PREVAILING AMBIENT CONDITIONS ON APHID POPULATION DENSITY AND TEMPORAL VIRUS DISEASE SPREAD IN THREE PEPPER (*Capsicum* spp. L.) CULTIVARS GROWN IN HUMID TRANSITION ZONE OF NIGERIA

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ABSTRACT

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Experiments were conducted in the early and late rainy seasons of 2013 to determine the effect of ambient conditions on aphid population density and spread of virus diseases in three cultivars of pepper (Rodo and Tatasai (C. annuum) and Sombo (C. frutescens). Seedlings were treated with cypermethrin while the controls were without treatment were transplanted to the field and laid out in a randomized complete block design with split plot arrangement and replicated three times. Leaf samples were taken and indexed for Pepper veinal mottle virus (PVMV) and Cucumber mosaic virus (CMV) using Enzyme-linked immunosorbent assay (ELISA). Data on rainfall, temperature and relative humidity were obtained from Agrometrological Station. Correlation analysis showed that vector population decreased with increase in rainfall and temperature at P ≤ 0.05.The lowest rainfall at less than 5 mm and the aphid population peak count of 230 coincided at 4 weeks after transplanting (WAT) in early season. Also,when rainfall was lowest at 3 WAT coincided with peak aphid population count of 150 in late season. The temperature of 25oC and relative humidity of between 70 and 80% in both seasons were observed during peak aphid populations.The highest weekly temporal spread of virus symptoms was 22.5% and 45.0% in the early and late seasons recorded the week after the peak aphid count was observed. PVMV and CMV incidences of 12.9% 7.4% and also 11.1% and 31.4% were detected in the early and late seasons. At such favourable ambient conditions recorded in this study, aphid vectors should be controlled to reduce the incidence of virus diseases on pepper.

INTRODUCTION

Pepper (*Capsicum* spp.) belongs to the family *Solanaceae* and Nigeria accounts for about 50% of African production (Erinle, 1989; Fajinmi, 2006) making Nigeria the largest producer of pepper in tropical Africa. Pepper provides nutritional and financial benefits to local farmers and it enhances food palatability and is also rich in vitamin C (Ali, 2006). They are consumed fresh and in a variety of processed products worldwide. In Nigeria, Pepper is cultivated principally in south western and northern parts of the country as well as in the northern guinea savannah and Sudan ecological zones (Erinle, 1989). However, there is a sizeable production of pepper in the rain forest and derived savannah of south western Nigeria (Opoku-Asiama *et al*., 1987; Fajinmi, 2010). According to FAOSTAT, (2013) Nigeria has a world share of 1.7 % in pepper production quantity. Cultivation of pepper has provided income to several farmers producing them even on a small scale level.

Nigeria produced about 60,000 tons of pepper in 2013 against the 65,000 tons produced in 2004. The production of pepper has several constrains, amongst which are losses to pests and diseases infestation (35- 90%, sometimes 100%) and a serious problem faced by farmers (RRA, 2007; Sutarya *et al*., 2009). The availability of commercial cultivars resistant to pests and diseases are still very limited and this may cause the farmers to rely more on the use of pesticides to control pests and diseases (Sutarya *et al*., 2009). Pests and diseases affecting pepper are numerous most causing severe economic damage, some of these diseases include: Bacterial spot, Bacterial Canker, Anthracnose, Fusarium wilt, Damping off, root knot, *Cucumber mosaic virus* (CMV), *Tomato mosaic virus* (ToMV), *Pepper mild mottle virus* (PMMV), and *Pepper venial mottle virus* (PVMV) (Valenzuela, 2011). Others include weeds, parasitic plant Dodder, cutworms, aphids, whiteflies, thrips, mites and pepper weevil (Sutarya *et al.,* 2009; Agbogidi and Okonmah, 2011. Different management tactics have been used to control pests and diseases of pepper, some have been effective, they include planting date and planting density (Duimovic and Bravo 1979), intercropping, spraying of insecticides, planting far away from tomato (AVRDC, 2004).

