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Full Length Research Paper

Effects of long-term cotton plantations on *Fusarium* and *Verticillium* wilt diseases infection in China

Lan Wang[1,2], Hongzu Feng[1], Liping Teng[1], Xiaofei Chen[1], Hongxia Dan[1], Shiqiong Lu[2], Jianmei Xu[2] and Ligang Zhou[2]*

[1]College of Plant Science, Tarim University, Alar 843300, Xinjiang, China.
[2]Department of Plant Pathology, College of Agronomy and Biotechnology, China Agricultural University (CAU), No. 2, Yuanmingyuan West Road, Haidian District, Beijing 100193, China.

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The population density of *Fusarium oxysporum f.* sp. *vasinfectum*, disease index of cotton fusarium wilt along with the relation of the wilt disease and pathogen density in long-term cotton plantations were studied by the methods of dilution plating and field investigation. Microsclerotium densities of *Verticillium dahliae* and disease index of verticillium wilt were also studied. The results showed that both pathogen densities were first increased and reached their maximum at the 10th continuous cropping year and then, gradually decreased. Very similarly, the disease indexes of fusarium and verticillium wilts exhibited the same variation trend, thereby, indicating that disease-suppressive soil may be formed in long-term cotton plantations that need to be further studied.

Key words: Long-term cotton plantations, continuous cropping, fusarium and verticillium wilt diseases, *Fusarium oxysporum* f. sp. *vasinfectum*, *Verticillium dahliae*, microsclerotia, population density, disease-suppressive soil.

INTRODUCTION

Xinjiang, a province in Northwest of China, is suitable for upland cotton (*Gossypium hirsutum* L.) cultivation owing to its exceptionally geographic and climatic conditions and has become the largest producer of cotton in China (Kaiyong et al., 2011). As plantation age (even more than 20 years in some areas) increases, monoculture cotton cultivation is increasingly practiced. However, the effects of such a system on soil pathogenic microbial properties and cotton diseases are not known for the long-term cultivation. Both *Fusarium oxysporum* and *verticillium dahliae* are widespread soil-borne pathogens that can cause cotton fusarium and verticillium wilts, two major vascular diseases in most cotton-growing regions of the world (DeVay et al., 1997; Cai et al., 2009). *V. dahliae* can survive over long periods in soil by producing microsclerotia in residual tissues of the infected cotton, which constitute the infective and spreading structures of the fungus. Microsclerotia are stimulated to germinate in rhizosphere soil or its vicinity by root exudates, penetrate plant roots, and move to the vascular tissues (Xiao and Subbarao, 1998; Xiao et al., 1998). *F. oxysporum* is often the most prevalent fungus found on the surface of live cotton roots. It also occurs in intact dead cotton roots which remain after minimum tillage practices. The isolates that cause fusarium wilt of cultivated cottons are assigned as *Fusarium oxysporum f.* sp. *vasinfectum*. They consist mainly of chlamydospores in plant debris (Bell et al., 2003). Interestingly, both fusarium and verticillium wilt diseases in Xinjiang long-term cotton plantations were not as serious as those in other Provinces (for example, Henan and Shandong) of China. The aim of this study was to investigate the effects of long-term cotton plantations on fusarium and verticillium wilt diseases along with their pathogen densities in Xinjiang of China in order to better manage these two cotton diseases.

*Corresponding author. E-mail: lgzhou@cau.edu.cn, lgzhou2002@yahoo.com, lgzhou2002@163.com. Tel/Fax: +86-10-62731199.

