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INTERACTIONS BETWEEN COMMON RUST, *UROMYCES APPENDICULATUS*, AND FUSARIUM WILT, *FUSARIUM OXYSPORUM* F. SP. *PHASEOLI*, ON COMMON BEANS

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ABSTRACT: The interactions between bean rust and *Fusarium* wilt were investigated in greenhouse experiments, using co-inoculations of common beans with *Uromyces appendiculatus* and *Fusarium oxysporum* f. sp. *phaseoli*. Disease and host parameter data were analyzed using regression equations and analysis of variance. The results revealed that pre-inoculation with *F. oxysporum* had antagonistic effects on rust development expressed as reduced pustule numbers and decreased pustule diameters. On the other hand, inoculation of *F. oxysporum* after *U. appendiculatus* did not affect bean rust development. Inoculation of *U. appendiculatus* had no effect on *Fusarium* wilt development or on host parameters.

Key words/phrases: Common bean rust; Disease interaction; *Fusarium* wilt.

INTRODUCTION

The effects of a disease complex on a host plant are usually estimated assuming that each disease acts independently. However, simultaneous occurrence of diseases on a single plant may result in additive, higher than additive (positive interaction) or lower than additive (negative interaction) effects on the host attributable to each pathogen (Waller and Bridge, 1984). In addition to effects on the host, the interactions among pathogens may enhance or impede activities of either or both pathogens (Bookbinder and Bloom, 1980; Bassanezi *et al.*, 1998). When a certain disease increases the severity of another disease, management of the one that stimulates the other may become more critical. On the other hand, when disease progress is reduced, a type of biological suppression is expressed. Hence, it is necessary to study the effects of simultaneously and sequentially occurring diseases. Johnson *et al.* (1986) and Harrison (1974) have stated that identification of the type of interaction is important, as the expected benefit from management of one pest is dependent on the level of the other pest. Interaction studies also have important implications for assessment and diagnosis of crop losses, as well as for the development of suitable

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management measures (Waller and Bridge, 1984); and its implication can lead to changing the risk assessment and economic decision criteria.

The interactions of diseases can have consequences in practical disease management. As reported by Firman (1972), when the black leaf streak pathogens, *Mycosphaerella fijensi* and *M. musicola*, the causes of Sigatoka disease on banana, were controlled, other cryptic foliar pathogens such as rust (*Uromyces musae*) became more evident, thereby making disease management decisions more complex. Bassanezi *et al.* (1998) further warned that changes in the disease dynamics of common rust due to *Bean line pattern virus* (BLPV) could jeopardize the accuracy of simulation models developed to predict common bean rust progress.

Common rust and *Fusarium* wilt are important foliar and soil-borne diseases of common beans (*Phaseolus vulgaris* L.), and are thought to cause a combined damage to their host. *Fusarium oxysporum* f. sp. *phaseoli*, through its wilting effect on leaves, is expected to hamper the development of rust on beans as the rust pathogen, *Uromyces appendiculatus*, requires vigorous green leaves for its sporulation (Cohen and Rotem, 1970; Wagner and Boyle, 1995). On the other hand, rust is assumed to divert host nutrients for its use, and deprive other plant parts of nutrients essential for their normal development, thereby weakening roots and exposing the host to further invasion by *F. oxysporum* f. sp. *phaseoli* (Lucas, 1998).

The objective of this study was to identify and quantify the type and magnitude of interactions occurring between common rust and *Fusarium* wilt diseases of common beans in affecting the dynamics of each disease on its host.

MATERIALS AND METHODS

Experiments on *F. oxysporum* f. sp. *phaseoli* pre-inoculation followed by *U. appendiculatus*

Plant material

Thirty seeds of common bean 'Dufrix' were sown in autoclaved sand in seed trays with a size of 45 x 25 cm size, which were then placed in a greenhouse at 22 ± 2°C. The greenhouse had a natural light of ten hours and supplemental light of two hours. When the primary leaves were fully expanded, 10 days after sowing (das), the roots were washed free of sand in running tap water, and about one centimeter of the root tips was cut off with a disinfected scissor.

