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A Survey of Five Plant Viruses in Weeds and Tobacco in Poland

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Abstract: Weeds may contribute to the spread of plant virus epidemics by acting as reservoirs of viruses or/and their vectors. The aim of this research was to study the prevalence of five viral pathogens in weeds in the fields of solanaceous crops in six provinces in Poland differing with soil and climate conditions. Most of the sampled sites were associated with tobacco production. The total number of 157 samples of tobacco and 600 samples of weeds were subjected to DAS-ELISA detection of tomato spotted wilt orthotospovirus (TSWV), cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco mosaic virus (TMV) and tobacco ringspot virus (TRSV). Twenty nine percent of samples of weeds were infected with at least one virus. TSWV and TMV were the most frequently detected in 17.5% and 14.7% of samples, respectively. In most provinces where infected tobacco was found, the same virus was also detected in weeds. Results of this survey are discussed in the context of the current status of virus epidemics in tobacco fields in Poland.

Keywords: plant virus diseases; potential weed reservoirs; virus detection; *Solanaceae*; solanaceous crops; *Nicotiana tabacum*

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1. Introduction

Weeds play an important role in plant virus epidemics in agroecosystems. They contribute to the spread of virus diseases in the crops because they can act as reservoirs of viruses and their vectors [1,2]. Most of the viral pathogens threatening cultivated plants have broad host ranges and naturally infect many species from at least a few families [3]. Therefore, they may thrive in alternative plant hosts, such as weeds, when annual crops are absent in the fields or when the resistant cultivars are grown. Surveys of viral infections in the local flora were performed in various cultivation areas around the world to monitor the disease incidence in different geographic regions or to identify important virus reservoirs and design effective disease control strategies (e.g., [4–6]).

Plants from Solanaceae family, including crops such as: tomato, potato, pepper and tobacco, are hosts of over 300 viruses from 39 different genera [7]. Some of these viruses were subjected to extensive research because of their impact on crop production. For example, tomato spotted wilt orthotospovirus (TSWV) causes significant yield reductions of vegetable and ornamental crops worldwide [8]. It is transmitted by several species of thrips. High fecundity and polyphagous nature of these vectors contribute to rapid spread of the disease and hinder its effective management. Moreover, TSWV host list includes more than 1000 plant species from 84 angiosperm families, with the strongest representation of *Asteraceae* and *Solanaceae* [9]. Therefore, as epidemics spreads to new areas, it is frequently detected in samples of native flora in the neighbourhood of crop fields.

Other important viral pathogens of solanaceous crops, such as cucumber mosaic virus (CMV) and potato virus Y (PVY), are transmitted by aphids and may interact synergistically with each other increasing the severity of disease symptoms [10]. Both viruses are

transmitted by aphids in a non-persistent manner that lowers the effectiveness of disease control by means of insecticides. CMV may also easily spread in agroecosystems due to its ability to infect many plant hosts (over 1200 species), including overwintering weeds or plants for which seed transmission of this virus was confirmed [10,11]. In contrast, PVY has a narrower host range, it infects mainly solanaceous crops and weeds belonging to a few plant families [3,6]. Weeds collected on the edges of potato fields in Poland, such as *Erodium cicutarium*, *Geranium pusillum*, *Lactuca serriola* and *Lamium purpureum*, have been confirmed as reservoirs of this virus [6].

In contrast to the three above-mentioned insect-transmitted viruses, tobacco mosaic virus (TMV) is easily spread by contact between plants and mechanical damage during agricultural treatments [12]. Moreover, it is one of the most persistent viruses; it remains infectious in a soil and post-harvest plant debits in the fields but also on the surface of the seeds which were not properly cleaned or disinfected. TMV is an important factor limiting tobacco production, but it also infects tomatoes, peppers and it is found in many weeds [3,13,14].

