

## ROLE OF THE APHID IN TRANSMISSION OF *VERTICILLIUM* WILT TO ALFALFA

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### Abstract

The survey which was carried out in alfalfa plantations at Ismailia Agricultural Research Station in seasons 1998/99, indicated that the incidence of *Verticillium* wilt ranged from 1.9 to 16.6%. The highest percentage was recorded in June, while the lowest was in February. No wilt symptoms were found in January. Aphids were found throughout the year and their population ranged in between 1.3 and 13.2 insect per plant. Highest population was found in March, whereas the lowest was in August and September. Natural percentage of field aphids contaminated with *Verticillium* ranged from 1.3 to 7.5 % and caused 16.7 and 33.3 % infection, while artificially ones (laboratory strain) resulted in 76.7 and 90% wilted plants for cut and uncut plants, respectively. Spraying cut and uncut plants with the spores of the fungus caused 43.3 and 6.6% wilted plants, respectively. Treatment with *Verticillium* spores reduced the length and weight of shoot and root as well as protein contents in cut and to a greater extent in the uncut plants. Both aphids (laboratory strain) free from *Verticillium* and the artificially contaminated caused greater reductions of the growth rates.

### INTRODUCTION

Importance of alfalfa *Medicago sativa* has increased in Egypt to meet the increasing of local consumption of forage crops. Alfalfa occupies an important position mainly in the newly reclaimed areas to improve soil type and to avoid the competition with other ordinary crops.

Due to its perennial nature, pest complex associated with it is varied and increased, specially late in the season, when the plants are left to produce seeds. El-Adawy *et al.* (1996) recorded *Aphis spp.*, *Hypera brunneipennis*, *Thrips spp.* and *Liriomyza congesta* as pest species on forage crops in Ismailia Governorate.

Alfalfa is also subjected to stand injury and yield loss due to diseases caused by vascular wilt pathogens. Atkinsin, 1981 ; Christine and French 1982 and Billar, 1989

reported that *Verticillium* wilt can drastically reduce yield and affect the quality of hay.

Recently, numerous investigators confirmed that some species of insects including pests, predators and pollinators are important vectors of spores of some pathogens (Harper and Huang, 1984; Huang *et al.*, 1986).

The objectives of this study are to:

- Clarify the role of aphids in dissemination of the *Verticillium* wilt and
- Determine the effect of each of the aphids and *Verticillium* on plants height, yield (fresh & dry matter) as well as protein contents.

## MATERIALS AND METHODS

**1. Survey :** Alfalfa (*Medicago sativa* L.) was seeded on the 10<sup>th</sup> of November 1998 in four plots (200m<sup>2</sup> each.) at the farm of Ismailia Agricultural Research Station. Regular agricultural practices were carried out. For monitoring aphids, 100 plants/plots were picked randomly (around and across each plot), at monthly intervals and directly prior to cutting. Samples were put in paper bags provided with a piece of cotton saturated with ether as anaesthetic to insects and then transferred to the laboratory. Alfalfa plants of each sample were carefully inspected and the number of prevailed aphids was recorded.

To estimate percentage of aphid contaminated with *Verticillium* spores, 100 aphids individual (from each plot) were separately collected, each in a glass tube by the aid of fine brush sterilized in alcohol. Aphids were killed individually by sterilized forceps, then each was placed on PDA (Potatoes Dextrose Agar) medium in petri dishes and incubated at 20°C for one week. The cultures were examined for *Verticillium albo atrum*. The percentage of contaminated aphids was calculated.

To estimate the percentage of infected plants with *Verticillium* wilt, 10 random wilted plants from each alfalfa plot, around and across each plot, were sampled monthly through the growing season. Stem segments of the wilted plants were surface sterilized in 5% sodium hypochlorite solution for two minutes then rinsed several times in sterilized distilled water and dried between two sterilized filter paper. The sterilized dry stems were cut into small pieces then placed on PDA medium in Petri dishes for one week at 20°C. The growing colonies were checked for *V. albo atrum*.

Pathogenicity technique of Boodly and Sel Drake (1977) was employed to evaluate the pathogenicity of the isolated fungus, from each of aphid and wilted plant.

## 2. Role of aphid in the transmission of *Verticillium* wilt to alfalfa :

Aphids used in the present study were collected from alfalfa plants and reared in muslin 10 cages (0.60 x 0.60. x 1.00 m). Each cage contained four pots, each planted with 10 alfalfa plants. The cages were kept in a controlled green house. Aphids colony was reared for 3 months away from any contamination at  $25^{\circ}\text{C} \pm 2$  and  $50 \pm 5$  % RH., cages showing wilt symptoms were excluded.