MATERIALS AND METHODS

Cotton plantations and soil materials

The upland cotton (*G. hirsutum* L.) plantations in this study are located in the Southwestern part (79°22' to 82°00'E, 40°20' to 41°30'N) of Xinjiang, China. The cotton plantations are in different cropping periods (that is, 0, 1, 2, 5, 10, 15, and 20 years) with very similar climate. In this area, continuous cotton (summer cotton-winter and fallow-summer cotton) sown after conventional tillage has been practiced. Cotton (*G. hirsutum* L.) seeds are usually planted in mid-April, harvested at the end of September, and finished at the end of October. Due to the development of tillage equipment, all post-harvest residues, since 1996 have been incorporated by ploughing (incorporation of cotton stalks to 20 cm depth, with chisel ploughing to 30 cm depth) at the end of November. It has been estimated that 6000 to 7500 kg/ha cotton residues are returned to the fields each year (Zheng et al., 2000). At the growth stages of pre-planting (May, 1st), seedling (June, 1st), bud (July, 1st) and flowering (July, 25th) of cotton in 2009, each soil sample consisted of five randomly chosen soil cores (sub-samples) from depths of 10 to 20 cm in the cotton plantations with different planting ages from 0 to 20 years. Five sub-samples were combined and mixed to represent a soil sample which was stored in sealed plastic bags at 4°C until required.

Detection of the pathogens of the soil samples

One part of each fresh soil sample was dried for 10 to 12 h to a constant weight at 105°C, and its water content was then estimated. The densities of *F. oxysporum* (mainly as chlamydospores) and *V. dahliae* (microsclerotia) in the soil samples were detected with the dilution plating method. Briefly, 10 g of fresh soil was added in a shake flask filled with 90 ml of sterilized water. After vigorously shaking for 10 min and then setting for 1 min, the supernatant was diluted with sterilized water to obtain a series of concentrations. The diluted suspension was spread on the selective medium for the growth of cotton fusarium wilt pathogen (Wen et al., 1993) or verticillium wilt pathogen (Hawke and Lazarovits, 1994; Wang et al., 2011). The fungal colonies (CFU per g of dry soil) were counted after four (4) days incubation for fusarium wilt pathogen and fourteen (14) days incubation for verticillium wilt pathogen at 25°C in darkness, respectively. The fungal densities in the soil samples were then determined. All the experiments were performed in triplicate.

Disease assessment of fusarium and verticillium wilts

At the growth stages of seedling (June, 1st), bud (July, 1st) and flowering (July, 25th) of cotton in 2009, fusarium wilt disease was assessed. Fusarium wilt disease severity was recorded on a scale of 0 to 4 according to the percentage of foliage with wilt symptom and/or plant dwarfed degree, in an acropetal progression (0 = no symptom; 1 = 1 to 10% foliage with wilt symptom; 2 = 11 to 25% foliage with wilt symptom; 3 = 26 to 50% foliage with wilt symptom, stunting; 4 = 51 to 100% foliage with wilt symptom, obviously stunting) (Gong et al., 2011).

At the growth stage of flowering (July, 25th) of cotton in 2009, verticillium wilt disease was assessed. Verticillium wilt disease severity was recorded on a scale of 0 to 4 according to the percentage of foliage affected by chlorotic, necrotic and wilt symptoms and/or defoliation, in an acropetal progression (0 = no symptom; 1 = 1 to 10% foliage affected; 2 = 11 to 25% foliage affected; 3 = 26 to 50% foliage affected; 4 = 51 to 100% foliage affected) (Huang et al., 2006). The percentage of fusarium or verticillium wilt disease index was determined using the formula:

Disease index (%) = [(∑scale × number of infected plants) / (highest scale × total number of plants)] × 100.

