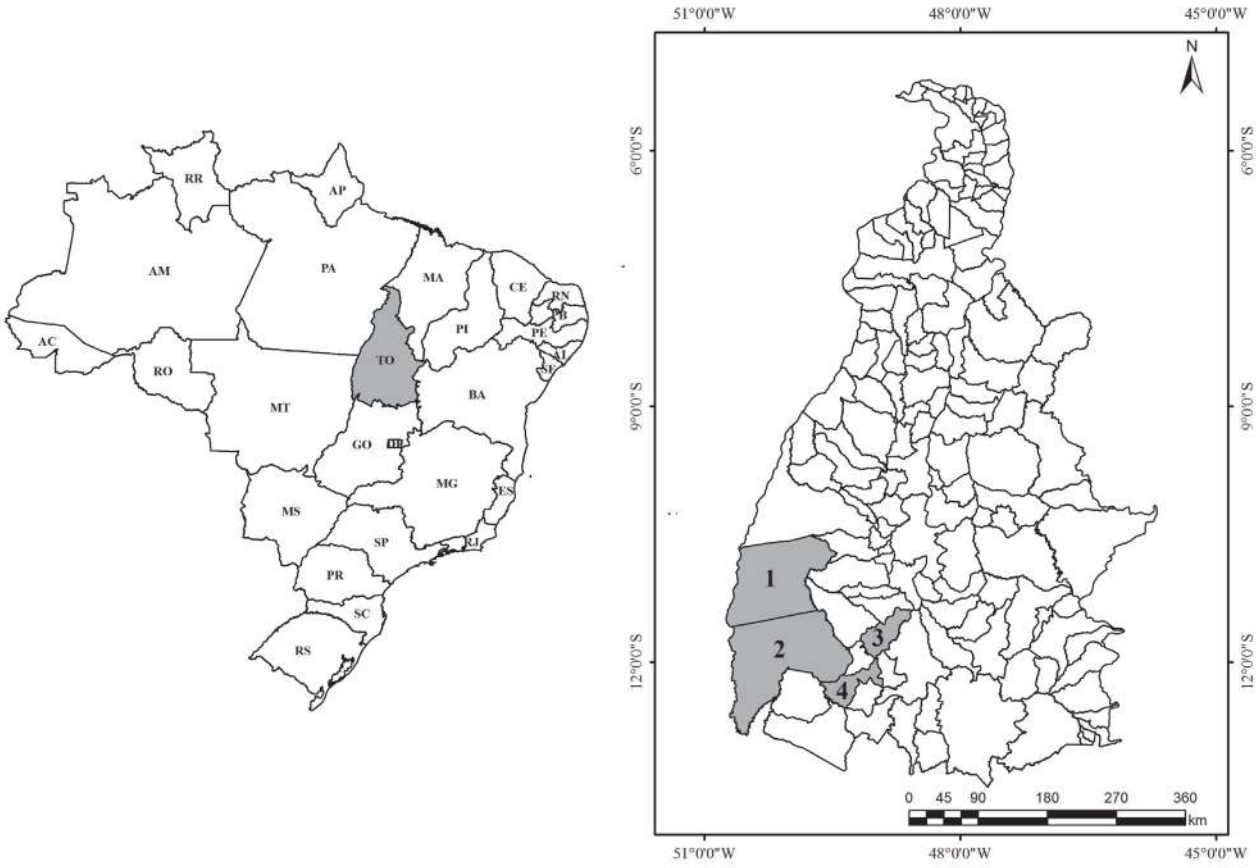
Weed collection and identification

Weed sampling was conducted during the growing season of watermelon, from July to September of two consecutive years, 2011 and 2012. A total of 346 samples exhibiting or not suggestive symptoms of viral infection was randomly collected from 29 fields at four watermelon-producing municipalities, Lagoa da Confusão (109 samples), Formoso do Araguaia (134 samples), Figueirópolis (37 samples), and Gurupi (66 samples). Weeds were collected usually, at the end of the growing season of the crop. Plant identification was done at the Department of Agronomy of the Federal University of Tocantins, at Gurupi, TO.

