PROGRESS WITH VERTICILLIUM WILT OF COTTON IN THE REPUBLIC OF SOUTH AFRICA

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Abstract

Verticillium wilt is currently the most important disease causing losses to the cotton crop in South Africa. Only defoliating type strains could be isolated. These isolates however, differed slightly in virulence to cotton. Strains collected from the Lower Orange River area were more virulent than any other isolates collected from the other areas. These strains also had different isozyme profile in the a-B-esterase systems. Indications are that these more virulent strains emerged due to selection pressure on the pathogen population, caused by monoculture of one cotton cultivar and several other agronomical practices. Progress are being made with a breeding programme for resistance to Verticillium wilt in South African cotton cultivars.

Introduction

Verticillium wilt is currently the most important disease causing losses to the cotton crop in South Africa. During the 1994/95 season 81 000 ha of cotton were produced under irrigation and dry land conditions. Of these, approximately 20 000 ha are now infested with the wilt pathogen, *Verticillium dahliae* Kleb. Although Verticillium wilt of cotton is widespread throughout the cotton production areas, the real problem is mainly concentrated in three of the major cotton producing areas. Irrigated cotton are severely affected by this disease. The disease is more prevalent in soils with a high clay or silt content. The three affected areas extends over two geographical and climatological regions.

Verticillium wilt was first reported in South Africa by Lister in 1959 (Lister, 1961). However, it was already observed in 1945 but was apparently confused with a physiological disorder, linked to a potassium deficiency syndrome (Lister, 1961). Earlier work on Verticillium wilt of cotton concentrated mainly on identification of the causal organism (Lister, 1961), the influence of Verticillium wilt on fiber quality (Van Heerden, 1964), survival of the pathogen in South African soils (Baard, 1979) and the irradiation of the pathogens from soils (Baard & Pauer, 1981).

Although Verticillium wilt of cotton was present all these years, it did not pose the threat to the cotton industry as it does now. The disease incidence and severity increased significantly since 1988 and reached epidemic proportions during the 1994/95 season. During this past season, fields infected up to 100% were not uncommon. This sudden increase in the occurrence of the disease prompted the cotton industry to request research on this problem. Consequently a breeding programme for resistance to Verticillium wilt in cotton was launched in 1991. This also raised questions regarding the population structure of *V. dahliae* in cotton soils and a research project dealing with this was launched in the 1994/95 season.

Materials And Methods

Investigation of the population structure of *V. dahliae* infecting cotton

Isolations. Isolations were made from the small veins of cotton leaves that showed symptoms of Verticillium wilt, petioles and from the soil. Corn meal agar was used for the tissue isolations while the soil extract agar of Isaac *et al.* (1971) with the modifications of Huismans *et al.* (1974) was used for isolation of the pathogen from soil. Quantitative determination of *V. dahliae* from soil was done with the wet sieve method of Ashworth *et al.* (1972), also using the modified soil extract agar.

Virulence and strain differentiation of the isolates. Pathogenicity of all isolates was tested on cotton cultivars Sicala, Acala OR 3 and Acala SJ5. Virulence of all isolates was tested on mentioned cotton cultivars as well as on Verticillium resistant and susceptible tomato cultivars.

Isozyme analysis to differentiate between populations. Isozyme extracts from fungal tissue were obtained using the protocol of Burdon and Roelf (1985). Five different isozyme systems, a-B-esterase, cellulase, oxidase, glycophospho-isomerase and catalase were tested on polyacrylamide gels in a Hoeffer Scientific Mighty Small gel apparatus and stained according to well established staining protocols (Burdon and Roelf, 1985). The relative mobility values were calculated to determine the position of the bands for all isolates.

Breeding for resistance

Inoculation and screening techniques

Cotton seedlings were inoculated with fa spore suspension of 1 x 10^6 conidia/ml using the stem puncture method of (Ashworth, 1983). Seedlings were also inoculated using the root-dip procedure, where the roots were clipped and dipped into a spore suspension of 1 x 10^6 conidia/ml inoculum. Seedlings were also planted in soil amende with 100 microsclerotia/g soil (Grisham & Anderson, 1983). Symptom development was monitored daily. Screening of breeding material was performed in wilt nurseries at two localities. Evaluation of resistance or tolerance was made by recording the number of plants showing visible symptoms every two weeks since the plating of the material. Percentage leaf area affected but the disease was

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also determined on an index scale of 1 to 5, where 1 represents no symptoms and 5 all leaves on a plant were affected and complete defoliation occurred. Yield losses were also determined as well as quality characteristics. From this data disease progress curves were calculated.

Breeding programme

The breeding programme is based on the pedigree selection method and was initiated during the 1990/91 growing season. The main purpose of this breeding programme is to evaluate available gene sources for resistance to Verticillium wilt and their suitability as sources for crosses. Inoculum density of the plots in the two field trails were determined to ensure uniform inoculum levels. Cultivars like Acala OR3, Acala SJ5 and Paymaster 404 were used in the crosses, as well as several other natural selections.