RESULTS AND DISCUSSION

Fusarium wilt pathogen density and disease index

F. oxysporum f. sp. *vasinfectum* densities in the soil samples from long-term cotton plantations with different cropping years are shown in Figure 1A. In the initial five (5) year's continuous cropping, the density of *F. oxysporum f. sp. vasinfectum* in the soil increased slowly. From five to ten (5 to 10) year's continuous cropping, the pathogen density increased rapidly, and reached the maximum at the 10th continuous cropping year that was 31.27×10^2, 42.05×10^2, 63.67×10^2, 74.60×10^2 CFU per g of dry soil at growth stages of pre-planting, seedling, bud, and flowering of cotton, respectively. From ten to twenty (10 to 20) year's continuous cropping, the pathogen density decreased gradually. At the 20th continuous cropping year, the density of *F. oxysporum f.* sp. *vasinfectum* in the soil was 11.51×10^2, 13.04×10^2, 27.62×10^2, 31.91×10^2 CFU per g of dry soil at growth stages of pre-planting, seedling, bud, and flowering of cotton, respectively, which was not significantly different ($p < 0.05$) from that at the 5th continuous cropping year. In different growth stages of each cropping year, the general trend of the density of *F. oxysporum f.* sp. *vasinfectum* in the soil was flowering stage > bud stage > seedling stage > pre-planting stage. Among them, the pathogen density at the flowering stage of each cropping year was the biggest which had its significant difference ($p < 0.05$) from that at any other growth stage of each cropping year.

Figure 1B shows the disease indexes of fusarium wilt in long-term cotton plantations with different cropping years. The disease indexes increased in continuous cropping cotton fields from 0 to 10 years, and then gradually decreased in the fields from 10 to 20 years. The disease index of fusarium wilt reached the maximum in the field with 10 year's continuous cotton cropping, which was 15.65, 12.18 and 52.73% at growth stage of seedling, budding and flowering, respectively. Among the three growth stages of each cropping year, the disease indexes at flowering stage were the highest that were obviously different from those at seedling or budding stage. By comparing Figures 1A and B, it was found that disease index of fusarium wilt was consistent with population density of *F. oxysporum f.* sp. *vasifectum* for each growth stage of each cropping year.

Microsclerotium density and verticillium wilt disease index

Figure 2 shows the disease index of verticillum wilt and

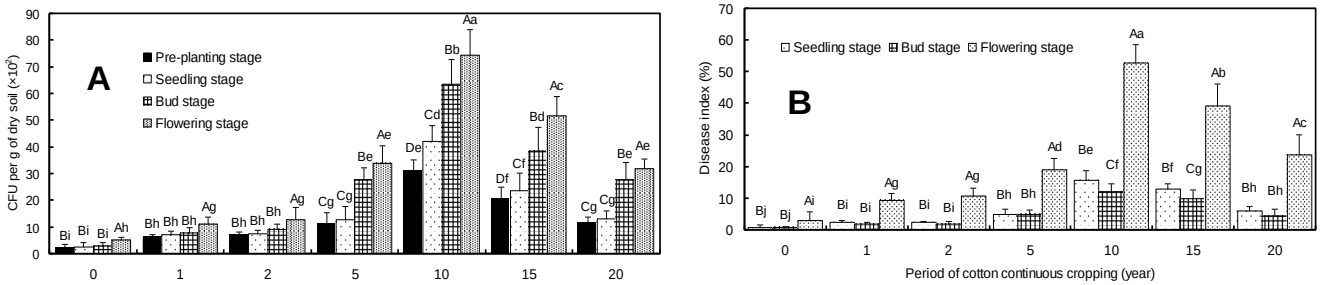

Figure 1. *Fusarium oxysporum f.* sp. *vasinfectum* densities in the soil samples (A) and the disease indexes of fusarium wilt (B) in long-term cotton plantations with different cropping years. Different small letters indicate significant differences among the treatments with different continuous cropping years at p = 0.05. Different capital letters indicate significant differences among different growth stages of each continuous cropping year at p = 0.05.

