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Study on the Role of Common Weeds in Survival of *Verticillium dahliae* the Causal Agent of Cotton Wilt Disease

[1]A. Heydari, [2]A. Ahmadi, [3]S. Sarkari, [3]H. Karbalayi Khiavi and [4]M. Delghandi
[1]Iranian Research Institute of Plant Protection, Tehran, Iran
[2]Neishabour Station, Razavi Khorasan Agricultural Research Center, Neishabour, Iran
[3]Moghan Agricultural Research Center, Moghan, Ardebil, Iran
[4]Agricultural Research Center of Razavi Khorasan, Mashhad, Iran

Abstract: This study was carried out to investigate the roles of common weeds in the survival of *Verticillium dahliae* and the incidence of cotton wilt disease in Moghan and Neishabour area of Iran during 2003-2005 cropping seasons. The design of the experiment was Randomized Complete Blocks (RCB) with ten treatments (No. of weeds) and four replications (No. of cotton fields). Populations of *V. dahliae* in the roots of weeds and their surrounding soil was determined every year and were compared in different treatments. The cotton wilt disease index was also evaluated in different fields in each experimental site. Results indicated that the fungal population was variable depending on weed species, experimental site and the year of study. In general, Pigweed (*Amaranthus retroflexus*), Nightshde (*Solanum nigrum*), Spiny cockleburr (*Xanthium spinosum*) and common purslane (*Portulaca oleraceea*) showed the highest fungal populations in their roots and surrounding soil. Disease index in Moghan cotton fields in different years varied and were higher than Neishabour fields.

Key words: Cotton, *Verticillium dahliae*, common weeds, Iran, Moghan, Neishabour

INTRODUCTION

Cotton is an important cash crop around the world including Iran. Cotton is cultivated in about 200000 ha in more than 15 provinces of Iran (Heydari *et al*., 2005; Naraghi *et al*., 2007). Harmful pests (insects, weeds and pathogens) are among the most important yield reducing agents in cotton fields (Batson and Blasengame, 1988; Bendict *et al*., 1989; Heydari *et al*., 2005; Heydari and Misaghi, 1998; Naraghi *et al*., 2007; Watkins, 1981).

Verticillium wilt disease is one of the most important diseases in cotton growing area of the world including Iran (Batson and Blasengame, 1988; Bendict *et al*., 1989; Lakutkin and Popov, 1976; Naraghi *et al*., 2007; Watkins, 1981). This disease is a soil-bon fungal disease caused by *Verticillium dahliae*. The fungus has a wide host range and can survive in the roots of many weed species (Bhat and Subbarao, 1999; Du Toit *et al*., 2005; Epstein *et al*., 2004; Ghalandar *et al*., 2004; Marios *et al*., 1982; Naraghi, *et al*., 2007; Resende *et al*., 1994; Wei *et al*., 1998). The fungus may attack and infect its host plant including cotton in all growth stages (Resende *et al*., 1994; Wei *et al*., 1998; Naraghi *et al*., 2007). The symptoms on cotton usually appear 5-7 weeks after planting. In some cases no symptom may appear before bolls are developed (Naraghi *et al*., 2007). Disease symptoms may vary depending on the growth stage, variety, environmental conditions and fungal races and include leaf chlorosis, vascular necrosis and wilting (Lakutkin and Popov, 1976; Naraghi *et al*., 2007). In severe cases the cotton plant may die as the result of complete wilting.

According to the results of previous studies the fungal agent of the disease can attack about 200 plant species from 40 families including many weed species (Bhat and Subbarao, 1999; Du Toit *et al*., 2005; Epstein *et al*., 2004; Ghalandar *et al*., 2004; Marios *et al*., 1982; Naraghi *et al*., 2007; Resende *et al*., 1994; Wei *et al*., 1998). The pathogen can survive in the root of these weeds without inducing and causing any symptoms (Lakutkin and Popov, 1976; Resende *et al*., 1994). More than 15 weed species have been reported as the host and vector of *V. dahliae*. Among these weeds some show the wilt symptoms but many others act just as the vector of the fungus without any diagnostic symptoms (Lakutkin and Popov, 1976; Resende *et al*., 1994). Some of the weeds species that have been reported to serve as the host of cotton wilt fungal agent include pigweed, nightshade, spiny cockleburr and common purslane (Lakutkin and Popov, 1976). A large number of weed

Corresponding Author: A. Heydari, Iranian Research Institute of Plant Protection, Tehran, Iran

species are grown in cotton fields in Iran which can potentially act as the host of *V. dahliae* and increase the incidence of verticillium wilt which is the most important and damaging disease in cotton fields in Iran.