In Nigeria, PVMV is one of the major constraints of pepper production (Alegbejo and Uvah 1987; Fajinmi *et al*. 2011). The virus causes severe and devastating destruction of pepper plants; and it has been reported that they are efficiently transmitted in nature by aphids, which are often difficult to control (Gebré-selassie *et al*., 1983; Atiri and Dele 1985; Fajinimi, 2006; Fajinmi and Odebode 2007. It has been observed that in aphid-borne viruses, about 5% of the infections occur during the primary spread where the virus is introduced to the plant by aphids that pick up the virus from an external source such as a reservoir host. The other 95% of the infections are believed to occur during the secondary cycle where the virus is spread by aphids that acquired the virus within planting (Horvath and Nienhaus 1982; Fajinmi, 2006). Moreover, precise information on population dynamics of aphid vectors alighting on crops is essential in the development of effective forecasting methods to avoid or reduce the spread of the virus (Byamukama, 2008). Spatial and temporal analysis of viral diseases can be used to test hypotheses on the dynamics of virus spread, including those concerned with the importance of primary inoculums and the mechanisms involved in the spread of the pathogen (Moreno *et al*., 2007).

This study looked at the correlation between weather factors and spread of virus diseases in different cultivars of pepper, the population of aphids and its effect on virus disease incidence with a means to establish a relationship between them which will help to formulate management strategies.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Teaching and Research Farm, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state located between latitude 7° 14′ N and longitude 3° 26′ E at about 32 m above sea level. The field experiments were conducted between May and August (Early season) and August – November (Late season) of year 2013 using three pepper cultivars.

Planting materials and field operations

The pepper seeds used for this study were sourced from the local market (Lafenwa market) in Ogun state. The pepper cultivars used were Rodo (*Capsicum annuum)*, Tatasai (*Capsicum annuum*) and Sombo (*Capsicum frutescens*). A 200 m^2 land was mapped out for the experiment. The land was ploughed and stumps as well as debris were removed. Marking out of plots was done. A 3 x 2 factorial experiment in randomized complete block design (RCBD) was laid out in a split plot arrangement with three replicates. The sub-plot size measured 2 m x 2 m with 1 m space between sub-plots. This was replicated three times resulting in a total size of 18 m x 10 m. A planting distance of 0.5 m x 0.5 m was used for inter and intra row spacing. The plot was weeded manually to ensure weed free plots. The main plot was the pepper cultivars while the sub plots where the pesticide spray regime. The factors included the cultivars of pepper; Rodo, Sombo, Tatasai with two levels of insecticidal application i.e. (i) No insecticidal application and (ii) Insecticidal application (Cypermethrin 10% EC at 0.002 a.i. per 2 litres of water every seven days).The treatment combinations were: Rodo with no Cypermethrin, Rodo with Cypermethrin, Sombo with no Cypermethrin, Sombo with Cypermethrin, Tatasai with no Cypermethrin, Tatasai with Cypermethrin.

Raising of pepper seedlings and field transplanting

The pepper seedlings used for the experiment were planted on 6^{th} of April and 12^{th} July for early and late season of 2013, respectively. The seedlings were raised for eight (8) weeks in the nursery before transplanting to the field. Insect free and insect proof cage (20 mesh per cm to prevent infestation by vectors) was used for the nursery. The seeds were planted in buckets filled with sterilized top soil. The young pepper seedlings were sprayed with insecticide (Cypermethrin 10% EC) at 0.002 a.i. per 2 litres of water to control insects and also the fungicide (Mancozeb 80% WP) at 2 kg ha^{-1} against Fungi was used. Wetting of the pepper seedlings with water was carried out twice daily. Disease-free eight weeks old pepper seedlings of each of the cultivars were transplanted into the field on 1^{st} of June and 6^{th} of September for the early and late season respectively according to the following treatments described previously. Each sub plot measured 2 m by 2 m with a plant population of 25 pepper stands. Each sub plot with the treatment combination was replicated three times.

Determination of temporal virus spread

The temporal spread of the virus diseases was calculated as weekly percentage incidence over time. This was determined by expressing the number of new infections per week as a percentage of the total number of plants in the plot (Ndunguru and Jeremiah 1999).

Determination of weekly aphid population density

Yellow bowls to attract aphid vectors were placed in each plot (Evans and Medler, 1966), and filled with a solution containing 98% water, 1.5% detergent and 0.5% formalin preservative (Alegbejo, 2001). Trapped aphid vectors were collected once in a week and the aphids counted while the solution was changed every week.