Forty other cages containing alfalfa plants, were left for 8 weeks before carrying out the experiment. The plants in 20 cages were cut to approximately 6 cm high by sterile shear. The other cages were left without cutting. Sixteen hundred individual aphids were allowed to move across a culture of *Verticillium albo atrum* so they get contaminated with fungal spores. Four cages with whole plants and four cages with cut plants, each was provided with 200 contaminated aphids. The same number of cages, were infested each with 200 aphids free from fungual contamination. Eight cages each was supplied with 200 contaminated aphids (field strain) and 8 cages were sprayed with suspension of *Verticillium albo-atrum* spores. The remaining cages were kept away from being infested with aphids or contaminated the fungus and served as control. All cages were kept at  $25 \pm 2^{\circ}\text{C}$  and  $60 \pm 5$  % R.H. Four weeks after treatments, severity of symptoms were scaled on an 1 to 5 according to Ireland and Leath (1987) where: 1= green leaves, 2 = general chlorosis of leaflet, 3= leaf necrosis and leaf curl on < 50% of stem, 4 = leaf necrosis and leaf curl on >50% of plant and 5= wilted stem with chlorosis, necrosis and leaf curl. All plants of each treatment were checked for the infection with the *Verticillium* wilt.

## 3. Effect of both aphids and *Verticillium* on alfalfa plants phenology

**a. On plant height (the length of the stem) :** Length of stem of each treatment was measured after 4 weeks from the treatment.

**B. On fresh yield :** Plants stems and roots were cut and each was weighted

**C. On dry matter :** Fresh samples were dried in an oven at  $70^{\circ}\text{C}$  to constant weight. Dry matter was determined according to the methods of Chapman and Pratt (1961)

**D. On protein content :** Nitrogen percentage was estimated by Kjeldahl method using Kjelte system (AOAC, 1954). Protein content was calculated by multiplying  $\text{N \%} \times 6.25$  where 6.25, is constant value.

## RESULTS AND DISCUSSION

Data in table 1 showed that the highest percentage of infected plants with *Verticillium* wilt were recorded in June and July (16.6 and 16.5%) respectively, whereas the lowest one was observed in February (1.9%). In January no infection was recorded. Aphids were found on the alfalfa plants throughout the year. Highest (13.2 insect/plant), and lowest (1.2 insect/plant) numbers were recorded during March and September; respectively.

Table 1. Percentage of naturally infected plants and aphids with *Verticillium albo atrum* in an alfalfa field at Ismailia in 1999.

Month	% <i>Verticillium</i> infected plants	Mean number of aphid / plant	% contaminated aphids
January	0	8.7	1.3
February	1.9	9.8	2.3
March	5.5	13.2	7.5
April	8.3	12.4	3.8
May	11.7	7.9	3.3
June	16.6	4.3	2.3
July	16.5	1.9	2.8
August	11.5	1.3	2.3
September	9.5	1.3	3.3
October	8.6	2.3	3.8
November	5.6	3.3	1.3
December	6.2	6.5	1.5
L.S.D.	2.217	5.11	

Percentage of the contaminated aphids with *Verticillium* spores, varied from 1.3 to 7.5 %. The highest percentage occurred during March, whereas the lowest was during January, November and December.

All isolations of *Verticillium albo atrum* from wilted plants and aphids were found pathogenic to alfalfa plants.

It is obvious that there was a gradual reduction in aphids population started from May, faced with gradually increase in *Verticillium* wilted plants. This may be due to the infection with *Verticillium* by aphids occurred early and disease development and symptoms expression took time, accompanied with the natural changes in the population of

aphids which had lower population. Harper and Huang (1984) showed that spores of *V. albo-atrum* are not produced on infected alfalfa plants in the early growing season. Also, the contaminated insect may be moved within the field and caused multiply infection.

Data in table 2 showed that aphids artificially contaminated with *V. albo-atrum* spores resulted in 76.7 and 90% infection on cut and uncut plants, respectively, while the field strain resulted in respective values of 16.7 and 33.3%. Spraying cut and uncut plants, with the spores of the fungus caused wilt infection ranged between 43.3 and 6.6 %, respectively.