Inoculum production and preparation

F. oxysporum f. sp. *phaseoli* was collected in Brazil from a common bean variety 'Carioca' in 2000. After the isolate was purified, it was stored at 4°C in PDA slants until it was used for the experiments. Pathogenicity of the isolate to variety 'Dufrix' was tested by inoculating the seedlings with *F. oxysporum* f. sp. *phaseoli* suspension adjusted at 10^6 spores ml^{-1}.

The isolate was grown on PDA plates at 24°C for 14 days. The plates were flooded with sterile water, and the suspension was passed through a four-layered sterile cheesecloth. The suspension was adjusted to spore concentrations of 10^2, 10^3, 10^4 and 10^5 *Fusarium* spores ml^{-1} using a haemocytometer.

A suspension of *U. appendiculatus* was prepared by mixing rust urediniospores with sterile water and two drops of Tween 20. The mixture was rotated at 5000 rpm for ten minutes, and passed through a double-layered cheesecloth. The suspension was adjusted to spore concentrations of $4 \cdot 10^2$, $4 \cdot 10^3$ and $4 \cdot 10^4$ urediniospores ml^{-1} with a haemocytometer.

Inoculations

Bean seedlings with cut root tips were dipped for ten minutes into the four spore suspensions of *F. oxysporum* f. sp. *phaseoli* (10^2, 10^3, 10^4 or 10^5 spores ml^{-1}) and in sterile water. After ten minutes, three seedlings were transplanted to 14-cm pots filled with sterilized soil. The pots were then moved into a greenhouse maintained at 22 ± 2°C. The pots were arranged in a randomized complete block design with four replications (one pot was considered as a replication). After the seedlings were well established, they were thinned into two per pot.

When the first trifoliolate leaves were half expanded, 11 days after *F. oxysporum* f. sp. *phaseoli* inoculation (26 das), the first trifoliolate leaves were inoculated with three concentrations of *U. appendiculatus* ($4 \cdot 10^2$, $4 \cdot 10^3$ or $4 \cdot 10^4$ urediniospores ml^{-1}) using hand sprayers. The control treatment was sprayed with sterile water. After inoculation, plants were placed in a humid chamber and kept for 24 hours in the dark. Thereafter, they were transferred to the greenhouse maintained at 22 ± 2°C and ten hours of natural and two hours of supplemental light. The pots were watered daily to maintain the soil moisture at field capacity.

Data collection

Disease assessment was carried out 37 das, corresponding to 17 days after

U. appendiculatus inoculation and 28 days after *F. oxysporum* f. sp. *phaseoli* inoculation. Wilt severity of plants was assessed using the 1 - 9 CIAT scale (van Schoonhoven and Pastor-Corrales, 1987). Rust severity of each leaflet was visually assessed with the descriptive key of Godoy *et al.* (1997). The mean rust severity of each plant was determined by dividing the sum of individual leaflet severities by the number of leaflets assessed. The number of pustules on a 3 by 3 cm leaf area was counted on the same day. This was done three times on the upper surface of each trifoliolate leaflet (on top, middle and bottom parts of each leaflet), making the total counted area 27 cm^2 per leaflet. In addition, one plant from each pot was cut at the soil level, dried in an oven at 65°C for 72 hours and dry weight was determined.

This experiment was repeated once with some adjustments. In the second run, a suspension of *U. appendiculatus* was adjusted to spore concentrations of $4 \cdot 10^2$, $4 \cdot 10^3$ or $4 \cdot 10^4$ urediniospores ml^{-1} with a haemocytometer. Bean seedlings with cut root tips were dipped for ten minutes into the three spore suspensions of *F. oxysporum* f. sp. *phaseoli* (10^3, 10^4 or 10^5 spores ml^{-1}) and in sterile water. The pots were arranged in a randomized complete block design with four replications (one pot was considered as a replication) in a greenhouse maintained at 22 ± 2°C. After the seedlings were well established, they were thinned into two per pot.

