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Epidemiology of Tomato Yellow Leaf Curl Virus in the Northern Regions of the West Bank, Palestine

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Abstract: A survey was carried out in 2011 to study the epidemiology of tomato yellow leaf curl virus (TYLCV) in tomato growing sites of the Tobas and the Jenin districts. The survey studied the population of the virus-inoculative whiteflies and the possible virus reservoirs throughout the year. The maximum TYLCV-inoculative whitefly population was recorded in Tobas, compared with the Jenin district in the different growing seasons of tomato. In the Tobas district, the inoculative whiteflies started to appear in March and reached the maximum value of 7% in July and August. In the Jenin district, the maximum inoculative whitefly population of 6% was recorded in August. Such whiteflies started to appear in May, which is two months after their appearance in the Tobas district. Furthermore, populations of inoculative whiteflies occurred when farmers started their tomato growing seasons. The appearance of inoculative whiteflies coincided with availability of the virus natural reservoirs which harbor the virus and support the vector during the crop-free period. Infected cheese weed mallows (up to 12%) and tobacco plants (up to 7%) were the virus sources for whiteflies throughout the year in the Tobas and the Jenin districts respectively. Infected volunteer tomatoes (up to 93%) play a secondary role in the virus epidemiology as they disappear during winter (crop-free period) in both districts.

Keywords – TYLCV; Inoculative whiteflies; Epidemiology; Palestine

Introduction

Tomato (*Lycopersicon esculentum*) is the most popular vegetable crop in Palestine. Its annual production is 204,000 metric tons which comprises about 32% of the total vegetable production in the country [1]. The crop is an important part of a diverse and balanced diet and provides colorful additions to any meal [2]. Also, Palestinian people use tomato fruit in their folkloric medicine [3].

Tomato yellow leaf curl virus (TYLCV) is the most devastating disease infecting tomato in many tropical and subtropical regions causing yield loss up to 100% [4, 5, 6]. Currently, in Palestine the virus is widely infecting tomatoes in northern regions of the West Bank including the Jenin and the Tobas districts. The highest rate of infection approaches 95% in the Tobas district during the summer growing season [7].

The virus has a quite broad host range, infecting plants belonging to solanaceae e.g. tobacco; malvaceae e.g. weed mallows; and lequiminosae e.g. beans [8, 9]. The virus, much like other Gemini viruses, is not seed-transmissible [10]. The causal agent of the disease has been isolated and identified as a single-strand DNA containing geminivirus species of the genus Begomovirus, and most of which posses a monopartite genome [11, 12].

The tropical whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is the known vector for TYLCV [13, 14]. The insect is the most noxious pest attacking field and greenhouse crops including tomato around the world [15].

Tomato production in Palestine does not reach its full potential due to the high rate of TYLCV infection which is the key factor responsible for continuous crop failure [1, 7, 16]. Whitefly population was reported to have two remarkable peaks in the northern regions of West Bank without paying any attention on the percentage of the inoculative whiteflies in the region [7]. Therefore, this research has studied the virus epidemiology in the northern regions of the west bank. Also, the research has studied the inoculative whiteflies and natural plant reservoirs which support the whiteflies before and during growing seasons. The northern regions were selected for this study as they are the major tomato growing sites in the country. The annual tomato production in these regions comprises 47% of the total tomato production in the West Bank [1].

Materials and Methods

Regions of study

Three regions were selected in the Jenin and the districts since they are the major contributors for tomato production in Palestine. The fields were selected in Al-Zababdeih and Qabatya regions to represent the Jenin district, whereas the fields of Al-Far'a region were selected to represent the Tobas district (Plate 1).

Plate 1: A map of the Jenin and the Tobas districts showing the studied regions

In addition the selected regions have the following weather conditions as reported by the Palestinian Meteorological General Directorate in 2011 (Table 1) [17].

Table 1: Meteorological conditions of the studied regions in 2011

Weather Attribute	Mean Value
Air temperature	21.8 Degree Celsius
Rainfall	336.5 Millimetre
Relative humidity	64%
Evaporation	2,102.7 Millimetre
Wind speed	6.6 km/hour

Growing seasons

Tomato is planted in the studied regions in different growing seasons including spring (March to April), summer (May to June) and autumn (July until September). The summer growing season is perhaps the most important as farmers usually cultivate tomato in larger areas. On the other hand, parts of the tomato-growing sites in the Jenin district are used during the summer season for tobacco cultivation which may increase the possibility for some residual tobacco plants to contaminate the soil as volunteers that harbour the virus in these sites.