Table 2. Percentages of infection and disease severity scores on alfalfa plants treated with contaminated and uncontaminated aphids and spores of *Verticillium albo-atrum*.

Treatments	Cut alfalfa plants		Uncut alfalfa plants	
	Percentage of infected plants.	Disease severity score*	Percentage of infected plants.	Disease severity score *
Artificial Contaminated Aphids	76.7	4.2	90.0	4.8
Aphids(field strain)	16.7	1.3	33.3	2.3
<i>V.albo atrum</i> spores	43.3	2.5	6.6	1.6
Uncontaminated aphids	0	1	0	1
Untreated	0	1	0	1
L.S.D.	34.9	0.836	24.2	0.456

\*According to the proposal of Ireland & Leath ( 1987).

No wilted plants were recorded when cages were not subjected to aphids or supplied with uncontaminated aphids. Percentage of wilted plants was higher on uncut plants when treated with artificially and field contaminated aphids. The contrary was obtained when plants were sprayed with the fungal spores, whereas percentage of the wilted plants were higher (43.3%) on cut plant than on the uncut ones (6.6%).

The result may be explained by that aphids feeding on the young leaflet are active on plants before being cut and frequently the infection increased, but after cutting aphids aggregate on the base of the shoot or may be winged and start to emigrate. The cut plants sprayed with the spores, show high percentage of infection as a result of the direct contact between the spores and the plants, which facilitating the spores gaining an access into the plants.

Data in tables 3 & 4 showed that all treatments affected the length, weight and protein contents of alfalfa plants, but the effect varied from one treatment to another.

Table 3. Effect of different treatments with *Verticillium* on length, weight (shoot & root), dry matter and protein content of cut and uncut alfalfa plants.

Treatments	Cut alfalfa plants					Uncut alfalfa plants				
	Plant length Cm.	Shoot Weight g.	Root Weight g.	% D.M	% protein content	Plant length Cm.	Shoot Weight g.	Root Weight g.	% D.M	% Protein content
Artificial Contaminated Aphids	7.7	2.2	10.4	18.7	18.0	13.1	1.8	10.3		17.6
Aphids (field strain)	9.2	5.3	10.1	16.0	18.7	21.1	3.1	12.2	0.7	19.8
<i>V.albo atrum</i> spores	20.0	7.8	10.1	16.0	21.3	22.1	3.5	9.9	4.5	21.3
Uncontaminated Aphids	9.5	4.0	11.3	14.1	21.3	27.7	11.5	9.6	13.8	20.1
Untreated	20.6	7.8	13.8	13.1	21.4	43.4	17.7	15.3	12.0	22.7
L.S.D.	2.803	2.02	1.75			4.43	2.56	3.08		

Table 4. Percentage change (+,-) of length, weight (shoot & root), dry matter and protein content of alfalfa (cut & uncut plants) in treated and untreated plants.

Treatments	Cut alfalfa plants					Uncut alfalfa plants				
	Plant length (cm)	Shoot Weight (gm)	Root Weight (gm)	% D.M*	% protein content	Plant length (cm)	Shoot Weight (gm)	Root Weight (gm)	% D.M*	% protein content
Artificial Contaminated Aphids	62.6	73.2	24.6	42.7	15.9	61.9	89.8	32.7	70.0	22.5
Aphids (field strain)	55.3	53.4	26.4	22.1	12.6	38.7	83.5	20.3	41.7	12.8
<i>V.albo atrum</i> spores	2.9	4.9	26.8	22.1	0.5	19.5	35.0	37.3	20.8	6.2
Uncontaminated Aphids	53.9	51.2	18.2	7.61	0.5	35.8	80.2	35.3	15.8	11.21

\* D.M = Dry matter (increase)

The artificially contaminated aphids caused reduction of 62.6, 73.2, 24.6 and 15.9 % of cut plant and 61.9, 89.8, 32.7 and 22.5% of the uncut plant in the plant height, shoot weight, root weight and protein content, respectively, whereas the field strain's respective value were 55.3, 53.4, 26.8 and 12.6 for the cut plant and 38.7, 83.5, 20.3 and 12.8 % of the uncut plant, respectively.

Spraying plants with *Verticillium* spores reduced the length, weight (shoot & root) and protein contents of the cut plants by 2.9 , 4.9 , 26.8 and 0.5 % , respectively, while correspondent values were 19.5, 35.0, 37.3 and 6.2% for the uncut plant, respectively. Uncontaminated aphids (laboratory strain) reduced the same parameters by 53.9, 51.2, 18.2 and 0.5 % in the cut and by 35.8, 80.2, 35.3 and 11.5 % in the uncut plants, respectively.

