Interactions of *Fusarium* and *Verticillium* Wilt with Root-Knot Nematodes in Cotton

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ABSTRACT

The association between root-knot nematode (RKN) and Fusarium wilt of cotton has been known since the 19th century but the effect of RKN infection on the Verticillium wilt has not been widely investigated. Two pot experiments were conducted to study the levels of interaction of each fungal species with RKN. Each pot (11x11x12 cm) had three plants with bamboo sticks (1x10cm) attached to the base of the stem for the application of the fungus without disturbing the root and the nematodes. The application of the fungi took place 30 days after the nematode invasion. Scoring of vascular browning on the xylem was recorded. The results showed that the incidence of Fusarium and Verticillium was increased significantly in presence of RKN in both experiments, but the increase in Fusarium was higher than in Verticillium.

Introduction

This work is a comparative study of the effect of root-knot nematode (RKN) *Meloidogyne incognita* on the host reaction to vascular invasion by *Fusarium oxysporum* (Schlecht) f.sp. *vasinfectum* (Fov) (Atkinson, 1892; Armstrong and Armstrong, 1960; Hillocks, 1984) Snyder and Hansen and *Verticillium dahliae* (Kleb) (Bell, 1992), in five cotton varieties differing in their relative susceptibilities to each pathogen and to root-knot-nematode (RKN).

RKN is a sedentary endoparasite of cotton roots, which induces the roots to develop knots or galls. The life cycle has several stages, but the only motile stage is the second stage juvenile that locates and invades roots. Subsequent stages occur in the root around specialized feeding sites.

Fusarium and *Verticillium* are fungi that cause diseases known as vascular wilts. The infection process is similar in both pathogens. The fungus enters the root and after reaching the vessels, sporulates and spreads through the whole vascular system, eventually blocking the vessels. A combination of vessel occlusion due to fungal mycelium, host defense mechanisms and the accumulation of mycotoxins bring about wilting.

It has been widely documented that RKNs enhance the susceptibility to Fov in a number of crop plants. This phenomenon has been widely reported but in the case of *Verticillium* wilt, root-knot nematodes show very little, if any effect on the incidence of wilt symptoms. Initially, researchers assumed that mechanical injury peroxidase in the xylem fluid of the stems. The investigation described here provides information on the stability of resistance to the two fungal diseases in fields infested with root-knot nematodes. This gives some insight into how nematodes affect the incidence from nematode penetration caused resistance breakdown to *Fusarium* wilt. Later work showed that nematodes can increase severe wilt even when nematode and fungal pathogens are spatially separated by inoculation onto different parts of the host. This implies that the effect of nematodes is systemic rather than confined to the roots. However, the mechanism of this systemic effect on host resistance mechanisms is not well understood.

Vascular resistance and its breakdown by RKN

The cytological and physiological changes that affect wilt resistance and are induced by root penetration by RKN are: 1) Formation of giant cells in the root tissue (galls). These cells, adjacent to the vascular elements, are the result of cell wall dissolution leading to large multinucleated bodies from which nematodes (and fungi) derive nutrients (Bird, 1961). 2) Increase of free amino acids and growth in lipids (malic, citric, fumaric, glyoxylic and glutamic acids) that could be a very good carbon source for fungal production of enzymes, (pectinases), capable of breaking down the vascular gels, or inhibiting occlusion (Gothoskar *et al.*, 1955; Pegg, 1981; Scheffer and Walker, 1953). 3) Decrease of sugar levels (glucose-t-P, glucose-6-P, fructose-t-P, and fructose-6-P) that occurs commonly in *Fusarium* wilt (Bell and Mace, 1981). The first step in understanding the role of the nematode in breaking the resistance of cotton to vascular invasion is to determine the effects of nematode invasion of the roots on the level of sugar, amino acids and

of the two vascular wilts, by inducing systemic changes on the physiology and morphology of cotton plants. in the longer term, this information will allow selection of vascular wilt resistant varieties in which resistance is not broken by nematodes and provides an

important data resource for breeding programmes in many cotton-growing countries.

Materials and Methods

Five cotton varieties were used, Auburn 56 (USA), Albar G501. Albar K602 (Zimbabwe), Makoka 78 (Malawi), (*Gossypium hirsutum*) (Upland cotton) and Seabrook SB 12 (*G. barbadense*) (American Egyptian cotton). All experiments were carried out in a greenhouse at a mean temperature of 25-27$_o$C, in 11x11x12cm pits with 3 plants per plot. Fungal experiments were carried out in growth chambers at 26$_o$C for Verticillium and at 28$_o$C for *Fusarium*. The experiment was a complete randomized block design for the varieties, with split-plot on the nematode factor. In each pot 8000 RKN juveniles were applied when the plants were 15-20 days old. Thirty days after RKN inoculation, equal quantities of fungal inoculum were applied in holes close to plants. The standard fungal inoculum was made by blending 15 Petri dish cultures, incubated for 7 days and diluted in two litres of distilled water. At sowing, bamboo sticks were placed next to the seed, to about 7cm depth and a universal bottle (8x2.5 cm) was placed to similar depth in the middle of the pot (Khoury and Alcorn, 1972a; Koury and Alcorn, 1972b). These were left in place until the fungal inoculum was applied in thehole left when they were removed and the holes were then filled with soil. Wilt incidence was estimated by the relative height of vascular browning in the xylem.

Results and Discussion

Based on the vascular browning score in the five varieties tested, RKN significantly increased disease severity for *Fusarium* wilt by more than 25% in the resistant variety ($p=0.0001$). *Verticillium* wilt was increased by a maximum of only 8% ($p=0.022$) (Figure 1). Although the pathogens have similar modes of infection and cause similar symptoms, they seem to interact differently with RKN. Work is continuing to determine the physiological basis for this difference in order to understanding the nature of nematode enhanced susceptibility to *Fusarium* wilt.

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Figure 1. Comprison of the level of *Fusarium* and *Verticillium* wilt incidence.