World Applied Sciences Journal 33 (1): 63-68, 2015
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2015.33.01.14566

Viral Infections in Potato Fields in Relation to Aphid Population

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Abstract: The performance of three potato varieties namely Granola, Diamant and Cardinal were evaluated in respect to potato leaf roll virus (PLRV) and potato virus Y (PVY) in relation to aphid population build up in the field. Data were collected on three growth stages of the plant namely early (emergence to 30 days after planting, DAP), mid (31 to 60 DAP) and late (61 DAP to 7 days before harvesting). The virus prevalence percentages in three potato varieties varied depending on early, mid and late stage of infection as well as potato varieties. It ranged from 15-34% for potato leaf roll virus (PLRV) and 22-51% for potato virus potato virus Y (PVY). Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) detected the viruses as PLRV and PVY. There was a strong and significant quadratic polynomial relationship ($y = -0.0125x^2 + 0.5482x - 3.7141$ & $R^2 = 0.5978$) between temperature and aphid population build up in potato field. The relationship between relative humidity and aphid population build up in the field was found significant but negatively correlated ($y = -0.0041x^2 + 0.6297x - 21.698$ & $R^2 = 0.5562$). The increase of aphid population in the field was positively correlated with the spread of PLRV and PVY in the potato field ($y = -0.7023x^2 + 1.1355x + 1.4692$ and $R^2 = 0.4089$). Among three potato varieties, Cardinal was more sensitive to both viruses, PLRV and PVY. In both cases the minimum virus prevalence were recorded in Granola. The virus prevalence was found higher at mid stage followed by late and early stage of infection in all the tested varieties. Together, these studies substantially increase our knowledge on environmental factors affecting aphid population and virus transmission in potato fields.

Key words: PLRV · PVY · Prevalence · DAS-ELISA · Aphid · Environmental factors

INTRODUCTION

Potato is one of the leading staple food crops of the world. The yield potential and food value compared to rice and wheat, potato is considered as a promising food crop to feed the hungry people of the world where food shortage is a chronic feature [1, 2]. The gradual loss of yield potential of potato has been recognized as one of the major constrains of potato cultivation [3]. Among the factors responsible for low yield of potato, virus diseases are regarded as most important ones [4]. Potato virus diseases have received very little attention because of the limited knowledge on viruses and lack of capacity to accurately detect and identify viral infections. This has resulted in growing problem of virus infection in the seed potato crop [5]. Among the virus diseases, potato leaf roll virus (PLRV) and potato virus Y (PVY) hve been considered the most important ones [4]. Both viruses are transmitted by aphid vectors (*Myzus persicae* and *Aphis gossypii*) in the potato field [6, 7]. Since both PVY and PLRV are aphid-transmitted viruses, the aphid populations in the field solely determine the epiphytotic development of these two viruses. The yield loss due to PLRV and PVY may vary from 20-85% [8]. Since these aphid-borne as well as tuber-borne viruses can be spread from infected plants to healthy plants and from season to season, control of these viruses is very difficult. Sowing of tolerant variety with enough knowledge on environmental conditions is a valid option for disease management as environmental conditions play a crucial role in vector population build up and development of these viral diseases in epidemic form. So it needs in depth investigation on the spread of important viral diseases of potato in relation to vector population. The present study

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was undertaken to find out the relationship of aphid population with the prevalence of PLRV and PVY on three potato varieties under field condition.

MATERIALS AND METHODS

Seed tubers of three potato varieties namely Granola, Diamant and Cardinal were included in this investigation. The third generation potato tubers were planted on 20 November in 2.0m X 2.4m unit plot maintaining 60 cm row to row and 20 cm plant to plant distance during 2006-2007 cropping season. Intercultural operation like earthing up, irrigation, weeding etc. were done as when necessary. The plants were inspected everyday morning to note the appearance and development of the symptoms of PLRV and PVY starting from plant emergence to harvesting. The virus was identified on the basis of field symptoms as described by Brunt *et al.* [9] and Hooker [4].