Data on ambient conditions or factors

Data on amount of rainfall, relative humidity, and temperature for the duration of the experiment were obtained from the Agro meteorological Centre, Federal University of Agriculture, Abeokuta (FUNAAB).

Collection of leaf samples showing virus- like symptoms for serological analysis

From each plot, three diseased leaf sample were collected. The samples were packed in a sample bag and well labelled and immediately transported to the laboratory of the Department of Crop Protection, FUNAAB. The samples were chopped into tiny pieces and preserved over Calcium chloride ($CaCl_2$) before the serological indexing of the viruses.

Preparation of leaf samples for Enzyme linked immunosorbent assay (ELISA)

Serological indexing was carried out at the Biotechnology laboratory FUNAAB. Preserved samples of approximately 0.1g were used for the test. These leaf samples were ground in phosphate buffer saline with 0.05% v/v Tween- 20 (PBS-T) at pH of 7.4 plus 2% polyvinyl pyrrollidone (PVP) using a sample extraction bag. The extracted saps were then transferred into a 1.5 ml microcentrifuge tubes to hold the saps.

Detection of CMV and PVMV in pepper leaf samples using DAS- ELISA

The sap of the 54 samples collected per season were used in Double Antibody Sandwich ELISA (DAS-ELISA) with *Pepper veinal mottle virus* (PVMV), *Cucumber mosaic virus* (CMV) specific polyclonal antibodies sourced from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany as described by Clark and Adams (1977). The ELISA plates were each coated with PVMV and CMV immunoglobulins (IgG) diluted in a coating buffer as specified by the manufacturer and incubated at 37 °C for 2 hours. The plates were then decanted and washed three times with PBS-Tween using washing bottle and were left full of buffer at 3 minutes intervals. The plates were blotted dry by tapping on tissue paper. Afterwards, 100 µl of the test leaf sap were pipette into each well and incubated overnight at 4 °C and washed again. Using a pipette, 100 µl of respective antivirus antibodies in conjugate buffer were added to respective wells and incubated at 37 °C for 2 hours. The plates were washed with PBS-T as earlier described before blotting dry on tissue paper. With a pipette, 100 µl of substrate buffer containing p- nitrophenyl phosphate (PNP) tablet at the rate of 0.5 mg per ml was added into the wells. The absorbance of the contents of the ELISA wells was read after incubation at room temperature at 1 hour using a Mindray MR 96-micro plate reader at 405 nm. Samples were positive to PVMV and CMV viruses if the absorbance reading were double that of healthy control samples.

RESULTS

Influence of ambient factors on aphid population density on the pepper cultivars in 2013

In the early season of 2013, the rainfall pattern was steady at about 10 mm (between 2 and 3 weeks after transplanting (WAT) and decreased between 3 and 4 WAT to its lowest peak at less than 5 mm of rainfall. At the same period, the aphid populations increased steadily from 100 at 2 to to 230 at 4WAT and the peak aphid population was at 4WAT when the rainfall was lowest. The temperature remained steady at 2 to 4 WAT at about 25º C as the aphid population increased. However as the temperature increased above 25ºC and also as rainfall increased from 0 to 15 mm at 4 to 6WAT, the aphid population also decreased. The relative humidity was relatively steady between 70 and 80% between 2 and 4 WAT when the aphid population was on the increase. As relative humidity increased from 5 WAT to 6 WAT from between 80 and 90% coincided with the decreasing aphid population (Fig. 1).