Tobacco (*Nicotiana tabacum* L.) is one of the most important non-food crops in Poland. In 2019, it was cultivated in the area of 16,000 ha and domestic production reached 33,200 tonnes [15]. According to FAOSTAT, Poland is the second largest producer of tobacco in Europe [16]. Cultivation of this crop is considered to be labour intensive but it is important especially in the economically underdeveloped parts of the country with poor soils and high unemployment rate [17]. Viral diseases caused by the above-mentioned pathogens may significantly lower yield and quality of tobacco leaves [12]. Here we aim at performing a survey of five viruses in weeds growing mainly in tobacco production fields located in regions differing largely with soil and climate conditions. The detected viruses will include TSWV, CMV, PVY, TMV and a virus which is less frequently observed in tobacco grown in Poland—tobacco ringspot virus (TRSV). Samples of leaves from infected plants will be retained for further research concerning tobacco breeding for virus resistance.

2. Materials and Methods

2.1. Sampling

We collected samples from July till September in one vegetation season, in tobacco production fields located in five provinces in Poland (KP, PD, DS, SK, LU; Figure 1; Table 1). These provinces are located in major tobacco cultivation regions differing with climate, soil and economic conditions. In addition, for comparative purposes, weeds were sampled in the area where tobacco was not grown, on Wolin island, in Zachodniopomorskie province (ZP). Differences in the number of sampled fields in six provinces resulted from uneven distribution of tobacco cultivation in the country and need to obtain permission from the farmers to include their field in this study.

Table 1. The number of sampled fields and plants from six provinces in Poland included in this survey.

Province Code	Province Name	Main Crops Cultivated in Sampled Fields	No. of Fields Sampled	No. of Collected Samples of:	
				Tobacco	Weeds
ZP	Zachodniopomorskie	potato	3	0	113
KP	Kujawsko-Pomorskie	tobacco, tomato	6	48	55
PD	Podlaskie	tobacco	2	0	55
DS	Dolnośląskie	tobacco	5	23	111
SK	Świętokrzyskie	tobacco	3	19	163
LU	Lubelskie	tobacco	8	67	103
Total			27	157	600

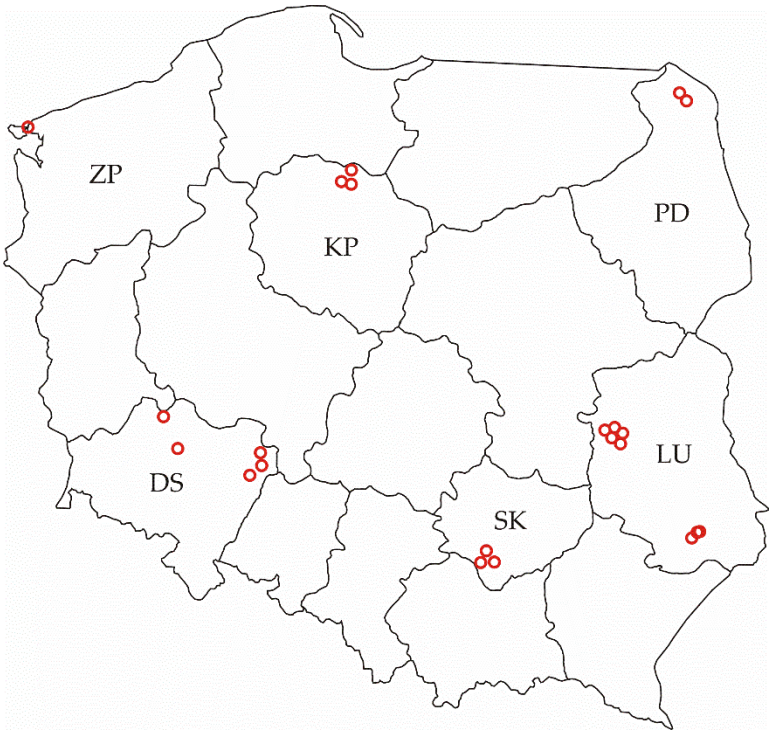

Figure 1. Map of 27 sampling sites included in this survey and located in six provinces in Poland: Zachodniopomorskie (ZP), Kujawsko-Pomorskie (KP), Podlaskie (PD), Dolnośląskie (DS), Świętorzyskie (SK) and Lubelskie (LU). Circles indicating closely located sampling sites overlap in some provinces. For the number of sampling sites see Table 1.