Virus identification

Serology assays

Virus identification in the collected samples was performed by Dot-ELISA (Clark and Adams, 1977) using polyclonal antisera against *Watermelon mosaic virus* (WMV), *Papaya ringspot virus* – type watermelon (PRSV-W), *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV), and *Zucchini lethal chlorosis virus* (ZLCV), kindly provided by Dr. Mirtes. F. Lima, Embrapa Hortaliças, Brasília, DF. Samples were prepared according to Banttari and Goodwin (1985), at a ratio of 1 g leaf tissue to 10 mL 0.5X Phosphate-buffered-saline (PBS) (0.08 M $NaH_2PO.4H_2O$; 0.02 M K_2HPO_4;



1- Lagoa da confusão; 2- Formoso do Araguaia; 3- Gurupi; 4- Figueirópolis.

Figure 1 **-** Tocantins State, Brazil, Map indicating the municipalities from which the virus isolates were obtained.

1.4 M NaCl; 0.02 M KCl; pH 7,4). Leaf extract (4 µL) was dotted onto nitrocellulose membrane (Hybond-C; GE Life Sciences, Rydalmere, Sydney), previously moistened with 0.5X PBS buffer. Membranes were dried at room temperature for about 30 minutes and then incubated in a blocking solution of 0.5X PBS containing 3% non-fat milk, in a shaker, for 3 hours. Membranes were then incubated with species-specific antibodies against each virus, at 1:1000 dilution. The secondary antibody, goat anti-rabbit IgG conjugated to alkaline phosphatase (Sigma) was diluted at 1:30.000. Membrane revelation was carried out in specific buffer [100 mM NaCl; 100 mM Tris-HCl; 5 mM $MgCl_2(6H_2O)$; pH 9,5] containing BCIP/NBT ("5-Bromo-4-chloro-3-indolyl phosphate/Nitro blue tetrazolium") (SIGMA FAST™). Leaves collected from zucchini (*Cucurbita pepo* cv. Caserta) plants that have been mechanically inoculated with each virus species and leaves from healthy plants, were employed, respectively, as positive and negative controls, in the assays.

Total RNA extraction and reverse transcription

Weed samples which were positive for viral infection in dot-ELISA, were submitted to reverse transcription and polymerase chain reaction (RT-PCR) for confirmation of virus presence in the plants. Total RNA was obtained from samples starting from 100 mg of frozen leaf tissue using the Plant RNA Purification Reagent kit (Invitrogen life technologies, USA), according to the manufacturer's protocol. Total RNA was eluted in 50 µL of RNAse free water and maintained at -80 °C until be used in RT-PCR reactions. cDNA was generated using the Superscript III (Invitrogen). The reverse transcription mixture consisted of 3 µL of total RNA, 1 µL dNTP (10 mM), 0.5 µL (200 U) of Superscript III, 5 µL 5X reaction buffer, 0.5 µL RNAse inhibitor (40 U) (Invitrogen), 2 µL (0,1 M) dithiothreitol (DTT), 1 µL random primers (20 mM), and sterile water to a final volume of 25 µL per reaction. The mixture was incubated at 37 °C for 50 min and then, at 80 °C for 15 min.

Designing of virus-specific primers

For virus detection, forward and reverse primers were designed based on virus genome sequences available at the GenBank database (National Center for Biotechnology Information [NCBI], https://www.ncbi.nlm.nih.gov). These primers targeted the gene encoding coat protein (CP) of PRSV-W (AY162218; AY010722; AY231130; EF183499; EF017707; NC001785; X67673; EU126128, EU475877, DQ374153; DQ374152), nuclear inclusion body (Nib) of WMV (NC006262; AY437609; EU660588; EU660584; EU660579; EU660590; EU660578, EU660589; EU660582; EU660580; EU660587; EU660586; EU660583; EU660581; EU660585; AB218280; AB369278; DQ399708), coat protein of ZYMV (AY279000; AY278999; AY278998; AY188994; AB369279; AB188116; AB188115; EF062583; EF062582; DQ124239; AF127929; NC003224; AF014811), coat protein of CMV (AM183116; AF127977; AF103991; NC001440; D10538, D10539; D00385; AJ585522; AJ831578; AJ304399; AB006813), and nucleoprotein (N) of (AF067069). The primer sequences, amplicon sizes, annealing temperatures, and corresponding regions in the genome of each target virus are listed in Table 1.