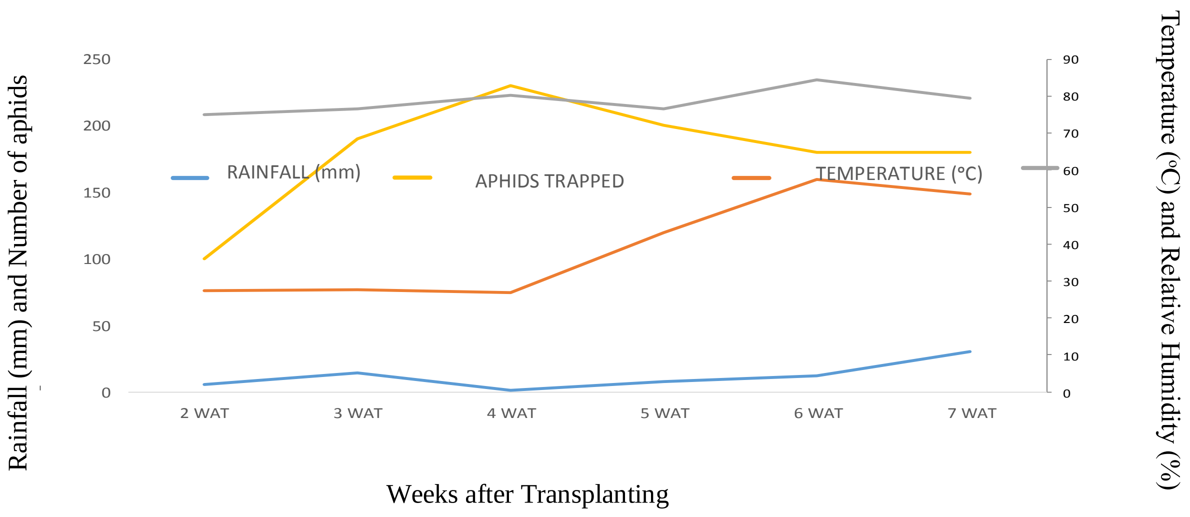

Fig. 1: Influence of ambient ecology on aphid populations trapped in pepper fields during the early season of 2013

In the late season, there was a sharp drop in rainfall from 2 WAT and it was lowest at 3 WAT. This also coincided with the peak aphid population at 3 WAT. The relative humidity was between 70 and 80% while the temperature was around 25º C at 3 WAT when the aphid population peaked (Fig. 2).
early season of 2013

Influence of aphid population on temporal virus spread in three pepper cultivars

It was observed that the percentage of virus infected plants increased weekly from 2 WAT and the highest percentage of infected plants was recorded one week after i.e the following week after the highest aphid count was recorded. The weekly percentage of infected plants decreased therafter as the aphid population also decreased (Fig. 3).