Occurrence of TYLCV in plant reservoirs

The selected fields were visited monthly for sample collection starting from January until December 2011. Leaf samples of volunteer tomato, tobacco and cheese weed mallow (*Malva parviflora*), the common weeds of tomato sites, were randomly collected from the top part of plants growing in approximately ten hectares of the studied area. Fifteen samples were collected from each plant type in every visit and prepared for polymerase chain reaction (PCR). Tobacco samples were collected only from the Jenin district because this plant was not found in any of the selected locations in the Tobas district.

DNA extraction

The method of DNA extraction was done according to [18]. The leaf samples were crushed in liquid nitrogen and extracted in 50 ml extraction buffer (200 mM tris-HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) then sodium acetate (3M) (pH 5.2) was added. After 5 min centrifugation, the supernatant was treated with chloroform-isoamylalcohol (24:1,v/v), then precipitated by isopropanol. After centrifugation at 13,000 rpm, the pellet was washed with 70% ethanol, dried and resuspended in 12 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0).

PCR testing

The PCR was employed as described by Navot *et. al.* (1992) using TYLCV-specific oligonucleotide primers [19]. Sub-genomic fragments of the virus genome were amplified. The primers were purchased from the Alltech Company, Paisley, UK. The primer sequences were from 5' to 3', P1V, ATACTTGGACACCTAATGGC, nucleotides (nt) 61-80, and P4C, TGGACATCTAGACCTAAG, nt. 2054-2071. The sequence of the P1V corresponds to the viron positive strand, whereas the P4C is complementary to the viron strand.

Development of inoculative whitefly population

The population trend of inoculative whiteflies was monitored throughout the year (January-December, 2011) in the studied areas. Whitefly samples were collected randomly from the top part of two hundred plant hosts scattered in one hectare of the tomato growing sites. The samples were collected using a cordless rechargeable vacuum cleaner adapted to collect insects into a plastic cylinder (vacuum sampling). The samples were transferred to laboratory within 2-3 h using icebox, identified and prepared for immuno-captured (IC)-PCR.

IC-PCR testing

The IC-PCR was employed as described by Sawalha (2009a) using TYLCV-specific polyclonal IgG [20]. IC-PCR was used because of a close relationship between the capsid's retention in the whitefly vector and the virus's transmissibility as reported by Sawalha (2009b) [21]. The antibodies were cross-absorbed overnight with acetone-washed non-inoculative whiteflies. The reaction was employed using TYLCV-specific oligonucleotide primers as pointed above.

Whitefly migration from plant reservoirs to tomato fields

An area of about 80 square meters of volunteer tobacco in Al-Zababdeih region, fully infested with whiteflies, was dusted with a day light fluorescent dust "Fire Orange," using a mechanical hand duster. Whitefly migration was recorded by positioning yellow sticky traps in tomato fields at various distances from the dusted plants. The traps were monitored daily for the appearance of fluorescent whiteflies [22].

In Al-Far'a region, a rectangular area of about 120 square meters of cheese weed mallow, 6 kilometers west of the main tomato production site was sprayed similarly with the fluorescent dust. The whitefly migration, from the infested area, toward the tomato fields was monitored daily as pointed above. The distance between the dusted areas of both districts was about 15 km.

Statistical analysis

Sample collection from the studied regions was done according to the standards of the Completely Randomized Design (CRD) where each reading was based on fifteen replicates from the same plant type. The Two-Sample Tests of Proportions (TSTP) was used to analyse of the data. The population difference of the inoculative whiteflies during the year was compared with the population at the starting points. The results were analysed using a level of significance when $\alpha = 0.05$ [23].

Results

PCR testing

A subgenomic fragment of TYLCV with a fragment length of 2027 base pair (bp) was amplified by a combination of P1V (20-mer primer from the intergenic region) with 18-mer primer (P4C). The PCR was able to detect TYLCV from both inoculative whiteflies and infected plants by producing clear DNA bands when electrophoresed in agarose gel for 90 minutes (Plate 2).