PCR

Species-specific primer sets were used individually to amplify fragments of the genome sequence of each virus, PRSV-W, WMV, ZYMV, CMV and ZLCV by RT-PCR and, then, confirm virus presence in the weed samples. The PCR reaction contained 0.4 µM of each forward and reverse primers, 0.2 mM of each dNTP of dNTP mixture, 1X PCR buffer, 2 mM of $MgCl_2$, 1 U of Taq DNA Polymerase (Invitrogen), 2 µL of cDNA as template and sterile water to a final volume of 25 µL. PCR was performed in a thermal cycler (Therm-1000-2, Axygen) under the following conditions: initial denaturation at 94 °C for 5 min followed by 35 cycles at 95 °C for 30 sec (denaturation), 52 °C for 1 min 30 sec (annealing), 72 °C for 4 min (elongation); and the final extension step at 72 °C for 8 min. Negative (total RNA extracted from a *Cucurbita pepo* cv. Caserta healthy plant) and positive (total RNA obtained from *Cucurbita pepo* cv. Caserta plants infected with each virus species) controls were used in all RT-PCR tests. The PCR amplification products were analyzed on 1.2% agarose gel electrophoresis, stained with ethidium bromide and visualized

Table 1 - Primers used for PRSV-W, CMV, WMV, ZYMV and ZLCV detection in weed samples, amplicon size and region in the virus genome

Virus[1]	Primer Sequences[2]	Product size (nt)	AT[3] (°C)	Region in the genome
PRSV-W	For: 5'TGGGTTATGATGGATGGGGA3'	398	52	Coat Protein
	Rev: 5'ATACCCAGGAGAGAGTGCAT3'			
CMV	For: 5'ACTNTTAACCACCCAACCTT3'	644	52	Coat Protein
	Rev: 5'TTAGCCGTAAGCTGGATGGA3'			
WMV	For: 5'TTRTTGTTGAATGCTGTCCT3'	535	52	Nuclear inclusion body
	Rev: 5'GCTGCACAAATTGCCTCAG3'			
ZYMV	For: 5'CATACATGCCGAGGTATGGTTT3'	644	52	Coat Protein
	Rev: 5'GTGTGCCGTTCAGTGTCTT3'			
ZLCV	For:5'GAGTTTCACTGTAATGTTCCATAGC3'	244	52	Nucleoprotein
	Rev: 5'AGYTTTGAGATGATCAGTGTTGT3'			

[1] PRSV-W = *Papaya ringspot virus*-type watermelon; CMV = *Cucumber mosaic virus*; WMV = *Watermelon mosaic virus*; ZYMV = *Zucchini yellow mosaic virus*; ZLCV = *Zucchini lethal chlorosis vírus*. [2] Primer sequences designed based on the sequences of each virus available in the GenBank. [3] Annealing temperature.

under UV light (Sambrook and Russell, 2001). The 1 kb PLUS DNA ladder (Invitrogen) was used to estimate the size of the amplicons in the gel.

Pathogenicity tests

Weed samples that tested positive for PRSV-W, WMV, ZYMV, CMV, and ZLCV (by Dot-ELISA) were mechanically inoculated onto plants of the following cucurbit species: ridged gourd (*Luffa acutangula* M. Roem.), bottle gourd (*Lagenaria vulgaris* L.), watermelon (*Citrullus lanatus* Thunb.), and zucchini (*Cucurbita pepo* L.). Extracts were prepared by gridding symptomatic leaves collected from the naturally-infected weed plants in a potassium phosphate buffer (0.01 M K_2HPO_4; pH 7.0; 0.1% Na_2SO_3) at a dilution of 1:10 (p:v; g mL^{-1}). Seedlings, previously dusted with carborundum 400 mesh, were inoculated twice, the first at the two true-leaf stage and the second 3 days after the first inoculation. Five plants of each cucurbit species were inoculated and a plant was maintained without inoculation; additionally, a plant was inoculated only with buffer as a mock control. Symptom expression was evaluated at 7, 14, 21, and 28 days after the second inoculation. The symptom rate was assessed according to Chung et al. (2007) as follows: chlorotic local lesion (CLL), light mosaic (LM), leaf deformation (LD), bubbles (B), severe mosaic (SM), and without symptoms (W/S). Finally, the inoculated plants were tested for virus infection by serology using antibodies against the same five viruses of interest.