The objectives of present study were to investigate the role of common weeds in the survival of *Verticillium dahliae* in the soil and the incidence and occurrence of cotton wilt disease in two major cotton growing area of Iran during three years (2003-2005).

MATERIALS AND METHODS

Cotton field selection: During Spring, 2003 and before cotton cultivation 4 cotton fields were randomly selected in Moghan and Neishabor area based on the history of verticillium wilt disease. In each field 10 common weeds were chosen to be studied. There were ten treatments (No. of weeds) and four replications (No. of cotton fields) in each experiment. Common weeds to be studied were a little different in Moghan and Neishabour area and were as:

Weeds (Moghan experiments): Red root pigweed (*Amaranthus retroflexus*), Nightshade (*Solanum nigrum*), Vulvetleaf (*Abutilon avicenae*), Goosefoot (*Chenopodium album*), Spiny cockleburr (*Xanthium spinosum*), Bindweed (*Convulvulus arvensis*), Pale flax (*Linum austriacum*), Nutsedge (*Cyperus rotundus*), Common purslane (*Portulaca oleracea*), Johnson grass (*Sorghum halepens*).

Weeds (Neishabour experiments): Red root pigweed (*Amaranthus retroflexux*), Prostrate pigweed (*Amaranthus* sp.), Nightshade (*Solanum nigrum*), Goosefoot (*Chenopodium album*), Spiny cockleburr (*Xanthium spinosum*), Bindweed (*Convulvulus arvensis*), Common purslane (*Portulaca oleracea*), Little mallow (*Malva* sp.), Wild rape (*Rapistrum rugosoum*), Hardheads (*Acropilton repens*).

Agronomic practices: Agronomic practices including land preparation and planting in each area was carried out according to the common procedures followed by cotton growers. In each area the commonly used cotton variety seeds were used for planting.

Fungal isolation and disease evaluation: In each field the above-mentioned weeds were collected at the end of cropping season and were carried to the laboratory. Roots of collected weeds and their surrounding soil were then weighed and the fungal population in each gram of root and soil were determined by preparation of suspension and culturing on Zapec medium and counting the fungal colonies after incubation. For evaluation of verticillium wilt disease incidence in each field, 100 cotton plants were selected randomly and were examined carefully based on their symptoms. Determination of disease index was carried out by scoring plants according to the 0-4 scale, where 0 was assigned to the plants with no symptoms and 4 for the plants which were completely wilted. After all plants were scored they were divided to five groups (N_1-N_5) according to their scores and the following formula was used for determination of average disease index (DI) in each field:

$$DI: [(N_1\times0) + (N_2\times1) + (N_3\times2) + (N_4\times3) + (N_5\times4)]/100$$

As was mentioned before, there were 10 treatments (No. of weeds) and four replications (No. of cotton fields) in each experiment. The design of the experiments was completely randomized blocks.

Statistical analysis: Collected data from different fields in all 3 years and 2 experimental sites were analyzed using Statistical Analysis System (SAS). Data were tested by analysis of variance (ANOVA) and means were compared by Duncan multiple range test.

RESULTS

Table 1 and 2 contain the results of this study in two experimental sites during 2003-2005. Results of the Moghan experiment are shown in Table 1. As this table shows in 2003 in Moghan area, there were various populations of *V. dahlia* in the roots and surrounding soils of common weeds. According to the results nightshade, common purslane and red root pigweed had the most fungal population in their surrounding soil, respectively (Table 1). In each gram of root the highest fungal populations belonged to common purslane, nightshade and red root pigweed, respectively (Table 1). Wilt disease index on cotton plants was evaluated 1.08 according to 0-4 scale (Table 1).