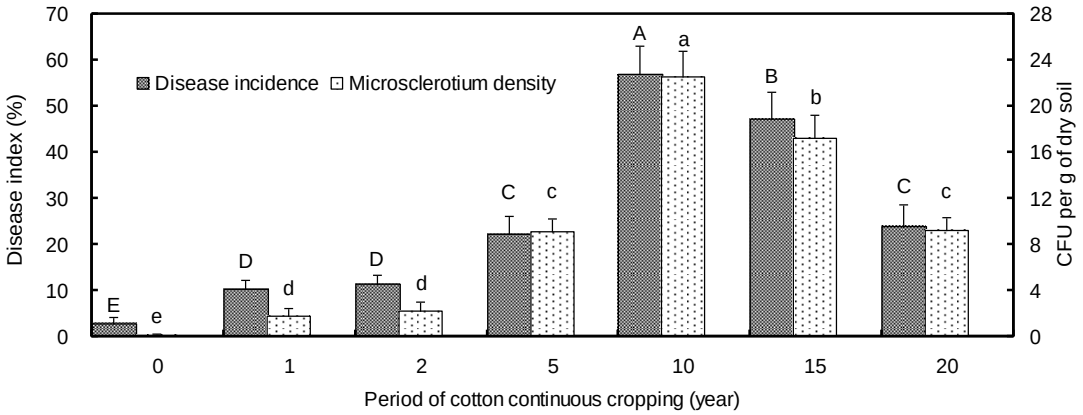

Figure 2. Disease index of verticillum wilt and microsclerotium density at flowering stage of cotton growth in long-term cotton plantations with different cropping years. Different small letters indicate significant differences of disease indexes among the treatments at p = 0.05. Different capital letters indicate significant differences of microsclerotium densities among the treatments at p = 0.05.

microsclerotium density at flowering stage of cotton growth in long-term cotton plantations with different cropping years. It was found that disease index of verticillum wilt was consistent with microsclerotium density in continuous cropping fields, and the trends of both verticillum wilt and its pathogen density were also consistent with those of both fusarium wilt and its pathogen density. Both disease index of verticillum wilt and microsclerotium density reached the maximum in the field with 10 year's continuous cotton cropping, which was 56.83 and 22.58% CFU per g of dry soil, respectively, at cotton flowering stage.

In summary, disease indexes of cotton fusarium and verticillium wilts were consistent with their pathogen population densities of the soils from this case study in Xinjiang of China. The disease indexes increased in continuous cropping cotton fields from 0 to 10 years, and then gradually decreased in the fields from 10 to 20 years. Among the different growth stages of each cropping year, both disease index and pathogen density were the biggest at flowering stage. In the middle or Eastern regions of China, continuous cotton cropping usually resulted in aggravation of soil-borne diseases and did not succeeded (for example, fusarium and verticillium wilts) (Ma et al., 1992; Zhu, 2007). However, in the Northwestern regions (for example, Xinjiang) of China, cotton continuous cropping can be achieved (Wang et al., 2011). Our study showed that both disease indexes and pathogen densities of fusarium and verticillium wilts gradually decreased after 10 year's continuous cropping, which indicated that disease-suppressive soils might be formed in the long-term cotton plantations (Mazzola, 2004; Kinkel et al., 2011). Several investigators have found that "disease-suppressive soil" phenomenon existed in long-term cotton plantations (Zhang and Li, 1980; Wu and Yan, 1985; Ma et al., 1992; Wang et al., 2008). Unfortunately, they did not compare cotton pathogen densities with the disease indexes of fusarium and verticillium wilts by using different continuous cropping field soils. Due to the continuous cotton cropping, specific

soil environment and rhizosphere conditions were formed that resulted in the decrease of pathogen densities of fusarium and verticillium wilt diseases. Kinkel et al. (2011) suggested a co-evolutionary framework for the study and management of disease-suppressive soil microbial communities. The action mechanism (such as, formation of antagonist microbes, production of antimicrobial components, and complex soil microbial communities) of the disease-suppressive soil against fusarium and verticillium wilt pathogens, the effects of the climates and cultivars on verticillium and fusarium wilt pathogens accumulation in soil should be further studied in detail (Elsas et al., 2008). Moreover, fusarium and verticillium wilts along with their pathogens in more than 20 year's cotton plantations should be further studied.

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