Statistical analysis

The number of samples collected for each weed species per municipality was compared to the total number of samples collected for each weed species by the Chi-square (χ^2) test at 1% or 5% probability.

RESULTS AND DISCUSSION

Twenty-two weed species sampled in watermelon fields at the State of Tocantins were classified in eight botanical families, as follows: Amaranthaceae (22), Euphorbiaceae (35), Solanaceae (128), Cucurbitaceae (27), Fabaceae (20), Boraginaceae (10), Malvaceae (46), and Asteraceae (58). Cutleaf groundcherry (65; *Physalis angulata*), apple of Peru (45; *Nicandra physaloides*) and bitter gourd (27; *Momordica charantia*) were the most frequently found weeds (Table 2).

Potyviruses (PRSV-W, WMV and ZYMV), a cucumovirus (CMV), and an orthotospovirus (ZLCV) were detected in weeds collected from several watermelon field locations: Lagoa da

Table 2 - Family, scientific name, common name and number of samples collected in commercial watermelon fields in four municipalities of the State of Tocantins, between 2011 and 2012 period

Family	Scientific name	Common name	Samples collected (no)				Weed Plants (Total number)
			Formoso do Araguaia	Lagoa da Confusão	Gurupi	Figueirópolis	
Amaranthaceae	*Amaranthus spinosus* L.	Spiny amaranth	00**	04*	09 ^{ns}	00**	13
	Amaranthus viridis L.	Slender amaranth	03^{ns}	00**	04 ^{ns}	02*	09
Euphorbiaceae	*Euphorbia heterophylla* L.	Wild poinsettia	07*	09*	04**	00**	20
	Chamaesyce hirta (L.) Millsp	Pillpod sandmat	08 ^{ns}	03*	00**	04*	15
Solanaceae	*Datura stramonium* L.	Jimson weed	02 ^{ns}	01 ^{ns}	02**	00*	05
	Nicandra physaloides (L.) Pers.	Apple of Peru	28*	17**	00**	00**	45
	Physalis angulata L.	Cutleaf groundcherry	32**	30**	03**	00**	65
	Solanum sisymbrifolium Lam.	Sticky nightshade	04*	06 ^{ns}	01**	02*	13
Cucurbitaceae	*Momordica charantia* L.	Bitter gourd	12*	09*	03**	03**	27
Fabaceae	*Senna obtusifolia* (L.) H. S. Irwin & Barneby	Sicklepod	00**	04*	07**	01**	12
	Desmodium tortuosum (Sw.) DC.	Dixie ticktrefoil	02 ^{ns}	00*	05 ^{ns}	01*	08
Boraginaceae	*Heliotropium indicum* L.	Indian heliotrope	04 ^{ns}	02*	00**	04 ^{ns}	10
Malvaceae	*Sida urens* L.	Tropical fanpetals	00**	03 ^{ns}	04 ^{ns}	01*	08
	Sidastrum micranthum (A.Saint-Hilaire) Fryxell	Dainty Sandmallow	00**	05 ^{ns}	01**	06 ^{ns}	12
	Sida spinosa L.	Malva- lacenta	02 ^{ns}	01 ^{ns}	02 ^{ns}	00*	05
	Sida rhombifolia L.	Cuban jute	11 ^{ns}	06*	02**	02**	21
Asteraceae	*Galinsoga quadriradiata* Ruiz & Pav.,	Shaggy soldier	04*	03**	02**	05*	14
	Pluchea sagittalis (Lam.) Cabrera	Wingstem Camphorweed	02 ^{ns}	01*	03 ^{ns}	00*	06
	Praxelis pauciflora (Kunth) R.M. King& H. Rob.	Praxelis	04**	05**	06**	03**	18
	Siegesbeckia orientalis L.	St. Paulswort	02 ^{ns}	00*	03 ^{ns}	01*	06
	Emilia fosbergii Nicolson	Florida tasselflower	07 ^{ns}	00**	05*	02**	14
Total			134 ^{ns}	109 ^{ns}	66*	37**	346

**, *: Indicates a significance difference between the sample collected in each municipality in relation to the total number of weeds collected within each family, as evaluated by the chi-square (χ^2) test, to 1 % and 5 % probability, respectively. ^{ns}: Not significant.