When the first trifoliolate leaves were half expanded, 11 days after *F. oxysporum* f. sp. *phaseoli* inoculation (26 das), the first trifoliolate leaves were inoculated with one of the three concentrations of *U. appendiculatus* ($4 \cdot 10^2$, $4 \cdot 10^3$ or $4 \cdot 10^4$ urediniospores ml^{-1}) using hand sprayers. The control treatment was sprayed with sterile water. After inoculation, plants were placed in a humid chamber and kept for 24 hours in the dark. Thereafter, they were transferred to the greenhouse maintained at 22 ± 2°C and ten hours of natural and two hours of supplemental light. The pots were watered daily.

Similar data collection procedure was followed as the first experiment for wilt severity, rust severity and pustule number. In addition, the diameter of 10-15 pustules was measured on two leaflets from each treatment. The measurement was done using Leica MZ 8 binocular fixed with a lens with graduated ruler.

Experiment on *U. appendiculatus* pre-inoculation followed by *F. oxysporum* f. sp. *phaseoli*

Similar plant material, pathogens and experimental design were used as in the *F. oxysporum* f. sp. *phaseoli* pre-inoculation experiment. The inoculation procedures were also similar except the sequence of inoculation. In this experiment, *U. appendiculatus* was inoculated first (27 das), followed by *F. oxysporum* f. sp. *phaseoli* (34 das). Disease assessment of rust severity and pustule number was carried out 31 and 17 days after *U. appendiculatus* inoculation, respectively. Wilt severity was assessed 32 days after *F. oxysporum* f. sp. *phaseoli* inoculation. The weight of oven-dried roots (at 65°C for 72 hours) of two plants was measured 58 das. This test was conducted only once.

Data analyses

For the mathematical analyses, all inoculum concentrations were log-transformed using log (x+1). Therefore, IC_{UA} and IC_{FOP} are the log-transformed inoculum concentration of *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli*, respectively.

The effects of the two inoculations on a measured variable y, for example the shoot dry weight, were checked by fitting the linear multiple regression function (1) with an interaction term to the observed data:

$$y = a - b\,IC_{UA} - c\,IC_{FOP} - d\,IC_{UA}\,IC_{FOP} \qquad (1)$$

If the variable y is the shoot dry weight, then the coefficient 'a' denotes an estimate of shoot dry weight of a healthy plant. The regression coefficients 'b' and 'c' represent the reduction in shoot dry weight due to *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* inoculations, respectively, and 'd' describes the combined effects of *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* inoculation.

When the observed surface corresponding to (1) was clearly non-linear, specific functions describing the trends of the observations were fitted to the data by non-linear regression analyses. For instance, the effect of inoculating *F. oxysporum* f. sp. *phaseoli* prior to *U. appendiculatus* on rust severity (RS) was described using the following regression equation:

$$RS = (a - b\,IC_{UA}\,IC_{FOP})\,(1 - \exp[1 - c\,IC_{UA}]) \qquad (2)$$

Here the second term is a monomolecular function of IC_{UA}, reflecting the fact that rust severity is not increasing linearly with increasing inoculum concentrations. The first term assumes that increasing the *F. oxysporum* f.

sp. *phaseoli* concentration reduces the rust severity for a given *U. appendiculatus* concentration.

The estimated surfaces were plotted in three-dimensional graphs using the Sigma plot regression procedure.

RESULTS

F. oxysporum f. sp. *phaseoli* pre-inoculation followed by *U. appendiculatus*

Rust severity

As expected, rust severity (RS) increased with higher inoculum concentrations of *U. appendiculatus* (Fig. 1a). On the other hand, RS drastically declined from relatively high levels on control plants (without *F. oxysporum* f. sp. *phaseoli* inoculation) to significantly lower levels on plants inoculated with higher *F. oxysporum* f. sp. *phaseoli* concentrations (Fig. 1a). Fitting the multiple linear regression equation (1) to the data resulted in:

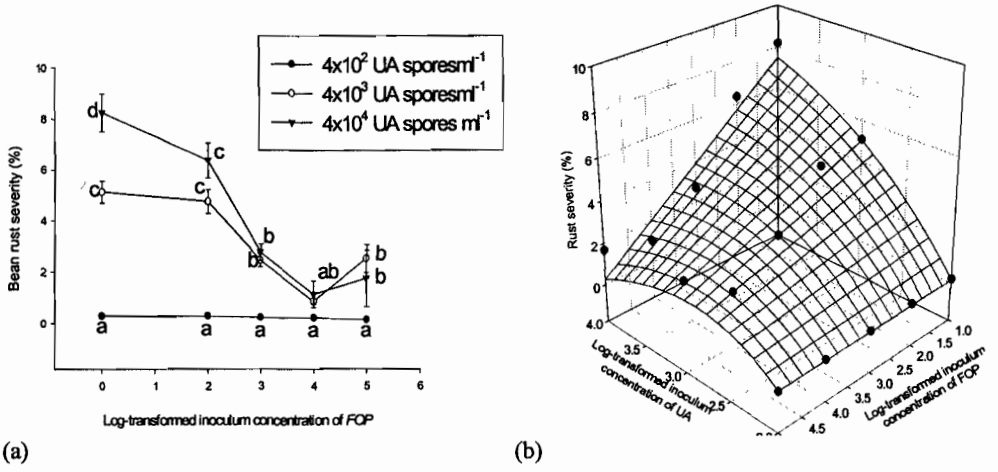
$$RS = -9.7827 + 3.9994\ IC_{UA} + 1.9161\ IC_{FOP} - 0.7457\ IC_{UA}\ IC_{FOP}$$

All regression coefficients are significantly different from 0 and the coefficient of determination R^2 is 86.7%. The positive coefficient of IC_{UA} reflects the increase in rust severity due to inoculation with *U. appendiculatus*. The two terms containing IC_{FOP} describe the effect of *F. oxysporum* f. sp. *phaseoli* concentration on common bean rust, which is negative because of the observed range of *U. appendiculatus* concentrations of the interaction term ($IC_{UA} \geq 2.6$).

From Fig. 1a it can be assumed that the rust severity is not increasing linearly with *U. appendiculatus* concentration. Therefore function 2 was also fitted to the data and resulted in:

$$RS = (15.0920 - 0.6017\ IC_{UA}\ IC_{FOP})\ (1 - \exp[1 - 0.3934\ IC_{UA}])$$

Again, the three regression coefficients are significantly different from 0, and the coefficient of determination is 90.3% (Fig. 1b).



(a) (b)

Fig. 1. (a) Effect of pre-inoculation of beans with *F. oxysporum* f. sp. *phaseoli* (11 days before *U. appendiculatus* inoculation) on rust severity, assessed 17 days after *U. appendiculatus* inoculation. Points followed by the same letter are not significantly different at p=0.05; (b) Response surface of rust severity data given by the equation: $RS = (15.0920 - 0.6017\ IC_{UA}\ IC_{FOP})\ (1 - \exp[1 - 0.3934\ IC_{UA}])$, $R^2 = 90.3\%$, n = 15 (Experiment 1).

The results of the repeated experiment (experiment 2) showed the same trends with respect to *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* inoculations. The maximum disease severity was 13.9% higher than that in the first experiment (8.3%). The coefficients of determination of the two functions were 96.2% for function 1 and 92.4% for function 2.

Number of pustules

For the polycyclic pathogen *U. appendiculatus*, the experiments were carried out in such a way that after inoculation, all bean leaves remained dry and therefore no secondary cycle from the first spore generation occurred. Hence, the number of pustules counted resulted from the initial inoculum *per se*.