Potato tubers were collected from the infected plants during harvesting. The infected tubers were spread over the floor for three months in diffused light for sprouting. Then, double antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) was performed of the sprouts against the antisera of Potato leaf roll virus (PLRV) and Potato virus Y (PVY) following the method as outlined by Clark and Adams [10] with some modifications. In this test, Patho Screen Kit presented by Agdia Incorporated, 30380 County Road 6, Elkhart, Indiana 46514 USA was used. The instructions written in the manual of Agdia Incorporated to perform the DAS-ELISA was followed. The sap was extracted from the sprouts of the tuber samples in extraction buffer at 1:10 ratio (tissue weight : extraction buffer volume). The extracted sap was poured @ 100µl per well of the ELISA plate which was precoated with virus specific IgG. The plate was then incubated in a humid box at room temperature for 2 hr. After incubation the plate was washed with washing buffer and then enzyme conjugate was dispensed @ 100 µl per well. The plate was incubated and washed following the same procedure mentioned before. The substrate solution was prepared following the instructions in the manual and used @ 100 µl per well. The plate was incubated in a humid box at room temperature for 60 minutes just after 60 minutes of incubation and 50 µl of 3 M sodium hydroxide were added to each well to stop the reaction. The plate was then observed visually to detect the development of yellow color incase of positive reaction. The optical density (OD) values were measured using a microplate reader (EAR 400 FW, SIT-LABINSTRUMENTS) at 405 nm wavelength.

Aphids were monitored by placing yellow traps in the field as suggested by Moericke [11]. Three yellow traps were placed in the experimental field to find out the aphid population and spread of these viruses from plant emergence to harvesting. Half of the yellow traps were filled with tap water and 2-3 drops of liquid trix were added to the water so that aphid could not fly after falling into the traps. The measurement of each yellow trap was $49.5\times32.5\times8.0$ cm^3 with an angle of 65°. The traps were placed above 1.0 m from the ground. The number of aphids fallen on the traps were counted every day between 9 to 10 AM up to the date of harvesting. The water of the traps was changed every day after counting the trapped aphids. Data on the temperature and relative humidity were collected from the nearest meteorological station. The data on the prevalence of PLRV and PVY was collected at three stages of the plant growth categorized as early (emergence to 30 days after planting, DAP), mid (31 to 60 DAP) and late (61 DAP to 7 days before harvesting) stage. The factorial experiment was laid out in the randomized complete block design with four replications. The means of different parameters were compared by Duncan's multiple range test (DMRT) at 5% level of significance using MSTAT-C software.

RESULTS AND DISCUSSION

Prevalence of PLRV and PVY: The results on prevalence of PLRV and PVY infection at three growth stages (early, mid and late) of three potato varieties observed in the experimental field are presented in Table 1. In all the three potato varieties, prevalence of PLRV and PVY infection was found significantly higher at mid stage as compared to late and early stage of plant growth. The maximum percent of PLRV and PVY infected plants were recorded as 34% and 51% from the variety Cardinal. In both cases minimum percent (15% and 22%) of infected plants were recorded in Granola. The results of the present study indicated that potato variety Granola performed better against the prevalence of PLRV and PVY infection compared to the others under field conditions. The prevalence of infection was found to be varied depending on the potato varieties (15-34% for PLRV and 22-51% for PVY) and stages of infection (3-17 for PLRV and 5-31 for PVY). Among the two viruses, the incidence of PVY was higher than that of PLRV in the same variety. This could be due to the fact that PVY is transmitted in non-persistent manner while PLRV is transmitted in persistent manner. Because of the short acquisition and inoculation feeding time, infective aphids are capable of

Table 1: Prevalence of PLRV and PVY on three potato varieties

	Percentage of prevalence									
	PLRV					PVY				
	Stages of growth					Stages of growth				
Varieties	Early	Mid	Late	Average	Healthy	Early	Mid	Late	Average	Healthy
Granola	3 f	8 bcd	4 ef	15	85	5 e	10 cd	7 de	22	78
Cardinal	7 cde	17 a	10 bc	34	66	8 cde	31 a	12 c	51	49
Diamant	4 ef	11 b	6 def	21	79	7 de	18 b	11 cd	36	64

Data with same letters in row or column (in each virus) are not significantly different at 5% level by DMRT among the treatment means of potato varieties, virus infection and infection interaction.