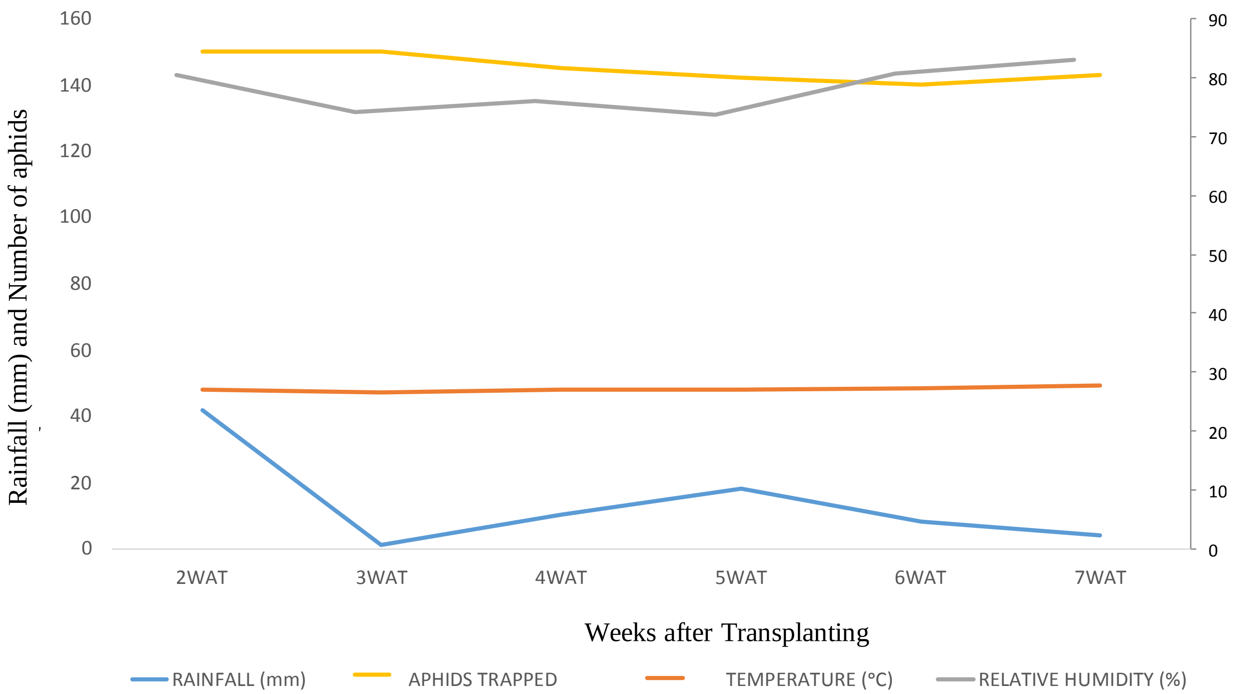

Fig. 2: Influence of ambient on population of aphids trapped in pepper fields in the late season of 2013

This trend was observed among the treatments (cypermethrin treated plants control). The percentages of infected pepper plants followed the same trend of increase and decrease over time.

In the late season, the highest weekly percentage of infected plants was recorded one week after the peak aphid population was recorded. The peak aphid population was recorded at 3WAT and the highest percentage of infected plants was observed 4 WAT. (Fig. 4).

Serological indexing of virus on pepper leaf samples

ELISA test was conducted and the results showed that 12.9% of the samples taken during the early season were infected with PVMV while 7.4% CMV was detected. In the late season 11.1% of the samples tested were infected with PVMV while 31.4% CMV was detected (Table 1).

Table 1: Detection of CMV and PVMV in pepper leaf samples collected in 2013

Season	No of samples indexed	CMV	PVMV
Early	54	4/54(7.41)	7/54(12.96)
Late	54	17/54 (31.48)	6/54 (11.11)

Cucumber mosaic virus (CMV), *Pepper veinal mottle virus* (PVMV)
* Figures in parentheses are percentage incidence.

DISCUSSION

Understanding virus, the way they spread, how they affect the plant can go a long way in formulating control measures to combat viral diseases. Ambient ecology cannot be ignored in epidemiology, as such, the ambient ecology of the farm area as well as the population of aphids trapped were noted in the study period. They were observed to influence the incidence of these virus diseases. The amount of rainfall was observed to affect the number of aphids trapped in the pepper fields, rainfall less than 10 mm seemed to favour higher aphid populations and vice versa, also peak aphid populations were recorded when the relative humidity was between 70 and 80%. Hasan *et al*. (2009) reported that high cloudiness, relative humidity, dew point favoured the aphid incidence and slight rainfall quickly declined the aphid population from the field. In West Africa, rainfall, temperature and wind were identified as key weather variables affecting virus diseases in cereal, vegetable and tuber crop production (Jerger, 2006). Ambient temperature of 25^{o}C favoured peak aphid populations in the field. Aphid transmission efficiency varies with temperature, with the highest temperature range from 20 to 25 °C (Jerger, 2006). Some researchers have reported lesser or higher temperature depending on the prevalent ambient ecology and aphid species. Muhmmad *et al*. (2010) reported higher maximum temperature of 30 °C, minimum temperature of about 13 °C, and relative humidity ranging from 60–70% were the most favorable environmental conditions for building up the aphid population beyond the economic threshold level while Kumar *et al*. (1997) reported that average temperature of 18.06 °C (Maximum 22.81and minimum13.31 °C) under the influence of high relative humidity with the range from 80.71% to 86.5% provided conductive conditions for aphid incidence .However Marek *et al.* (2014) reported that the population dynamics of aphids shows periodic fluctuation as a result of a factor like temperature.

In the early and late seasons it was observed that the higher the number of aphids trapped, the higher the disease incidence the following week. This study further observed that the week with the highest number of aphids trapped had the highest infection of which the symptoms became obvious in the following week. Prasannath *et al*. (2014) in their study observed a significantly positive relationship between the incidence of virus diseases and vector population. Similar reports by Rahman *et al*. (2006), Gupta (2000) and Paul (2002) have reported an increase of whitefly population and its positive correlation with the spread of tomato yellow leaf curl virus disease (TYLCVD) under field conditions. The high temperature could make the aphids hide under the leaves due to the intensity and the scorching heat. At such moment moving around of the aphid vector is reduced and thus aphids' population is reduced resulting in lower disease incidence (Kaushik, 2011).