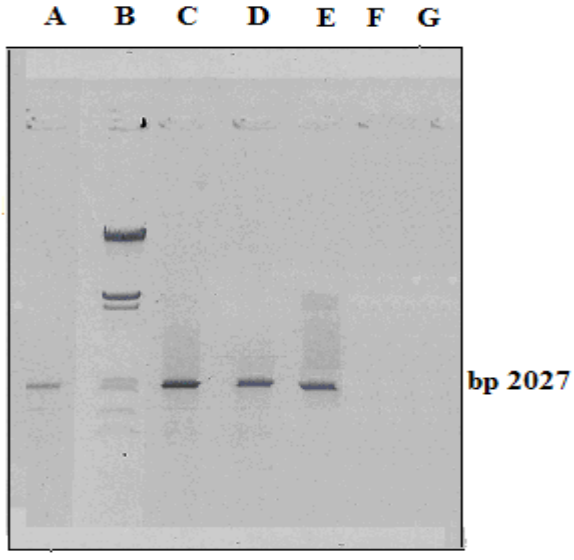


Plate 2: Agar gel electrophoreses of amplified PCR products of TYLCV DNA from infected plants and inoculative whiteflies using P1V and P4C primers.

Lane A: Inoculative whitefly
Lane B: DNA size marker (*Lambda Hind III Eco R1*, 123-21226 bp).
Lane C: Infected tobacco sample
Lane D: Infected tomato sample
Lane E: Infected cheese weed mallow samples
Lane F: Non inoculative whitefly

Lane G: Sap mixture of healthy tobacco, tomato and cheese weed mallow plants.

Occurrence of TYLCV in plant reservoirs

PCR tests showed that the virus was infecting cheese weed mallows throughout the year in Al-Far'a region. In addition, the virus infection of volunteer tomatoes started from May and increased to reach the maximum value during August. The maximum infections of both volunteer tomato and cheese weed mallow were 93% and 12% respectively (Fig 1).

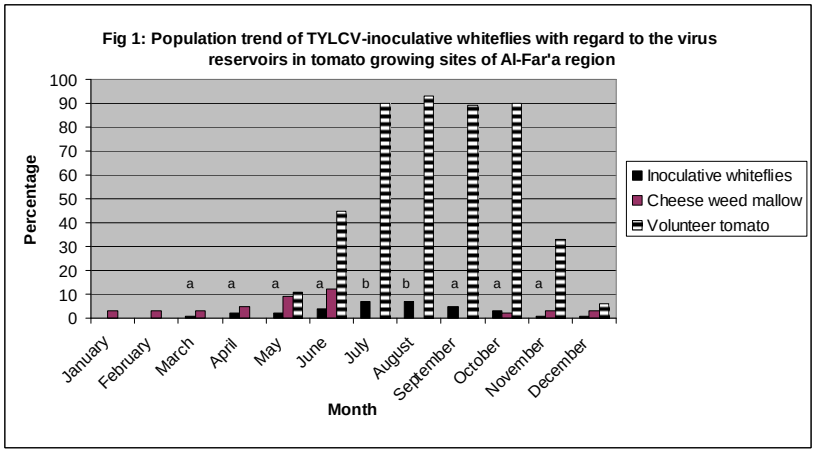
A different situation was recorded in both Al-Zababdeih and Qabatya where the infected volunteer tobaccos appeared throughout the year. The maximum rate of infections for such virus reservoirs in these regions were 7% and 6% in Al-Zababdeih and Qabatya respectively. In the case of cheese weed mallows of both regions, the first virus infection was recorded in March and increased to the maximum value of 12% in June. In both regions, the first record of virus infection of volunteer tomatoes was in May. The virus infection of those plants increased in October to the maximum rates of 75% and 74% in Qabatya and Al-Zababdeih respectively (Fig 2 and 3).

Development of inoculative whitefly population

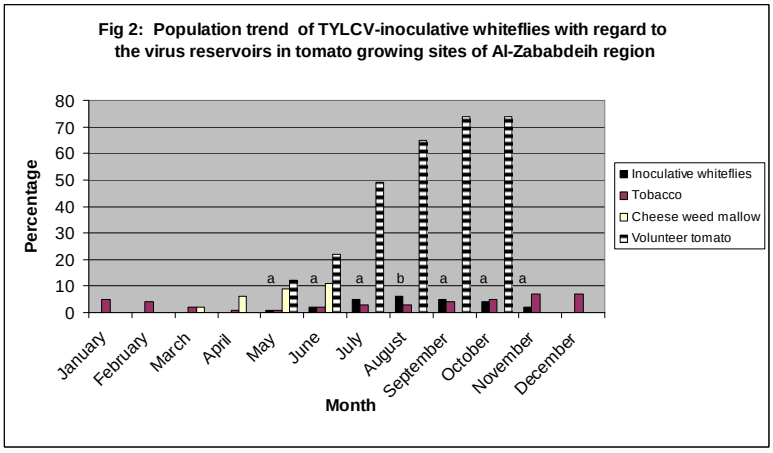
The results showed that inoculative whiteflies in Al-Far'a region started to appear in March and increased rapidly until they reached the maximum population of 7% during July and August. Furthermore, the inoculative whiteflies in this region remained until the end of growing season in December. Statistical analysis revealed a significant difference of the inoculative whiteflies population in July and August compared with the population at the starting point in March (Fig 1).