Table 2: Results of DAS-ELISA

	Reaction against the antibody of			
	PLRV		PVY	
Varieties	VO	OD	VO	OD
Granola	+	0.61-1.25	+	0.83-1.08
Cardinal	+	0.68-1.75	+	0.92-2.48
Diamant	+	0.83-1.88	+	0.88-1.51
Positive control	+	1.88-2.52	+	1.92-2.68
VO = Visual observation	+ = Positive response		PLRV = Potato leaf roll virus	
OD = Optical density			PVY = Potato virus Y	

acquiring PVY within seconds to minutes and able to transmit within seconds of probing an uninfected plant [12, 13]. Among three potato varieties, Cardinal was more sensitive to both the viruses. Similar observations on varietal performance against PLRV and PVY were noted by Brunt *et al.* [9] and Lakra [14].

Serological Detection: The results of Double Antibody Sandwich Enzyme-Linked Immuno-Sorbent Assay (DAS-ELISA) for the detection of PLRV and PVY from the infected samples are presented in Table 2. It was observed that infected samples in all varieties have given positive response (+) against the antibody of PLRV and PVY. The results revealed that OD value 0.61 was the minimum which showed yellow coloration visually and regarded as positive reaction against PLRV. In case of PVY, the positive reaction was observed with the OD value 0.83. However, OD value ranged from 0.61 to 1.88 for virus infected samples under investigation and 1.88 to 2.68 for positive control, respectively. ELISA has been used widely to detect PLRV and PVY coat protein in infected plants since years [10, 15].

Spread of the Virus in the Field

Relationship of Temperature and Humidity with Aphid Population Build-up in the Field

Temperature: The average temperature in the potato field was 20.5°C when the experiment was started (December 12, 2006) which dropped down to 14.8°C in the next consecutive 30 days and then increased to 24.4°C in the following 60 days. In this period, aphid population declined from initial 96 at the first 10 days to 48 at the 4th 10 days and then increased to 516 in the 8th 10 days. After that aphid population again gradually declined to 118 in the subsequent 20 days (Fig. 1A). A quadratic polynomial relationship between temperature and aphid population build up in the potato field was observed as it is indicated by the equation $y = -0.0125x^2 + 0.5482x - 3.7141$ ($R^2 = 0.5978$) where the R^2 value was high and the relationship was strong (Fig. 1B). Temperature is the most important environmental factor that affects aphid behavior, development and reproduction. The development of aphid populations influenced by temperature was demonstrated by many scientists [16-18]. The temperature ranges from 20-25°C for the optimum development of aphid. Increasing temperature accelerates development, reproduction capacity and also enhances migration but reproduction declined and completely ceased at 30°C [19, 20].

Relative Humidity: The relative humidity percentages in the potato field during the experimental period varied from 70.9 to 88.3% (Fig. 2A). Number of aphid gradually decreased from 96 to 48 in the next 30 days which increased to 516 in the subsequent 40 days and then declined again to 118 in the next 20 days. In Fig. 2B, the

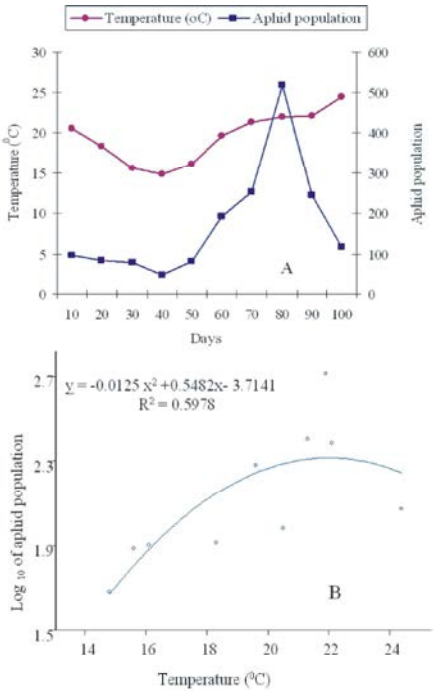

Fig. 1: Aphid population influenced by temperature (December 12, 2006 to March 13, 2007) (A) and relationship between temperature and aphid population (B) in the potato field.