The ELISA result confirmed the presence of CMV and PVMV in some samples collected and tested. However, not all the samples that showed virus-like symptoms tested positive to PVMV and CMV. The thought of other viral infections cannot be over ruled as there were other aphid transmitted virus infections that have been reported in pepper (Ayo-John, 2012; Arogundade *et al*. 2014). Over 68 viruses have been reported to infect pepper from various locations all over the world (Pernezny *et al*. 2003), more than half of these are transmitted by aphids while other vectors include nematodes, thrips, leafhopper, beetle, fungi or contact through the soil (Parisa *et al*. 2014). Although feeding by aphids directly affects plants, the most damaging effects are caused by the pathogens they transmit as they move among host plants (Radcliffe and Ragsdale, 2002; Ng and Perry, 2004; Hogenhout *et al*., 2008). Aphids were observed in the cypermethrin applied plots also with viral disease incidences. Aphids are difficult to control because they are transmitted in a non-persistent manner within a few seconds of the aphid probing even before feeding thus making the management of the virus diseases difficult (Fajinmi *et al*., 2007). The aphids transmit PVMV and CMV viruses in a non-persistent manner, within few seconds successfully. The aphids are therefore able to transmit the virus resulting in symptom appearance later before it is killed by the cypermethrin applied.

Forecasting disease development is important in controlling virus vectors and diseases in the field. Mathematical models and decision-support schemes have been developed for forecasting and control of virus diseases. The decision support system captures the effect of weather on the development, reproduction, movement and survival of aphids and it is used to predict how much secondary spread of the virus from the initial foci of infection has occurred (Jerger, 2006). The present knowledge of weather forecasting with decision support systems can also be used to predict vector occurrence and spread with a view to suggest to the farmers when to apply control measures. This will also require the development of man power and capacity building by integration and or reintroduction of plant disease epidemiology into the curriculum for higher degree learning in plant pathology, agriculture and related disciplines. At such favourable ambient conditions recorded in this study, aphid vectors should be controlled to reduce the incidence of virus diseases on pepper

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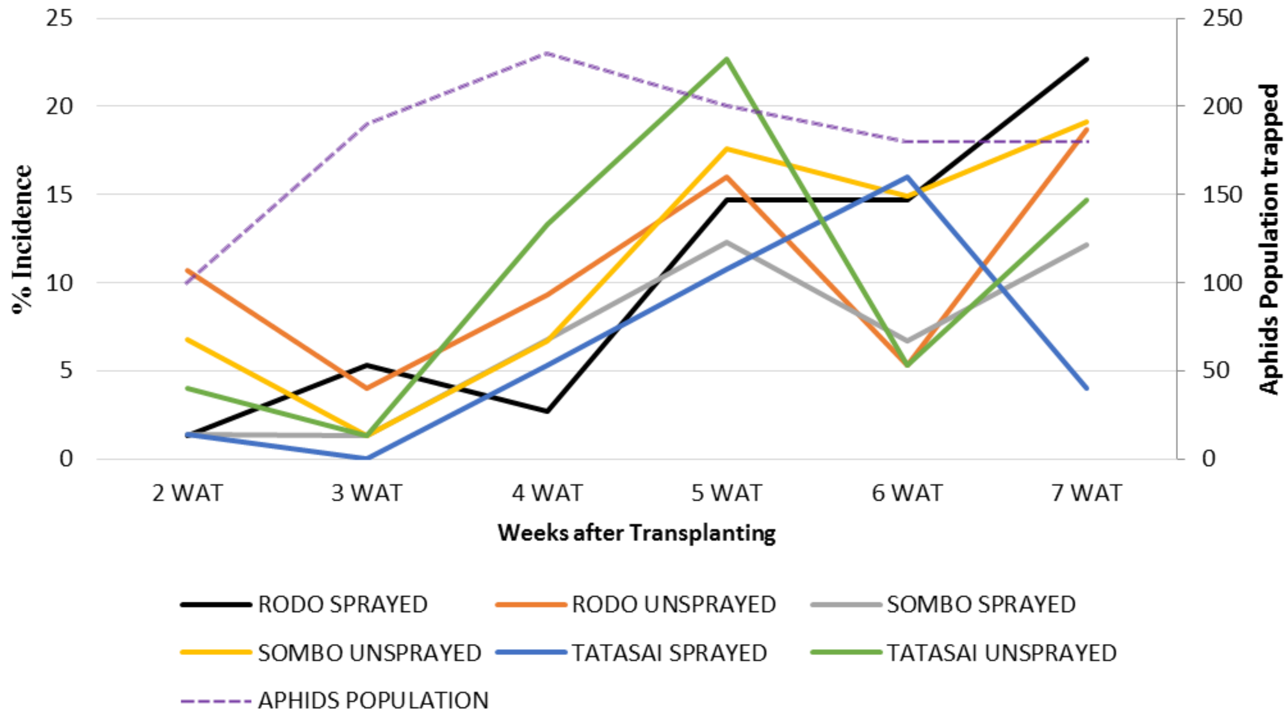