Like the rust severity, the pustule number (PN) also increased with higher *U. appendiculatus* concentrations and decreased with increasing *F. oxysporum* f. sp. *phaseoli* concentrations, except for the highest inoculum concentration of *F. oxysporum* f. sp. *phaseoli* (Fig. 2a). When the multiple linear regression equation (1) was fitted to the data, all regression coefficients were significantly different from 0 and the coefficient of determination R^2 was 85.9%:

$$PN = -115.3699 + 49.0768\ IC_{UA} + 22.3853\ IC_{FOP} - 8.7420\ IC_{UA}\ IC_{FOP}$$

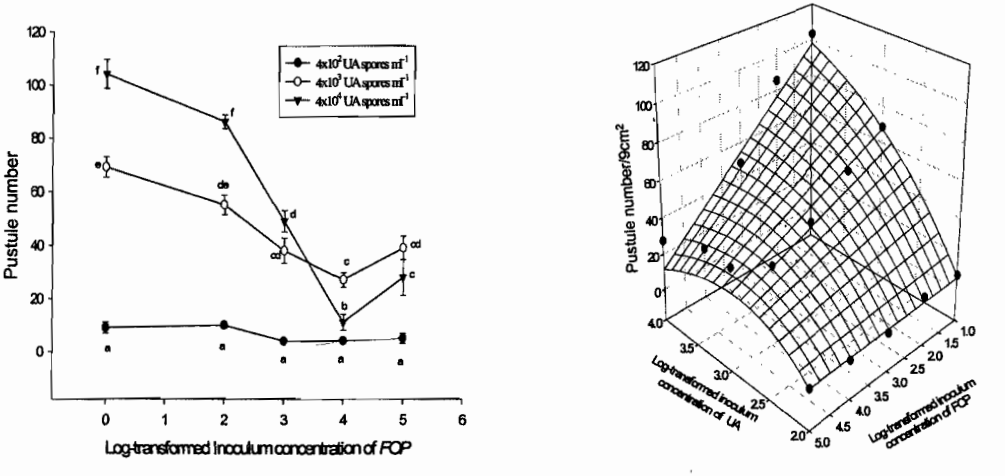
The positive coefficient of IC_{UA} reflects again the increase in pustule number due to the *U. appendiculatus* inoculation. The two terms containing IC_{FOP} describe the effect of *F. oxysporum* f. sp. *phaseoli* concentration on pustule number, which was negative because IC_{UA} was higher than 2.6.

Assuming once more that the pustule number did not linearly increase with *U. appendiculatus* concentration, function (2) was also fitted to the data and resulted in:

$$PN = (186.6258 - 6.8282\ IC_{UA}\ IC_{FOP})\ (1 - \exp[1 - 0.4049\ IC_{UA}])$$

All regression coefficients were significantly different from 0, and the coefficient of determination was 90.9% (Fig. 2b).

The pustule number in the second experiment showed the same trends with respect to *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* inoculations. The pustule numbers were higher in this experiment reaching a maximum value of 475 pustules for $IC_{UA} = 4.6$ and $IC_{FOP} = 0$ instead of 104 pustules for the same inoculations in the first experiment. The coefficients of determination of the two functions were 93.9% for function 1 and 87.7% for function 2.



(a) (b)

Fig. 2. (a) Effect of pre-inoculation of beans with *F. oxysporum* f. sp. *phaseoli* (11 days before *U. appendiculatus* inoculation) on the number of rust pustules on 9 cm^2 leaf area, assessed 17 days after *U. appendiculatus* inoculation. Points followed by the same letter are not significantly different at p=0.05; (b) Response surface of pustule number described by the equation: $PN = (186.6258 - 6.8282\ IC_{UA}\ IC_{FOP})\ (1 - \exp[1 - 0.4049\ IC_{UA}])$, $R^2 = 90.9\%$, n = 15 (Experiment 1).

Pustule size

Similar to the pustule number, the pustule diameter (recorded only in the second experiment) decreased with higher inoculum concentrations of *F. oxysporum* f. sp. *phaseoli*. In addition, the pustules were smaller with increasing *U. appendiculatus* concentrations, but the reduction was not as great as that caused by higher *F. oxysporum* f. sp. *phaseoli*. The pustule diameter on plants inoculated with 10^5 spores ml^{-1} *F. oxysporum* f. sp. *phaseoli* were reduced by 38.0, 39.3 and 49.9% for *U. appendiculatus* concentrations of $4{\cdot}10^2$, $4{\cdot}10^3$ and $4{\cdot}10^4$ spores ml^{-1}, respectively, compared to those not inoculated with *F. oxysporum* f. sp. *phaseoli* (Fig. 3).