Confusão, Formoso do Araguaia, Figueirópolis, and Gurupi. Out of 346 weed plants collected, only 36 samples classified in four species were positive for viral infection: *Amaranthus spinosus* (3), *Nicandra physaloides* (9), *Physalis angulata* (18) and, *Heliotropium indicum* (6) (Table 3). Weed samples identified as positive by serological approach also tested positive in RT-PCR assays for the same virus species amplifying DNA fragments of the expected sizes (Table 3, Figure 2). Positive and negative controls for each virus, reacted as expected, in dot_ELISA, as well as, in RT-PCR assays. Negative samples by serology and RT-PCR based methods corresponded to plants belonging to 17 weed species.

Solórzano-Morales et al. (2011) also confirmed viral infection in plants of five weed species by RT-PCR using specific primers. They detected *Tomato chlorotic virus* (ToCV) infection in plants of *Ruta chalepensis* (Rutaceae), *Icosandra phytolacca* (Phytolacaceae), *Plantago major* (Plantaginaceae), and *Brassica* sp. (Brassicaceae).

Symptoms, including light and severe mosaic, leaf deformation and blistering, were observed in weed plants infected with virus, singly or in multiple infections with ZYMV and WMV. Interestingly, an asymptomatic Indian heliotrope plant (1/10 plants) tested positive for ZYMV based on serological and molecular results. Two Sicklepod plants (Fabaceae) (2/7) collected in watermelon fields, at Gurupi showed leaf deformation symptoms, but none of them tested positive for the antisera tested by dot-ELISA nor to any virus-specific primers by RT-PCR, suggesting that those symptoms may be caused by other biotic or even abiotic factors.

Virus-like symptoms including mild to severe mosaic, chlorotic local lesions, leaf deformation and blistering were induced in plants of different cucurbit species in response to virus inoculation. Leaf deformation was induced in plants of all cucurbit species, while blistering was observed in *Luffa acutangula*, *Lagenaria vulgaris and Citrullus lanatus*, and severe mosaic was verified in *Luffa acutangula*, *Lagenaria vulgaris* and *Cucurbita pepo*. Reduction in leaf size and mosaic occurred only in *Citrullus lanatus* plants (Table 4 and Figure 3). These symptoms confirmed the importance

Table 3 - Serological evaluation of weed species collected in the State of Tocantins, between 2011 and 2012 using polyclonal antibodies against PRSV-W, WMV, ZYMV, CMV and ZLCV: county localization, weed species, number of samples tested and positive samples

	Virus-positive samples (nº)*						
County/weed species	Total number of samples	PRSV-W	CMV	WMV	ZYMV	ZLCV	Infected samples
Lagoa da Confusão							
Amaranthus spinosus	4	-	-	-	3	-	3
Nicandra physaloides	17	2	-	-	2	-	4
Physalis angulate	30	3	-	1	7	-	11
Formoso do Araguaia							
Nicandra physaloides	28	1	-	2	2	-	5
Physalis angulate	32	2	1	-	2	2	7
Heliotropium indicum	4	-	-	-	3	1	4
Figueirópolis							
Heliotropium indicum	4	-	2	-	-	-	2
Total	119	8	3	3	19	3	36

* PRSV-W= *Papaya ringspot* virus – type watermelon; WMV = *Watermelon mosaic* virus;, ZYMV = *Zucchini yellow* mosaic virus; CMV = *Cucumber mosaic* virus; ZLCV = *Zucchini lethal* chlorosis virus.