We collected leaf samples of tobacco plants showing symptoms of viral diseases. In case of weeds, we collected plants irrespective of the presence of the symptoms, because as Cooper and Jones [18] pointed out, in such plants viral infections are often symptomless or associated with only slight abnormalities. We collected samples of weeds representing species which were the most common in the field and growing among the crop plants or in a direct surrounding of the field. Species assigned to a category of weeds included wild plants and volunteer crops which were growing in the field but were not the intended crop. Full list of sampled species is provided in Table S2. The collected weeds were subjected to species determination using available taxonomic keys.

2.2. Virus Detection Using DAS-ELISA

Virus detection was performed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) [19]. This method was selected because it is robust in allowing detection of several viruses for a large number of samples [20]. First leaf samples (approx. 250 mg per plant) were ground using TissueLyser II homogenizer (Qiagen). Then, immediately extraction buffer was added, and the resulting suspension of ground plant material was subjected to DAS-ELISA detection of five viruses using reagents and analysis recommendations provided by Bioreba (Reinach, Switzerland). PVY was detected using a cocktail of monoclonal antibodies complementary to isolates from all common strains of the virus (Bioreba catalogue number: 112912). Polyclonal antibodies were used for detection of TSWV, CMV, TMV and TRSV (Bioreba catalogue numbers: 190115, 160615, 190412, 152215, respectively). According to manufacturer's information, tests for TSWV may show a weak reaction with isolates of impatiens necrotic spot virus; while the ones detecting TMV may react with three other tobamoviruses.

Uninfected plants were used as negative controls. Positive controls for detection of TMV and TRSV were obtained from Bioreba (catalogue numbers: 190453 and 152253, respectively) while for detection of the remaining three viruses infected tobacco leaves

originating from our own collection were used. Final results of DAS-ELISA assays were collected by measurement of absorbance at 405 nm, after 90 min incubation, by means of Tecan Sunrise microplate reader (Tecan, Männedorf, Switzerland). All absorbance readings were corrected for blank containing only buffers and no plant material. Tested samples were considered positive if their corrected absorbance value was higher than twice the corrected value of negative control.

Statistical analysis of the frequency of virus detection in different groups of weeds was done using Pearson's Chi^2 tests by means of software Statistica v.13 (TIBCO Software Inc.).

Based on DAS-ELISA results, we selected 43 samples of virus infected tobacco and subjected them to RT-PCR detection of the same five viruses to confirm the obtained results. Samples with confirmed detection of one virus were retained for use as inoculum in greenhouse inoculation tests performed to select virus resistant breeding lines of tobacco.

2.3. Virus Detection Using RT-PCR

Leaf material of selected samples was ground in liquid nitrogen and then subjected to RNA extraction using RNeasy PowerPlant® kit (Qiagen, Hilden, Germany). Then cDNA synthesis was performed using random hexamers and SuperScript® III Reverse Transcriptase (Invitrogen) following the manufacturer's instructions. For PCR amplification of the five viruses Platinum™ Green Hot Start PCR 2× Master Mix (Invitrogen™, Thermo Fisher Scientific) was used. TSWV and TMV were amplified using primers and PCR thermal conditions according to Liu et al. [21]. The remaining three viruses, PVY, CMV and TRSV, were detected using protocols described by Chikh Ali et al. [22], Zhang et al. [23], Jossey and Babadoost [24], respectively. PCR products were separated and visualized on 2% agarose gel stained with ethidium bromide. Their size was estimated by comparison with 100 bp ladder.

3. Results and Discussion

Among the 600 collected samples of weeds, 176 (29.3%) were infected with at least one virus (Table 2). In three provinces, all sampled tobacco plants with disease symptoms were infected at least with one of the viruses detected in this study, while in KP province, symptoms observed on majority of the collected samples must have been caused by other pathogens (Table 3).