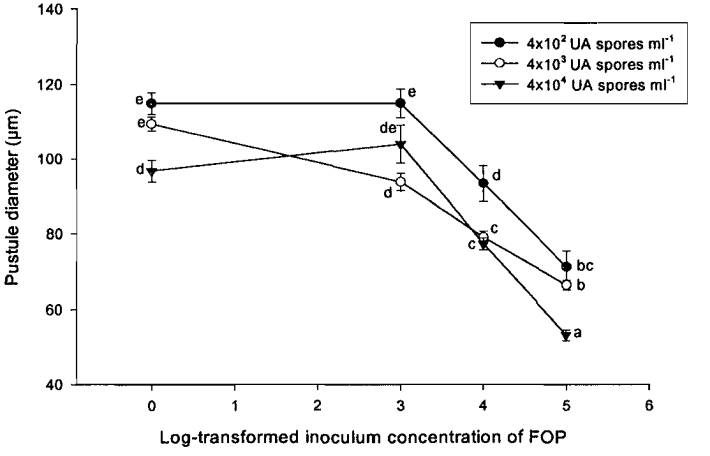


Fig. 3. Effect of pre-inoculation of beans with *F. oxysporum* f. sp. *phaseoli* (11 days before *U. appendiculatus* inoculation) on the diameter of rust pustules, assessed 17 days after *U. appendiculatus* inoculation. Points followed by the same letter are not significantly different at p=0.05 (Experiment 2).

Analysis of variance using the SAS programme showed that the reduction due to higher *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* concentrations was significant. Similarly, the interaction effect of the two diseases was also significant ($p < 0.0001$).

Fusarium wilt

Fusarium wilt on common bean 'Dufrix' was expressed in the form of wilting, chlorosis and stunting of leaves and plants. The disease also caused premature defoliation, and in some cases, premature death.

The wilt severity obviously increased with higher *F. oxysporum* f. sp.

phaseoli concentration, while the effect of post-inoculation with *U. appendiculatus* was not uniform. In the first experiment, the wilt severity ranged from 1.0 (no symptom) on all plants inoculated with 10^2 spores ml^{-1} *F. oxysporum* f. sp. *phaseoli* concentration to 8.25 on plants co-inoculated with *F. oxysporum* f. sp. *phaseoli* and *U. appendiculatus* concentrations of 10^4 and $4 \cdot 10^2$ spores ml^{-1}, respectively (Fig. 4). The post-inoculation with increasing concentrations of *U. appendiculatus* did not cause a clear effect on wilt severity.

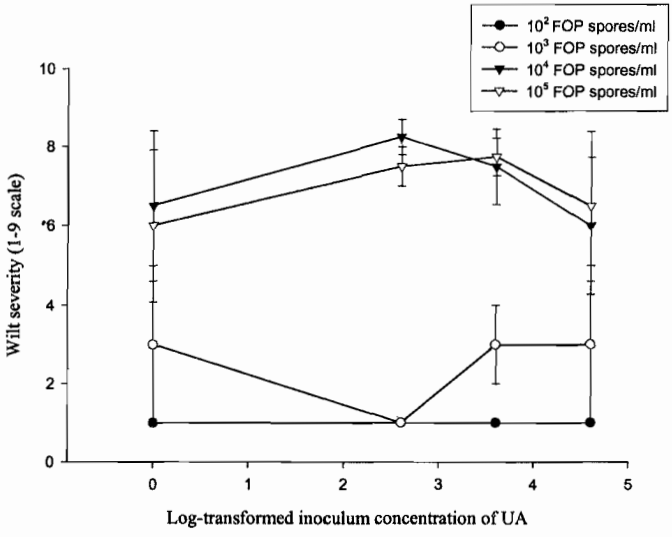


Fig. 4. Effect of post-inoculation of beans with *U. appendiculatus* (11 days after *F. oxysporum* f. sp. *phaseoli* inoculation) on wilt severity, assessed 28 days after *F. oxysporum* f. sp. *phaseoli* inoculation (Experiment 1).