In both Al-Zababdeih and Qabatya regions, the whiteflies started to appear in a low population during May then increased to reach the peak during August. The maximum whitefly population was 6% in both regions. Statistically, the significant difference of the whitefly population was recorded only in August when compared with the population at the starting point in May.

A sudden spike of inoculative whitefly population was recorded after the first tomato transplantation during May in the studied regions. In addition, the population of inoculative whitefly started to decrease after October in both districts (Fig 2 and 3).



* Similar letters indicate no significant difference for inoculative whitefly population



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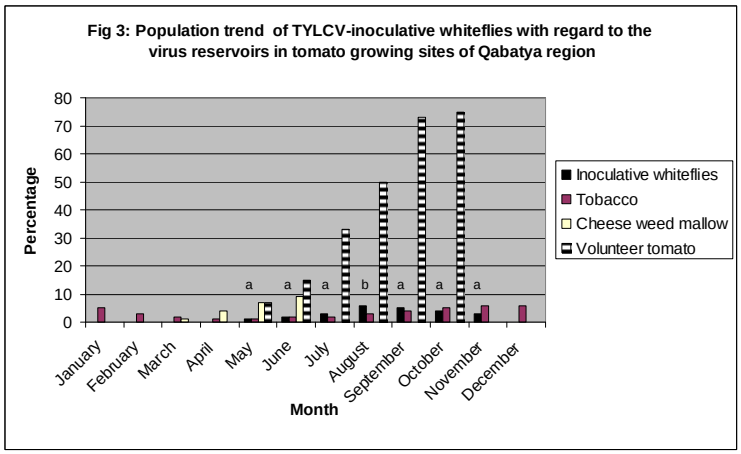
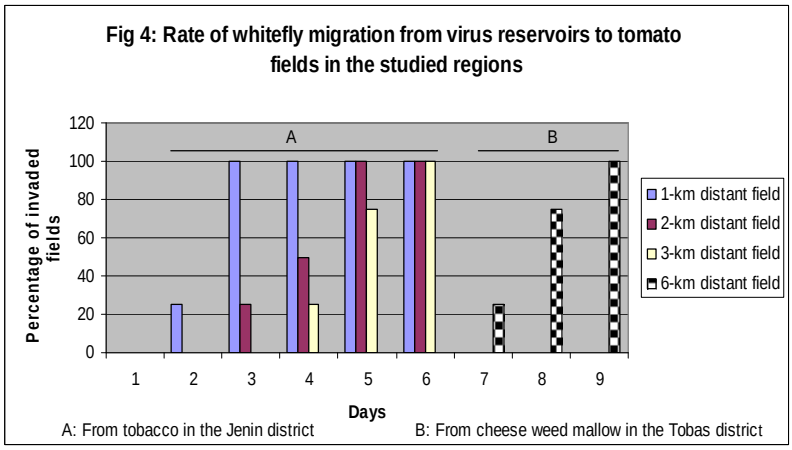


* Similar letters indicate no significant difference for inoculative whitefly population



Whitefly migration from plant reservoirs to tomato fields

The results showed that fluorescent whiteflies started to approach tomato fields, at a distance of 1-km from the dusting site, two days after dusting in the Jenin district. In addition, the whiteflies needed three and four days to reach fields 2- and 3-km away from the dusted volunteer tobacco. In the Tobas district, the fluorescent whiteflies crossed a distance of 6-km and started to approach tomato fields one week after dusting. There was no record whatsoever of overlapping between the two dusted sites in the two districts that are 15 km apart and separated by huge mountains and populous urban areas (Fig 4).