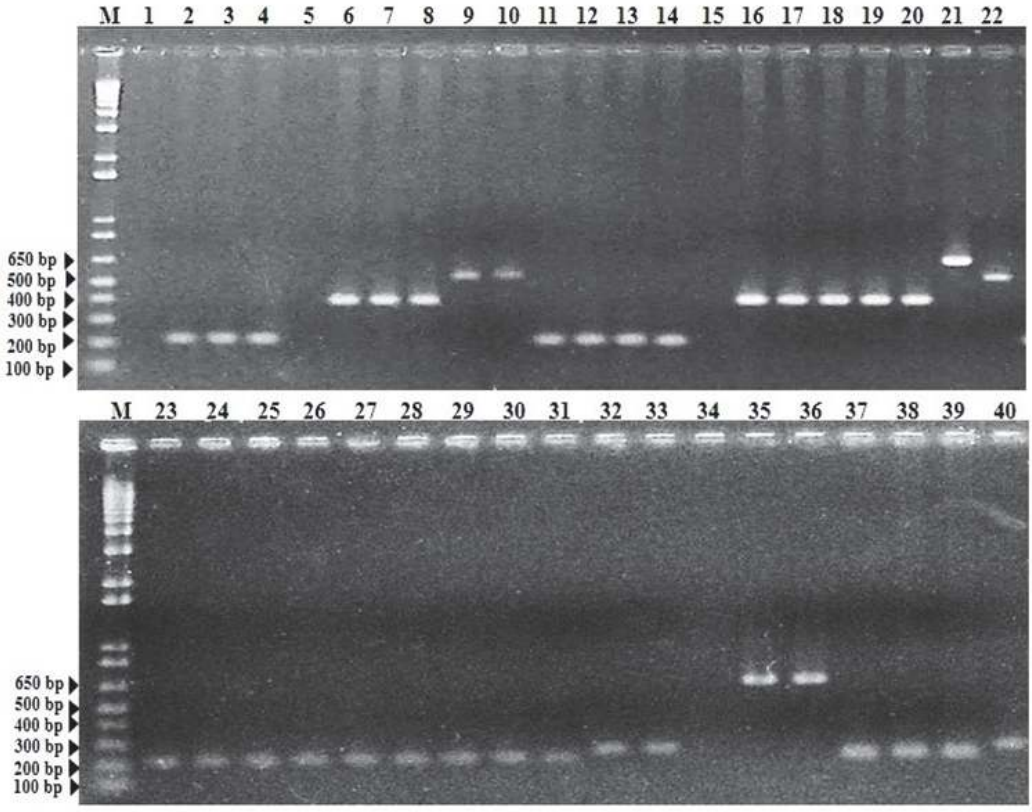


Figure 2 - (A-B). RT-PCR detection of *Papaya ringspot virus* – type watermelon (PRSV-W), *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV), and *Zucchini lethal chlorosis virus* (ZLCV) using total RNA obtained from field-grown weed plants and species-specific primers (A) M - 1 kb Plus DNA ladder (Invitrogen); Lane 1- healthy *Amaranthus spinosus;* Lanes 2-4- *A. spinosus* infected with ZYMV; Lane 5- healthy *Nicandra physaloides*; Lanes 6-8- *N. physaloides* infected with PRSV-W; Lanes 9-10- *N. physaloides* infected with WMV; Lanes 11-14- *N. physaloides* infected with ZYMV; Lane 15- healthy *Physalis angulata*; Lanes 16-20- *P. angulata* infected with PRSV-W; Lane 21- *P. angulata* infected with CMV; Lane 22- *P. angulata* infected with WMV; (B) Lanes 23-31- *P. angulata* infected with ZYMV; Lanes 32-33- *P. angulata* infected with ZLCV; Lane 34- healthy *Heliotropium indicum*; Lanes 35-36- *H. indicum* infected with CMV; Lanes 37-39- *H. indicum* infected with ZYMV; Lane 40- *H. indicum* infected with ZLCV.

Table 4 - Evaluation of cucurbit species to *Papaya ringspot virus* – type watermelon (PRSV-W), *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), and *Zucchini lethal chlorosis virus* (ZLCV) isolates obtained from virus-infected weed plants, 21 days post inoculation

Cucurbitaceae species	PRSV-W		CMV		ZYMV		WMV		ZLCV	
	Symptoms	Dot-ELISA	Symptoms	Dot-ELISA	Symptoms	Dot-ELISA	Symptoms	Dot-ELISA	Symptom	Dot-ELISA
Luffa acutangula	LD, SM, B[(1)]	+[(2)]	LD, SM, B	+	LD, SM, B	+	LD, SM, B	+	LD, B	+
Lagenaria vulgaris	LLC, LM	+	SM, B	+	LD, SM, B	+	LD, SM, B	+	LM, B	+
Citrullus lanatus	LD, SM, B,	+	LD, LM, B	+	LD, SM, B	+	LD, LM, B	+	LD, LM, B	+
Cucurbita pepo	LD, SM	+	LD, LM, B	+	LD, SM	+	LM	+	LD, SM	+

[(1)] LLC–chlorotic local lesion; LM–light mosaic; LD–leaf deformation; B–bubbles; SM–severe mosaic. [(2)] (+) positive samples for virus infection.

