

Research article

Fusarium verticillioides: A New Cotton Wilt Pathogen in Uzbekistan

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Abstract

An increase in wilt has been observed in cotton fields in Uzbekistan. This prompted us to conduct a survey of Uzbek cotton fields for wilt symptoms over a five year period beginning in 2007. Twenty-four regions with different soil types and ecologies were screened. In 9 regions, over 45% of the plants demonstrated wilt symptoms and over 60% of the plants showed wilt symptoms in 4 of these regions. During this screening, we identified a new pathogen affecting cotton in Bukhara province that was identified as *Fusarium verticillioides* (Sacc.) Nirenberg (*Syn. F. moniliforme* Sheld.). Identification was based on mycological studies and genetic analysis. In greenhouse studies, this pathogen was shown to be more aggressive on cotton than the *Fusarium oxysporum* f. sp. *vasinfectum* that is endemic to Uzbekistan, but is not more aggressive on tomato, potato, eggplant and pepper.

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Introduction

During the last 10 years commercial cotton cultivars grown in Uzbekistan have experienced an increase in wilt disease resulting in reduced yields and fiber quality. These losses have been attributed to the soil borne fungal pathogens *Verticillium dahliae* Kleb. and *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Snyder et Hansen. This was thought to be a recurrence of an epiphytotic event that occurred during the 1960 to 1970 decade in Uzbekistan. During Soviet times, cotton was grown as a mono-culture in Uzbekistan. This permitted an accumulation of pathogenic propagules in the soil. After independence, cotton has often been rotated with wheat. This may

occasion the development of new more aggressive pathogenic strains. Regardless of the reason, these new strains is adversely affecting recently released cotton varieties in Uzbekistan [1, 2].

Other countries have also reported new virulent biotypes of *Fusarium*. For example, in the USA a new aggressive race of *F. oxysporum* f. sp. *vasinfectum* (F.o.v.) has been identified in California [3]. In Australia, an aggressive F.o.v. biotype was identified in 1992-1993 [4]; in spite of stringent attempts to contain the pathogen, it rapidly spread from the original site of identification to adjacent locations. Both of these F.o.v. biotypes infect cotton growing in alkaline clay soils without the intervention of a nematode. Some lines tolerant to these pathogens have been identified, but they are not resistant. To investigate the occurrence of wilt pathogens in various cotton growing regions of Uzbekistan, a study was conducted in cotton fields in 24 provinces or independent Republics in Uzbekistan during 2007 to 2011.

Materials and Method

Survey of Fields and Isolation of Pathogens: Over a period of five years we surveyed cotton fields for wilt disease in in Andijan, Ferghana, Namangan, Tashkent, Syrdarya, Jizzakh, Samarkand, Navoi, Bukhara, Kashkadarya, Surkhandarya, and Khorezm provinces and the Republic of Karakalpakstan. The percent of plants with wilt symptoms was ascertained by the method of M.R. Unusov, et al. [5]. Thus, diseased plants with wilt symptoms were selected and the organisms were isolated in pure culture by the method of M.K. Hohkryakov [6] using Czapek and wort agar. Thirteen isolates were recovered from different varieties of wilted cotton plants. For mycological analysis, sections of roots, stems, stalks and leaves from diseased plants were placed in a moist chamber and then placed in Czapek and wort agar (pH 7-8) [6], and incubated at 26°C for 5-7 days. Plants that were approximately three weeks old were artificially inoculated according to the method of Sidorova [7]. That is, a syringe was used to place a 10 μ L drop of suspension of the inoculum (1×10^6 propagules per mL) on the stem at the first internode, and the needle was used to puncture through the drop into the xylem vessels.

Results and Discussion

Table 1 shows the data on the susceptibility of cotton varieties growing in different regions of Uzbekistan. Different cotton varieties are grouped together by color. Variety C-6524 was the most widely grown with plantings in 9 regions. The number of diseased plants in these regions was high, ranging from 20 up to 64%. An-Bayaut-2 was the second most widely grown with plantings in 5 regions. It showed the smallest number of diseased plants (1 to 10%) among all of the varieties in most regions. The exception was in the Karmana region where the number of diseased An-Bayaut-2 plants was 48%. Bukhara-6 was also planted in this region and many of these plants (57%) exhibited disease symptoms. Thus, the relatively low number of An-Bayaut-2 plants exhibiting disease symptoms in 5 regions, probably reflects a relatively low disease pressure in these regions, rather than disease resistance. The major exception to the high number of plants showing disease symptoms was in the autonomous Republic of Karakalpakstan where plants were sampled in two regions. Variety C-4727 was grown in these regions, and no plants showed disease symptoms. Variety C-4727 was not grown in any other region, but cotton disease losses in the autonomous Republic of Karakalpakstan is always very low. Khorezm province and the Republic of Karakalpakstan, showed a very low incidence of wilt; *Alternaria* was the dominant species identified. Thus, the observation of no plants exhibiting disease symptoms may be due to drought and extremely high temperatures in 2011. A low wilt incidence (1 to 2%) has previously been reported in these areas. An-Bayaut-2 is generally considered to be resistant to wilt pathogens. However, the high incidence of An-Bayaut-2 plants showing disease symptoms may indicate that a unique pathogen population is developing with significantly higher inoculum levels and/or an aggressive biotype.

Specific sites such as the Robiya Mukhammadieva fields in the Kattakurgan region and the Sulton ota Narpay field in the Narpay region showed a significantly higher number of diseased plants (54 % and 63%) compared to other parts of the country. Omad cotton was grown on both of these sites. In addition, in the Bukhara province severe wilt lesions were observed on the variety Bukhara-6 with 45 to 50% of the plants showing wilt symptoms.