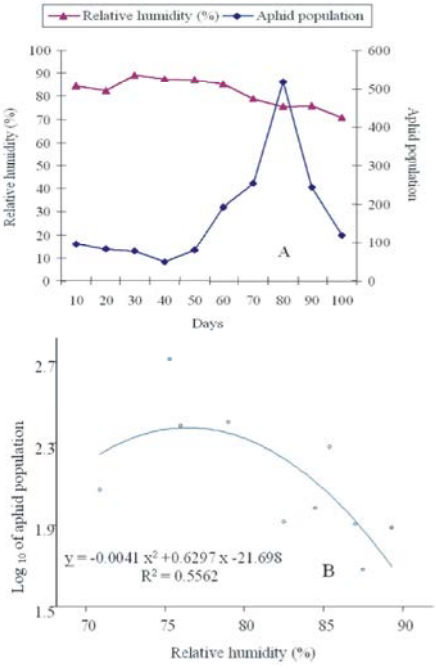

Fig. 2: Aphid population influenced by percent relative humidity (December 12, 2006 to March 13, 2007) (A) and relationship between percent relative humidity and aphid population (B) in the potato field.

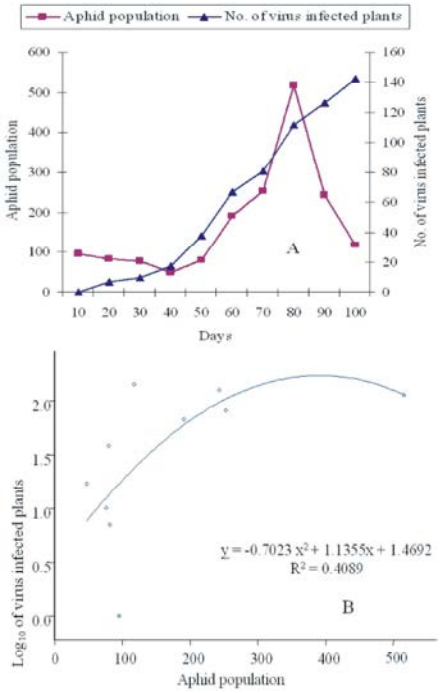

Fig. 3: Spread of PLRV and PVY infection in potato field in relation to aphid population during December 12, 2006 to March 13, 2007 (A) and relationship between aphid population and virus infection (B).

equation $y = -0.0041x^2 + 0.6297x - 21.698$ ($R^2 = 0.5562$) indicates a quadratic polynomial relationship between relative humidity and aphid population build up in the potato field. The relationship was somewhat significant but showed a negative trend indicating the negative effect of relative humidity on the aphid population build up in the field. The result is so far in agreement with the findings of Niaz and Ayub [21] and Karim *et al*. [22].

Relationship Between the Aphid Population Build-up and the Spread of PLRV and PVY in the Potato Field: The numbers of aphid caught in the potato field in every 10 days are presented in Fig. 3A. The results obtained in the present study demonstrated that the presence of increased number of aphid increased the number of PLRV and PVY infected plants in the potato field with few exceptions, while the number of aphid population gradually increased up to 516 and declined to 118. This might be due to the maturity of the plant, which did not favor the aphid. Whereas a steady increasing trend observed in respect of disease spread during the study period. This is due to increasing the population of viruliferous aphid and continuous symptom expression of previously inoculated plants in the field.

A quadratic polynomial relationship between aphid population build up and spread of PLRV and PVY in the field was found as indicated by the equation, $y = -0.7023x^2 + 1.1355x + 1.4692$ ($R^2 = 0.4089$) where the R^2 value indicates that about 40.89% of the disease spread can be explained by the aphid population (Fig. 3B). It is clear from this study that increase of aphid population is positively correlated with the spread of PLRV and PVY in the field. Also, Lakra [14] and Basky [23] showed that high aphid populations had significant effects on the proportion of PVY and PLRV infection in seed potato fields.

Results of the present investigation clearly show that none of the three potato varieties had adequate level of tolerance against PLRV and PVY though the prevalence varied from 15-34% for PLRV and 22-51% for PVY depending upon potato varieties. Virus prevalence was found higher at mid stage followed by late and early stage of infection in all varieties. The temperature and relative humidity were strongly related with the aphid population on build up in the field. Moreover, the increase of aphid population in the field was significantly correlated with the spread of PLRV and PVY.

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