In 2004 among 10 common weeds, common purslane, red root pigweed and bindweed had the highest population of *V. dahliae* in 1 g of their surrounding soil (Table 1). The highest fungal population in weeds roots in this year belonged to common purslane, wild pigweed and nightshade, respectively (Table 1). Cotton wilt disease index in this year was evaluated 1.54 which was higher than 2003 (Table 1).

In year 2005 as the Table 1 shows in Moghan area there were various populations of *V. dahlia* in the roots and surrounding soils of common weeds. According to

Table 1: *Verticillium dahlia* population in each gram of surrounding soil of common weeds, each gram of their root and cotton wilt disease index in Moghan field experiments

	Year 2003			Year 2004			Year 2005		
Weeds	[1]Fungal population in g soil	[2]Fungal population in g root	[3]Disease index	Fungal population in g soil	Fungal population in g root	Disease index	Fungal population in g soil	Fungal population in g root	Disease index
Red root pig weed	330.0ab	18.50b	1.08	353.0ab	25.500b	1.54	387.0b	29.0b	2.25
Night shade	371.5a	19.50b		295.5bcd	20.000b		363.0b	25.5c	
Velvet leaf	263.5cd	8.0cd		273.0cde	7.50cd		340.5bc	20.5c	
Goose foot	258.5cde	5.0cd		227.0ef	8.00cd		292.0cd	15.5d	
Spiny cockle burr	230.0cde	8.0cd		246.5cdef	4.50de		287.0cd	13.0de	
Bind weed	286.5bc	8.5cd		306.5bc	11.500c		248.0d	10.0e	
Pale flax	259.5cde	5.0cd		204.0fg	2.00de		194.5e	1.0f	
Nut sedge	210.0de	0.00d		163.0g	0.000e		180.0e	1.5f	
Comon purslane	369.5a	33.00a		379.5a	32.500a		493.5a	37.5a	
Johnson grass	193.5a	1.0cd		232.5def	2.50de		185.5e	0.5f	

[1]: No. of *V. dahliae* sclerotia in 1 g surrounding soil of weed roots, [2]: No. of fungal infection (colony) in 1 g of weed roots, [3]: Index of cotton wilt disease in the field based on 0-4 scale, In each vertical column, Values followed by the same letter(s) are not statistically different ($p<0.05$) according to Duncan Multiple Range test

Table 2: *Verticillium dahlia* population in each gram of surrounding soil of common weeds, each gram of their root and cotton wilt disease index in Neishabour field experiments

	Year 2003			Year 2004			Year 2005		
Weeds	[1]Fungal population in g soil	[2]Fungal population in g root	[3]Disease index	Fungal population in g soil	Fungal population in g root	Disease index	Fungal population in g soil	Fungal population in g root	Disease index
Red root pig weed	303.0abc	28.5abc	0.49	276.0b	23.0cde	0.32	290.0bcd	35.0c	0.41
Prostrate pig weed	313.0ab	23.5bcde		263.0b	24.0cde		285.0bcd	20.0de	
Bind weed	231.0cd	14.0bcde		233.0b	12.005e		248.00e	13.0e	
Common purslane	325.0ab	39.5ab		321.0a	36.500b		322.0abc	48.0b	
Little mallow	270.0bc	11.0de		260.0b	13.50de		265.0de	17.0e	
Night shade	371.0a	50.0a		336.0a	51.500a		347.00a	62.0a	
Spiny cokleburr	346.0ab	43.5a		327.0a	49.500a		324.0ab	47.5b	
Goose foot	287.0bc	24.0abcd		268.0b	25.5cde		294.0bcd	29.0cd	
Wild rape	186.0d	2.0e		177.0c	0.000f		175.00f	1.5f	
Hard heads	274.0bc	11.5cde		264.0b	14.50cd		278.0cde	13.0e	

[1]: No. of *V. dahliae* sclerotia in 1 g surrounding soil of weed roots, [2]: No. of fungal infection (colony) in 1 g of weed roots, [3]: Index of cotton wilt disease in the field based on 0-4 scale, In each vertical column, Values followed by the same letter(s) are not statistically different ($p<0.05$) according to Duncan Multiple Range test

the results, common purslane, red root pigweed and nightshade showed the most fungal population in their surrounding soil and roots. Disease index on cotton plants in 2005 was evaluated 2.25 according to 0-4 scale, which was higher than previous years (Table 1).