Fig. 3: Influence of Aphids population on the percentage of virus infected plants of three pepper cultivars during crop growth on the field in early season 2013

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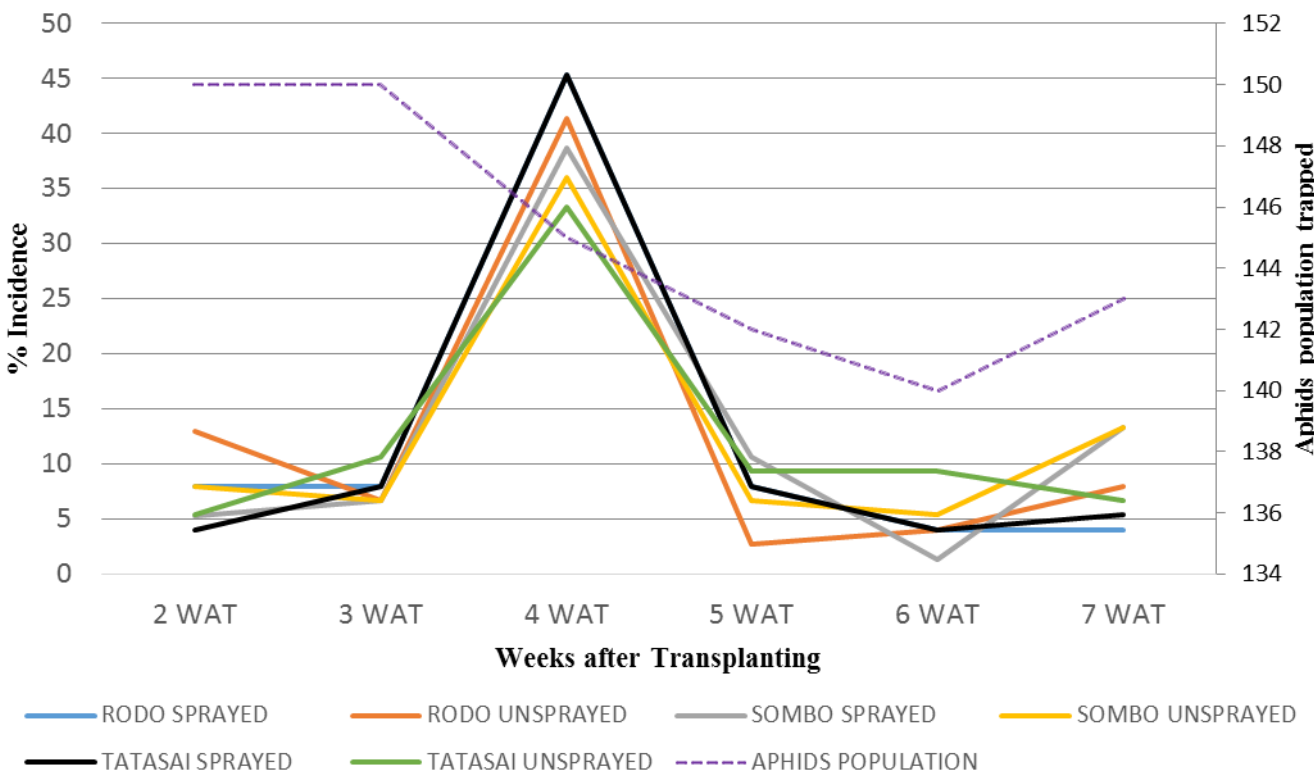

Fig. 4: Influence of Aphids population on the percentage of virus infected plants of three pepper cultivars during crop growth on the field in late season 2013

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