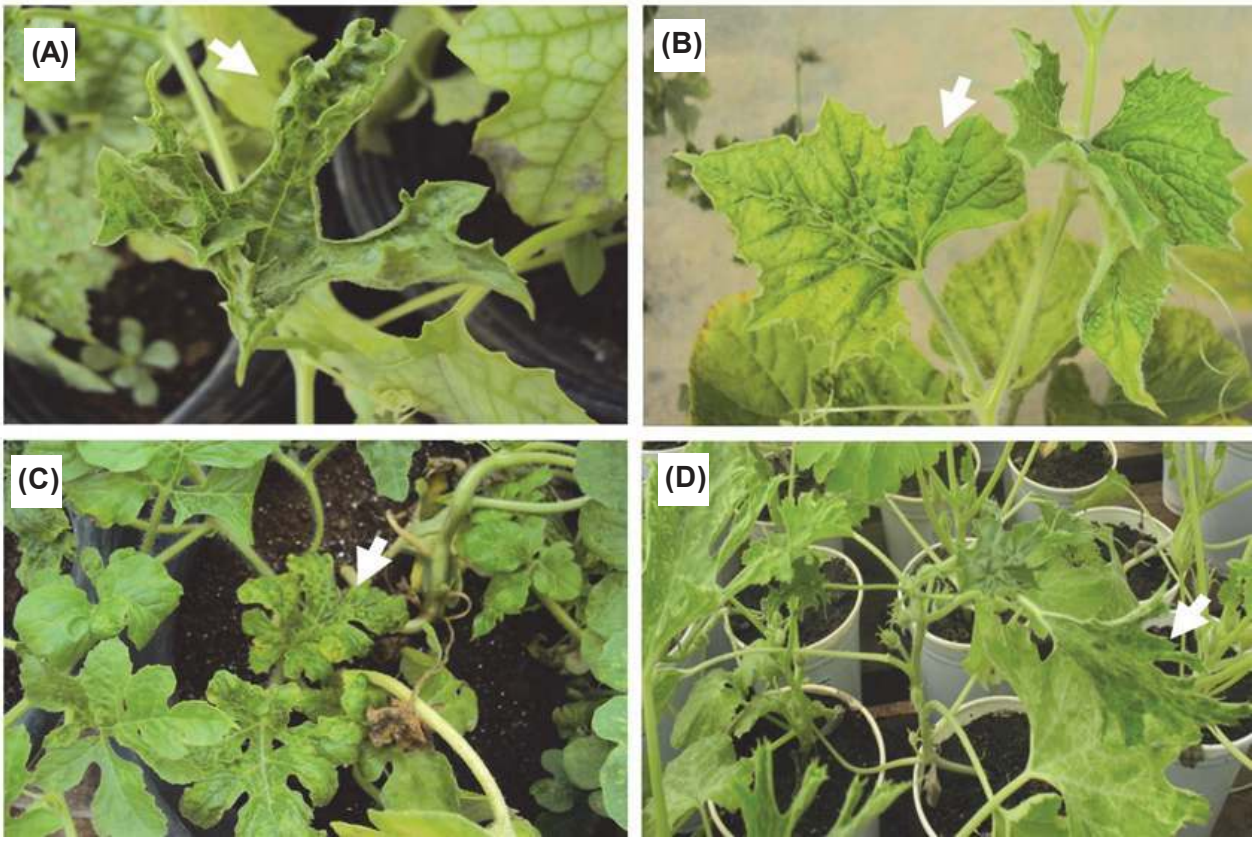


Figure 3 - Disease symptoms in the curcubit species caused by PRSV-W, CMV, WMV, ZYMV, and ZLCV. (A) *Luffa acutangula* showing leaf deformation, blistering, and a severe mosaic; (B) *Lagenaria vulgaris* showing leaf deformation, blistering, and a severe mosaic; (C) *Citrullus lanatus* showing leaf deformation, blistering, reduction in leaf size, and a severe mosaic; (D) *Cucurbita pepo* showing leaf deformation and a severe mosaic.

of infected-weed plants as virus reservoirs, favoring secondary spreading by insect vectors to infect cucurbits in the field.