The results of Neishabour experiments are shown in Table 2. As the table indicates in 2003, among 10 common weeds, nightshade, spiny cockleburr and common purslane had the highest population of *V. dahliae* in 1 g of their surrounding soil and 1 g of their root as well (Table 2). Wilt disease index was evaluated 0.49 in 2003 (Table 2).

According to Table 2 in 2004 in Neishabour area there were various populations of *V. dahlia* in the roots and surrounding soils of common weeds. According to the results, the highest fungal populations in each g of surrounding soil and root belonged to nightshade, spiny cockleburr and common purslane, respectively (Table 2). Disease index on cotton plants was measured as 0.32 according to 0-4 scale (Table 2).

The results of Neishabour experiment in 2005 indicates that among 10 common weeds, nightshade, spiny cockleburr and common purslane had the highest population of *V. dahliae* in one gram of their surrounding soil (Table 2). However, the highest fungal population in the roots in this year belonged to nightshade, common purslane and spiny cockleburr respectively (Table 2). Wilt disease index in the fields in 2005 was evaluated 0.41 (Table 2).

DISCUSSION

The overall results of this study show that some of the common weeds in cotton fields can act as the potential host for *Verticillium dahliae* and play important role in the survival of this pathogenic fungus which is the causal agent of cotton wilt disease. Our results agree with those of some previous studies (Du Toit *et al*., 2005; Epstein *et al*., 2004; Ghalandar *et al*., 2004; Marios *et al*., 1982; Naraghi *et al*., 2007; Resende *et al*., 1994; Wei *et al*.,

1998). Some weeds can play important roles in the survival of *V. dahliae* in cotton fields (Resende *et al*., 1994; Wei *et al*., 1998). In their study however goose foot and little mallow were shown to be the most important hosts of the fungus whereas in present study common purslane, nightshade, pigweed and spiny cockleburr were the most susceptible weeds. The differences in the results could be due to the differences in fungal isolates and environmental conditions.

A significant point that can be derived from the results of this study is the relationship between inoculum potential and disease incidence (Agrios, 1988; Batson and Blasengame, 1988; Berger *et al*., 1986). As a principle of plant pathology for occurrence of a disease in a given area on a given crop the diseases pyramid which include a pathogen (inoculum density), a susceptible host, conducive environmental conditions and sufficient time should be completed (Agrios, 1988). Lack of some or one of the above-mentioned factors may results in absence of the disease (Agrios, 1988). For example in this study in almost all experiments there were a high populations of *V. dahliae* in the surrounding soil of the weeds but the infection rates in their roots were low. This indicates that in addition to high fungal populations other factors are also required for fungal infection and diseases incidence.

In another part of the study especially in Neishabour field experiments a high fungal population in the surrounding soil and the roots of common weeds were observed but this high population did not result in a high disease incidence (index) in these experiments. These findings indicate that for occurrence of cotton wilt disease in the fields the presence of high fungal population (inoculum density) is not enough because may be one of the other factors is not present. In Moghan field experiments despite lower population of *V. dahliae* higher disease index was observed and this could be due to the conducive environmental conditions and the higher degree of susceptibility of cotton variety planted in this area.

Results of the study varied in different years and different experimental sites. In comparison, wilt disease incidence was higher in Moghan fields than those of Neishabour. In Moghan experiments wilt disease incidence increased year by year. The observed differences could be due to the various environmental conditions (rain fall), different cotton varieties (susceptibility to wilt disease) and perhaps different *V. dahliae* isolates. The above factors have been shown to play important roles in the incidence of wilt disease in cotton fields (Batson and Blasengame, 1988; Berger *et al*., 1986).

CONCLUSIONS

Based on the overall results of this study it is concluded that some common weeds can play important roles in the survival of *V. dahliae* and the occurrence and incidence of cotton wilt disease in the field and therefore effective weed management strategies should be employed particularly in those cotton growing area where susceptible varieties are planted and a high populations of *V. dahliae* in the soils are detected. The role of environmental conditions including soil moisture in the interactions among common weeds, cotton plant and pathogenic fungus (*V. dahliae)* should also be taken to the account.

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