Table 2. Results of DAS-ELISA detection of five viruses in the samples of weeds collected in six provinces in Poland. Shaded cells indicate the detected presence of the same virus in samples of tobacco in the same province.

Province Code	No. of Tested Samples	Percentage (%) of Samples of Weeds Infected with:						
		TSWV	TMV	PVY	CMV	TRSV	At Least 1 Virus	More Than 1 Virus
ZP	113	7.1	1.8	0.9	2.7	2.7	7.1	3.5
KP	55	10.9	9.1	10.9	10.9	20.0	30.9	14.5
PD	55	9.1	1.8	1.8	0.0	1.8	10.9	3.6
DS	111	22.5	12.6	6.3	0.9	0.0	37.8	4.5
SK	163	15.3	0.6	2.5	0.6	7.4	17.2	6.7
LU	103	35.0	63.1	7.8	1.9	2.9	72.8	32.0
Total	600	17.5	14.7	4.5	2.2	5.0	29.3	10.5

In most provinces where a particular virus was found in tobacco, the same virus was also detected in weeds (Tables 2 and 3). This observation indicates that there is a possibility that weeds may contribute to virus epidemics in tobacco fields by acting as virus reservoirs. However, a high virus prevalence in tobacco does not always correspond to high prevalence of the same virus in weeds. Interesting discrepancies are found for TMV detected in provinces SK and DS. They may be explained by poor transmission of this virus between weeds and tobacco. TMV can be transmitted with seeds which were not properly cleaned and easily spread on the field by agricultural treatments leading to touching or

mechanical damage of adjacent tobacco plants. Such treatments increase transmission between tobacco plants rather than transmission between tobacco and weeds.

Table 3. Results of DAS-ELISA detection of five viruses in the samples of tobacco collected in four provinces in Poland.

Province Code	No. of Tested Samples	Percentage (%) of Samples of Tobacco Infected with:						
		TSWV	TMV	PVY	CMV	TRSV	At Least 1 Virus	More Than 1 Virus
KP	48	4.2	6.3	4.2	6.3	4.2	12.5	8.3
DS	23	100.0	0.0	8.7	0.0	0.0	100.0	8.7
SK	19	100.0	36.8	0.0	0.0	0.0	100.0	36.8
LU	67	65.7	85.1	9.0	4.5	0.0	100.0	61.2
Total	157	56.1	42.7	6.4	3.8	1.3	73.2	34.4

Of the five tested viruses, TSWV and TMV were the most common, found in 105 (17.5%) and 88 (14.7%) samples of all tested weeds, respectively (Table 2). These two pathogens were also the most frequently detected in tobacco; of the 157 samples of plants with disease symptoms, 88 (56.1%) and 67 (42.7%) were infected with TSWV and TMV, respectively (Table 3). There remaining three viruses were less frequently detected, found in 2.2–5.0% of weeds and 1.3–6.4% of tested tobacco samples. Although, TRSV may locally occur at higher frequencies (it was found in 20.0% of weeds in KP province) what can be explained by its higher dependence on local soil environment because this virus is transmitted by nematodes.

RT-PCR confirmed the results of serological analysis of the selected 43 tobacco samples. PCR product of size expected for TSWV was amplified for 41 out of 42 samples that tested positive in DAS-ELISA test. TMV specific product was detected for 23 samples while 17 of them had this virus detected in serological test. These results show that false positive results due to cross-reaction of antibodies in DAS-ELISA in our study are unlikely. For TMV, RT-PCR appeared to be a more sensitive detection method compared to DAS-ELISA. In case of other viruses only small discrepancies were found between the two detection methods (Table S1). For 17 out of the 43 tested tobacco samples single infection with only TSWV was confirmed. These samples will be retained in the long-term collection in −80 °C and used in the future as inoculum for TSWV inoculation tests necessary to select tobacco breeding lines resistant to this virus.