Results

Population structure

59 isolates of *V. dahliae* were collected from all the cotton producing areas in South Africa. All isolates were recovered from the three problematic areas. Two other isolates, deposited in the National Fungi Collection of South Africa during 1989 were also retrieved. These two isolates were collected in the area of the Lower Orange River area and Limpopo Valley. They were isolated from cultivars Acala OR3 and Sicala.

The pathogenicity and virulence of all these isolates were tested on several cotton and tomato cultivars. The virulence study indicated that all 61 isolates belong to the defoliating strain group. However, virulence differences within this group, were detected on cotton. The isolates collected from the Lower Orange River area, designated as D1, were more virulent, i.e. causing defoliation more rapidly than the isolates form the Limpopo Valley and Loskop Irrigation Scheme (designated D2). Surprisingly, the D1 isolates also differed from the ones obtained from the National Fungi Collection.

All isolates were investigated for isozyme variation. Variation was demonstrated only in one of the five systems tested. The isolates from the Lower Orange River area had a different banding pattern than all other isolates in the a-B-esterase system.

The quantitative analysis of *V. dahliae* in cotton soils revealed that only one strain of pathotype (D2) of the pathogen exists in the cotton soils of the Limpopo Valley and Loskop Irrigation Scheme. However, both pathotypes D1 and D2 co-exist in the soils of the Lower Orange River area. The quantitative analysis of this population demonstrated a population composition of D1:D2 in a ratio of 9:1.

Resistance breeding

The breeding programme was only initiated in the 1990/91 season, thus going on for five generations. Initially, screening for resistance was only done in one wilt nursery in the Lower Orange River area, but now we have evaluated and correlated several greenhouse inoculation techniques with field data from two localities. The stem-puncture procedure gave the most consistent results and correlated 96% with field evaluations. After five generations, we now have three breeding lines which show potential as possible Verticillium wilt tolerant cultivars. They have been selected from random Acala OR3 crossing

Discussion

The epidemical occurrences of Verticillium wilt in cotton in South Africa can probably be ascribed to cultural practices followed by farmers in the three problematic areas. In the area of the Lower Orange River, only one cotton cultivar, Acala OR3, is produced. This cultivar has been cultivated since its release in 1980. Farmers in this area have very small farming units and cannot afford to follow an extensive crop rotation system which can reduce the inoculum density of the pathogen. Furthermore, farmers plant wheat, which in itself has the potential to reduce the soil inoculum density, but fail to control weeds. May of these weeds are susceptible to and are excellent hosts for the pathogen. consequently this increases the population of the pathogen in the soil. Furthermore, the cultivation of only one cultivar are conductive for selection pressure on the pathogen. The fact that there is a mixture of two sub-strains of a defoliating strain in the soil is indicative of this. This is also supported by the difference between these isolates ant the one deposited in our National Fungi Collection. Although the Limpopo Valley is now also fa one cultivar area - this was not the case until recently. The problem in this area is that farmers have ratoon cotton. This create an ideal milieu for increasing inoculum density. It can be possible that one can also expect a change in the population composition as in the area of the Lower Orange River in the near future. In the area of the Loskop Irrigation Scheme, farmers produce cotton in rotation with several vegetable crops. However, many of these vegetables, such as potatoes, tomato, chili pepper, safflower, beetroot, eggplant and sweet potato are susceptible to Verticillium wilt - causing an increase in inoculum density.

Poor weed control in cotton and other rotation crops seems to be an enormous problem. Many of the weeds occurring in the cotton production areas of South Africa are susceptible to *V. dahliae* and act as superb hosts for the pathogen and as source of inoculum.

Furthermore, farmers tend to plough in crop residues only shortly before the next crop and as such not leaving enough time for complete decomposition of residues.

Although we now have three promising breeding lines which exhibit some tolerance to Verticillium wilt, we are also trying to select resistance within locally adapted and desirable cultivars. Field screening in wilt nurseries will now be supplemented with greenhouse evalations. The fact that there are populations differences in the different cotton areas complicates the breeding and selection process in wilt nurseries. Caution should be exhibited when conclusions are being made and every aspect of the population structure should be taken into account. This also indicates the importance of constant monitoring of the population structure of the wilt pathogen throughout the cotton production areas of South Africa. The use of wilt nurseries are very advantageous, but dependency on results only from them are very dangerous - as demonstrated with the study of the population structure where within the span of five years a shift in population composition occurred.

As we only initiated the breeding programme in 1990, and the supportive projects in 1994, many of these results are preliminary. As far as the population structure is concerned, we will continue the investigation, but will employ techniques such as RFLP's in a PCR system and extend the investigation to resemblances or differences with potato and tomato isolates. The quality of the promising breeding lines will have to be evaluated very critically and possibly back crosses with desirable cultivars will have to be made.

Literature Review

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