Percentage of infected plants was high for ZYMV (52.7%) followed by PRSV-W (22.2%), while CMV, WMV, and ZLCV showed the same percentage of infected weeds (8.3% - 4). Studies performed in Oklahoma, United States revealed that the incidence of PRSV-W in weeds collected from cucurbit-growing areas was higher compared to ZYMV (Ali et al., 2012). According to these same authors, variation in virus incidence in different regions can be explained by factors such as initial inoculum sources, host species and insect vectors occurring in cucurbit commercial areas.

The results presented in the present study indicate that the weeds *A. spinosus*, *N. physaloides*, *P. angulata* and *H. indicum* serve as alternative hosts for cucurbit-infecting viruses contributing as inoculum sources to secondary dissemination, for infection of watermelon fields, in the State

of Tocantins. Furthermore, cutleaf groundcherry stood out as the most abundant weed (50%) in watermelon fields and plants were infected by different virus species (PRSV-W, CMV, ZYMV, WMV and ZLCV). Moreover, these results indicate that cutleaf groundcherry plays an important role in viruses' epidemiology, acting as virus source from which secondary spread to infect watermelon fields can occurr. These data reinforce the need of employing efficient management control strategies to weed elimination within, as well as, in the surrounding areas of watermelon fields (Table 4) aiming at reducing virus source and then, the chances of infection of the crop.

Other studies involving different crops have also shown the importance of weeds as potential virus reservoirs to infect crops that are economically important and contributing to disease occurrence during the growing season, and also to virus dissemination (Ali et al., 2012; Papayiannis et al., 2011; Papayiannis et al., 2012; Solórzano-Morales et al., 2011; Asala et al., 2014). Additionally, weeds can still withstand drought in the field, and survive in the absence of preferred hosts, becoming an important initial source of virus inoculum, which can be spread not only to commercial crops, but also to infect other weed plants after harvesting periods (Asala et al., 2014).

In the present study, there were differences regarding the number of samples collected in the different geographical areas within the State of Tocantins. Collections of weed plants at Lagoa da Confusão and Formoso do Araguaia watermelon fields resulted in the highest number of samples (Table 2). In addition, plants also had the highest infection rates, 50% and 44.4%, for Lagoa da Confusão and Formoso do Araguaia, respectively (Table 3). On the other hand, the percentage of infected plants from fields at the municipality of Figueirópolis (Table 3) was only 5.5%; while at Gurupi no virus infection was detected in weed plants sampled. The continuous watermelon cultivation in the municipalities of Lagoa da Confusão and Formoso do Araguaia may be a determinant factor for weed infection in the field all over the year, as has been shown in the present study, then, perpetuating virus inoculum.

Watermelon crop is of great economic importance to the State of Tocantins, especially at Lagoa da Confusão and Formoso do Araguaia that are the main watermelon-producing regions of the state. From these municipalities, watermelon fruits are commercialized in the domestic markets located mainly in the states of Minas Gerais, São Paulo and Goiás. In the last years, watermelon cultivation in the State of Tocantins has been facing a serious restriction due to virus occurrence which is considered as the main limiting factor for watermelon production with significant impact on the local economy (Chaves et al., 2013). The detection of PRSV-W, CMV, ZYMV, WMV and ZLCV infecting weeds plants, as reported in the present study, indicate that these plants contribute for maintaining virus inoculum available in the absence of cucurbits in the field, and increases the chances of perpetuating pathogens survival, leading to watermelon infection and yield losses. Thus, in order to efficiently control virus reservoirs and reduce secondary spreading, it is mandatory that watermelon growers of the State of Tocantins adopt efficient control strategies, regionally, such as destroying weeds within and at the surroundings of watermelon fields and thus, reducing the chances of virus infection of the crop during the growing season.

This research indicated that *Amaranthus spinosus*, *Nicandra physaloides*, *Physalis angulata* and *Heliotropium indicum* can be naturally infected by PRSV-W, WMV, ZYMV, CMV and ZLCV in the field and thus, acting as virus reservoirs to infect watermelon fields in the State of Tocantins. The identification of the virus species infecting weeds contributes to the knowledge of disease epidemiology which is important to enable a proper weed and virus disease management.

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