Mixed infections (with more than one virus per plant) were detected for 63 (10.5%) tested weeds and 54 (34.4%) tobacco samples. The most frequent coinfection was identified for the viruses that were the most frequently detected; based on DAS-ELISA results, 37 weeds and 43 tobacco plants were infected with both TSWV and TMV. However, there were also plants that tested positive for all five viruses (Table S2). Weeds with such coinfections belonged to Asteraceae family and were identified as *Achillea milefolium*, *Sonchus oleraceus* and *Crepis tectorum*. Generally, viral infections and co-infections were more frequently detected for weeds from this family compared to other families (Table 4).

Viral infections with at least one virus were more frequently detected for annual weeds compared to long lived species (Table 5). This result is surprising because annual species have less time to accumulate pathogens. However, annual weeds tend to be efficient colonizers, hence they may grow closer to crop plants in a field and exchange viral pathogens with them.

Table 4. Number and percentage of weeds infected with one or more than one virus and belonging to Asteraceae family vs. other families.

Family	No. of Plants	Number and Percentage (%) of Weeds Infected with:	
		At Least 1 Virus	More Than 1 Virus
Asteraceae	170	64 (37.6%)	30 (17.6%)
Other families	430	112 (26.1%)	33 (7.7%)
Pearson's Chi^2	Chi^2	7.909	12.894
	df	1	1
	p-value	0.0049 *	0.0003 *

* statistically significant $p < 0.05$.

Table 5. Number and percentage of infected weeds with different life cycle: annuals vs. perennials.

Life Cycle #	No. of Plants	Number and Percentage (%) of Weeds Infected with:	
		At Least 1 Virus	More Than 1 Virus
Annuals	349	114 (32.7%)	39 (11.1%)
Perennials	251	62 (24.7%)	24 (9.6%)
Pearson' chi^2	chi^2	4.467	0.404
	df	1	1
	p-value	0.035 *	0.524

Category annuals includes plants which were not reported to live longer than one year. All other plants with longer or variable life span are included in perennials category * statistically significant $p < 0.05$.

This study provides valuable information on the prevalence of important viruses threatening tobacco fields in different regions of Poland. TMV was the most frequently detected in LU province (Tables 2 and 3), where the highest number of tobacco farms and approximately half of domestic tobacco cultivation area are located [17]. This result confirms observation that tobacco mosaic is the most frequently observed in tobacco in the south-east of Poland [12]. TMV is a very persistent virus; for many years after harvest it may remain infectious in the soil, plant debris or even in water from irrigation ditches surrounding fields [12,25]. There are very few TMV resistant tobacco cultivars and they are not commonly grown in Poland [26]. Growing the same susceptible crop in subsequent years may lead to accumulation of virus inoculum in the field. Therefore, crop rotation is recommended as a highly effective method of controlling TMV in tobacco cultivation [12].

Detection of TSWV in all regions of the country, including fields in remote areas in ZP province, confirms ubiquitous nature of the virus. It is also present in weeds even in the coldest, north-east area (PD), where TSWV symptoms were never observed in tobacco fields. The spread of TSWV epidemics in this crop in Poland is observed since 1950, when it was first observed near town Zamość (LU province); by 2007 it was recorded in the whole southern part of the country [27]. Recent observations of tobacco fields, regularly performed every year, showed that TSWV is nowadays quite common in Kujawsko-Pomorskie (KP) province but still absent in Podlaskie (PD) province (M. Przybyś–unpublished data). This study showed that TSWV is present in weeds in PD province, therefore local conditions affecting vector transmission could be investigated to explain low incidence of tomato spotted wilt in tobacco in this area.

TSWV is a very destructive virus for tobacco fields. Simultaneous infection of young plants may lead to complete loss of the crop [12]. Currently, there are no TSWV resistant tobacco cultivars suitable for production. Therefore, in the face of spreading TSWV epidemics, breeding efforts leading to obtaining such cultivars should be intensified. The collection of TSWV infected samples suitable for inoculation tests, acquired within this study, will be used for this purpose.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11081667/s1, Table S1: List of tobacco samples included in a survey of five viruses in Poland, Table S2: List of weeds included in a survey of five viruses in Poland.

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