The different treatments increased the percentage of dry matter of alfalfa plants, it ranged between 7.6% and 70% according to the treatment and plant organ examined.

The results showed that *Verticillium* wilt was absent throughout January although the captured aphids were contaminated with the fungus.

It is obvious that all treatments reduced the length and weight of shoot and root as well as the protein content. This may result from the aphids feeding on the top of the plant, thus keeping the length shorter than normal. Klostermeyer (1962) reviewed that high levels of pea aphid, *Acyrtosiphon pisum*, feeding have been associated with cessation of apical growth. The dryness of plant tissue as a result of infection with *Verticillium* also led to the same result. The lesser reduction on root weight than on shoot may be attributed to that the damage is mainly directed to the shoot rather than the root system. The increase in dry matter percentage may be attributed to the reduction in water content as a result of aphids feeding and water deficits in plant parts as a result of *Verticillium* infection which may lead to the destruction of the whole plant. Data reported herein showed that the alfalfa aphid play an important role as a vector of *Verticillium albo atrum* to alfalfa.

The obtained data are in agreement with that obtained by Rassoulia (1989) who reviewed that *Aphis pisum* and *Therioaphis trifolii* reduced foliage yield by 27.7 - 75.5 and 20.51 % respectively, while *T. trifolii* reduced the protein by 25.5 % compared with uninfested plants.

In conclusion, alfalfa aphids play an important role in transmission of *Verticillium* spores. Both reduce the yield and protein content so, it becomes necessary to protect alfalfa plant against aphids infestation and to avoid the aggravation of infection with *Verticillium*. It is better to avoid the use of chemical pesticides to control the aphids, because the contaminated aphids that may be present in a low proportion is enough to transmit the fungus spores and eradication of the pest by using pesticides is impossible. So, cutting is the ideal methods to control the pest when necessary. If there is a neces-

sity to control aphids throughout the period before collection of seeds when cutting is stopped, natural or bio substances can be used and seed dressing can also be made out. The obtained results are in agreement with that obtained by Christan (1982), who reviewed that *Verticillium* conidiospores were observed on some aphids killed and incubated on selective medium.



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## دور المن فى نقل مرض ذبول الفرتسيليوم للبرسيم الحجازى

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اتضح من خلال الحصر الذى اجرى على البرسيم الحجازى فى محطة البحوث الزراعية بإسماعيلية فى موسم ٩٨-١٩٩٩، ان الذبول الفرتسيليومى تراوح وجوده من ١,٩ إلى ١٦,٦٪ وقد سجلت أعلى إصابة فى شهر يونيو وأقل إصابة فى شهر فبراير. ولم توجد أعراض للمرض فى شهر يناير. وجد المن طوال الموسم وتراوح تعداده من ١,٢ إلى ١٢,٢ حشرة / نبات. سجل أكبر عدد من الحشرات فى شهر مارس بينما وجد أقل عدد فى شهر سبتمبر. تراوحت نسبة المن الملوث بجراثيم الفرتسيليوم بين ١,٢ إلى ٧,٩٪.

ظهرت أعراض الذبول الفرتسيليومى على النباتات التى عرضت لحشرات ملوثة بالفطر أو التى تم رشها بجراثيم الفطر. ولقد تسبب المن الملوث (سلالة حقلية) فى نسبة إصابة ١٦,٧-٣٣,٣٪. بينما المن الذى تم تلويثه صناعيا فقد سبب نسبة إصابة ٧٦,٦ - ٩٠٪ لنباتات تم حشها أو التى لم تحش على التوالي. سبب رش النباتات التى تم حشها أو التى يتم بجراثيم الفطر حدوث ذبول تراوحت نسبته من ٤٢,٣ إلى ٦,٦٪. كما أنها أدت إلى قصر أطوال النباتات ونقص أوزانها (سوق، جذور) والمحتوى البروتينى للنباتات التى تم حشها أو التى لم يتم حشها. أحدثت كل من سلالة المن المعملية الخالية من فطر الفرتسيليوم و السلالة الحقلية نقصا فى العناصر التى تم ذكرها سابقا وفى معدلات النمو المختلفة للنباتات سواء التى تم حشها أو النباتات التى لم يتم حشها.