From 2002 to the present, we observed an increase of *Fusarium* wilt on medium-fiber cotton varieties, especially the varieties grown in Bukhara province. This was especially true during the years 2007 to 2011 when other varieties of medium-fiber were also strongly affected. In the beginning, we assumed that the infection agent was *Fusarium oxysporum*, since the parent variety Bukhara-6 is a long-fiber cotton, and genetically they are considered to be relatively more susceptible to *Fusarium* wilt. However, when we isolated the pathogen from diseased plants of Bukhara-6 collected from the Bukhara region, we found a new pathogen, which has now been identified as *Fusarium verticillioides* (Sacc.) Nirenberg (*Syn. F. moniliforme* Sheld.). We verified the pathogenicity of this isolate by stem puncture. Plants inoculated by this method in the greenhouse exhibited wilt symptoms similar to those observed in the field.

Mycological analyses showed that the aerial mycelium of the fungus is well developed, fluffy, white, then pink and white. Microconidia were fusiform-ovoid, unicellular or with a partition, in chains or false heads, usually scattered in the aerial mycelium in the form of a powder. Single-celled micro conidia were - 4 - 18 x 1.5 - 4 μ m, with a partition - 9 - 30 x 2 - 5 μ m, 3 - 20 - 60 x 2 - 4.5 μ m, 5 - 37 - 70 x 2 - 4.5 μ m, with walls 7 - 58 - 90 x 2.5 - 4.5 μ m. The fungus was subsequently subjected to genetic analysis in the Institute of Genetics and Plant Experimental Biology ASU. The genes from NIR and rDNA were sequenced and found to be analogous to those of *Gibberella fujikuroi* (Sawada) Ito, which is the sexual stage of *Fusarium moniliforme* Sheld.

Pidoplichko [8], and Bilai [9] previously reported that *Fusarium moniliforme* Sheld. can affect cereals, grains, cotton and other crops. The appearance of this new pathogen may be the result of rotation of wheat with cotton. During Soviet times, cotton was grown in Uzbekistan as a mono-culture. Since independence, cotton is often rotated with wheat. The field from which the *F. verticillioides* was isolated had been rotated with wheat in 2010. Thus, an isolate that was originally a pathogen on wheat may have adapted to cotton.

To investigate the virulence of *F.o.v.* and *F. verticillioides* on other plants, we artificially inoculated tomato, potato, eggplant, Bulgarian pepper, and cotton (C-6524) with these pathogens. Results of this study are shown in Table 2. The artificially infected cotton plants showed almost identical symptoms compared to those observed in the field for both pathogens. Infected tissues from each of these plants were placed in moist chambers, and the pathogens were re-isolated and examined microscopically to verify the presence of the original inoculated isolates.

Table 1: Percent of diseased cotton varieties showing wilt symptoms in different regions of Uzbekistan during 2007 to 2011 (different shading indicates identical varieties).

Year Surveyed	Provinces	Regions	Varieties	Diseased plants (%)	Area, hectare
2007	Namangan	Uychi	Andijan-35	62	20
		Namangan	Namangan-77	68	4
2007	Andijan	Shahrikhan	C-6524	33	28
		Oltinkul	C-6524	30	28
2007	Ferghana	Bagdad	C-6524	64	6
		Uch-kupruk	C-6524	20	5
		Rishtan	C-6524	28	7.9
2008	Tashkent	Yangiyul	C-6524	34	62
			C-6524	62	2
		Chinaz	C-6524	40	45
			C-6524	30	9
2008	Syrdarya	Mirzaabad	AN-Bayaut-2	2	12
			AN-Bayaut-2	5	25
		Ok-oltin	AN-Bayaut-2	3	10
			AN-Bayaut-2	10	21
2008	Jizzakh	Mirzachul	AN-Bayaut-2	1	18

			AN-Bayaut-2	5	25
			AN-Bayaut-2	2	9
		Dustlik	AN-Bayaut-2	3	25
			AN-Bayaut-2	2	22
2009	Navoi	Karmana	AN-Bayaut-2	48	6
			Bukhara-6	57	9
2009	Bukhara	Gijduvan	Bukhara-6	45	67
			Bukhara-6	50	2
2010	Kashkadarya	Yakkabag	Bukhara-6	23	20
		Kamashi	Bukhara-6	17	10
2009	Samarkand	Narpay	Omad	63	9
		Kattakurgan	Omad	54	15
2010	Surkhandarya	Denov	Namangan-77	15	15
		Oltinsoy	Bukhara-102	10	42
2011	Khorezm	Bagat	Mekhnat	30	11
2011	Republic of Karakalpakistan	Hodjely	C-4727	0	27
		Ellikkala	C-4727	0	13

Table 2: Plants affected by wilt pathogens found in Uzbek cotton fields.

Plants	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	<i>Fusarium verticillioides</i>
	Tashkent population	Bukhara population
Cotton C-6524	+ ^a	++
Tomato	+++	+++
Potato	+++	+++
Eggplant	++	++
Bulgarian pepper	+	+

^a + no external wilt symptoms; ++ external wilt symptoms; +++ plants dead.

Conclusion

We have established for the first time in Uzbekistan that medium- and long-fibred cotton varieties are affected by a new pathogen, *Fusarium verticillioides* (Sacc.) Nirenberg. Thus, cotton varieties in Uzbekistan are affected by three fungal wilt pathogens *V. dahliae* Kleb., *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyder et Hansen. and *F. verticillioides* (Sacc.) Nirenberg. Furthermore, the observations that the variety that most consistently showed the lowest percent of diseased plants in 5 provinces (i.e., An-Bayaut-2) still had a high percent of diseased plants (i.e., 48.2%) in Navoi Province where apparently disease pressure was apparently more severe. Thus, new disease resistant germplasm is needed to combat these pathogens.

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