Discussion

The current research highlights the essential role of inoculative whiteflies and virus plant reservoirs in the epidemiology of TYLCV in tomato growing sites. The population of those vectors is affected by the natural host plants which act as epicenters that enhance the virus outbreak in the region. The presence of virus reservoirs in the tomato growing sites especially volunteer tomato and tobacco plants may be attributed to poor agricultural practices, reckless behavior of traditional farmers, planting tobacco in tomato growing sites and extensive tomato cultivation. The suitable agricultural land in this region is very limited and farmers tend to use such available area extensively. As a result of that several crops especially tomato and tobacco are present in the same field or nearby fields. On the other hand, most farmers use traditional methods and practices as the majority of them are neither literate nor educated using experience passed down from their ancestors over the decades [24]. So, the competence of those reservoirs to maintain the virus and give a primary source of inoculum to newly established tomato crops is reasonable. Such a situation becomes more probable when those virus reservoirs are present in the same area with tomato crops. Although there is no definitive threshold number of those plant reservoirs, when their number becomes high enough they act as a potential source of the virus to tomato crops [4]. The infection of those crops may be enhanced by the favorable weather conditions of the region which activate the whitefly vectors for longer periods during the year [17]. Such conditions may also increase the possibility of virus transmission to natural host plants ([25, 26]. Al-Musa (1986) reported that volunteer tomatoes growing near hedges surrounding citrus groves and along irrigation canals are reservoirs of TYLCV in the Jordan Valley [27].

Although volunteer tobacco plants have a lower rate of infection, their presence throughout the year enables them to be a potential primary virus source in the Jenin district. In this regard, Ioannou and Hadjinicolis (1991) isolated TYLCV from tobacco planted near tomato fields in

Cyprus. Thus, the crop was considered a secondary host plant that could potentially act as a natural reservoir for TYLCV [28]. Al-Musa (1986) reported that tobacco serves as an important reservoir for TYLCV in the Jordan Valley [27]. On the other hand, cheese weed mallows were important virus reservoirs that play a significant role in the virus epidemiology in both districts. The plants survive and harbor the virus during the crop-free period of winter which enables them to be a primary virus source to newly established tomato fields. They start their active growth in the spring which is concomitant with the increase in the whitefly population and the tomato-transplanting period [7]. Although those tobaccos and cheese weed mallows were growing not far from the tomato-growing areas, the whiteflies could move this distance and transmit the virus to tomato fields. In this regard, Cohen *et. al* (1988) reported that whiteflies could transmit the TYLCV from *Cynanchum acutum* virus source, 7 km away from the tomato-growing sites in the Jordan Valley [22].

Despite the fact that low level of inoculative whiteflies was detected in tomato growing sites, they have a critical importance in the virus epidemiology. However, rough estimation showed that even if the percent of inoculative whiteflies is low during the population peak, 2,000-20,000 whiteflies land weekly on each square meter, thus ensuring high TYLCV infection within a short time (two months). A possible explanation for the relatively low percentage of viruliferous whiteflies within the field population may be due to the periodic acquisition phenomenon [29]. In this regard, Cohen *et. al*. (1988) reported that only 5.4% of the whitefly population collected on *C. acutum* near tomato fields was inoculative in the Jordan Valley [22]. A low percent of inoculative whiteflies (0.18-0.67%) was found also in the case of African cassava mosaic virus (ACMV) [30]. The jump in the population of inoculative whiteflies when farmers started their tomato growing seasons may be attributed to multiplication of the virus sources. In such a case, severe qualitative and quantitative yield reduction may reach 100% [4, 31].

Conclusion and recommendations

Recognition of factors involved in the epidemiology of TYLCV may lead the way to efficient control of the disease by tackling the cycle at its weakest point. So, it is extremely important to be aware of the naturally infected hosts of TYLCV in order to be able to take control of and manage the virus. Ioannou (1987) observed a significant decrease in the disease incidence when the sources of inoculum were eliminated from tomato growing sites [32]. Therefore, the removal of volunteer tomato and tobacco plants, as well as cheese weed mallows from tomato growing sites is necessary to control the disease. In addition, controlling of those plants in March to May before the beginning of inoculative whitefly appearance may limit the spread of the virus into tomato fields. On the other hand, spraying pesticides to control the vector should be started with the time of tomato transplantation in the Tobas district while such a step can be delayed about one month after the crop transplantation in the Jenin district. Implementing the aforementioned procedures may provide a satisfactory level of disease control and prevention.

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