The same trends were also observed in the second experiment, in which the lowest *F. oxysporum* f. sp. *phaseoli* inoculation level did not cause any disease, while the highest inoculation level resulted in a wilt severity of 9. Once more, *U. appendiculatus* inoculations had no effect on wilt severity.

Shoot dry weight

The mean shoot dry weight (SDW) of the 20 inoculation combinations in experiment 1 ranged from 1.8 to 4.5 g/plant. The effects of both inoculations were rather weak. According to the regression analyses using equation (2), *F. oxysporum* f. sp. *phaseoli* and the interaction between *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* had no significant effects on SDW, while

U. appendiculatus inoculations significantly reduced SDW according to the equation: SDW = 3.69 – 0.1822 * IC_{UA} (R^2 = 29.2%).

In the second experiment, SDW was generally lower, varying from 0.4 to 3.0 g/plant. Here *F. oxysporum* f. sp. *phaseoli* inoculations clearly reduced SDW, while the effects of *U. appendiculatus* inoculations were variable (Fig. 5). The statistical analyses revealed that *U. appendiculatus* inoculation as well as the interaction between both inoculations had no significant effects on SDW, but *F. oxysporum* f. sp. *phaseoli* inoculations significantly lowered the weight: SDW = 2.57 - 0.3241 * IC_{FOP} (R^2 = 64.9%).

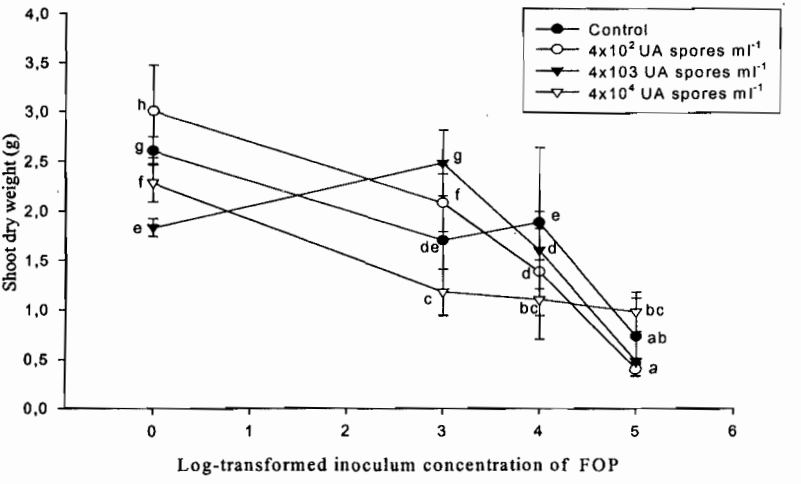


Fig. 5. Effect of post-inoculation of beans with *U. appendiculatus* (11 days after *F. oxysporum* f. sp. *phaseoli*) on shoot dry weight, measured 45 das. Points followed by the same letter are not significantly different at p=0.05.

U. appendiculatus pre-inoculation followed by *F. oxysporum* f. sp. *phaseoli*

Rust severity

Unlike the *F. oxysporum* f. sp. *phaseoli* pre-inoculation experiment, *F. oxysporum* f. sp. *phaseoli* post-inoculation did not affect rust severity. As expected, rust severity increased with higher *U. appendiculatus* inoculum concentrations. The mean rust severities, assessed 17 days after inoculation with *U. appendiculatus* concentrations of $4 \cdot 10^2$, $4 \cdot 10^3$ and $4 \cdot 10^4$ spores ml^{-1}, were 0.007, 1.33 and 10.22%, respectively. The severities reached 0.21, 2.63 and 14.97% 31 days after inoculation with the same *U. appendiculatus* concentrations (Fig. 6).

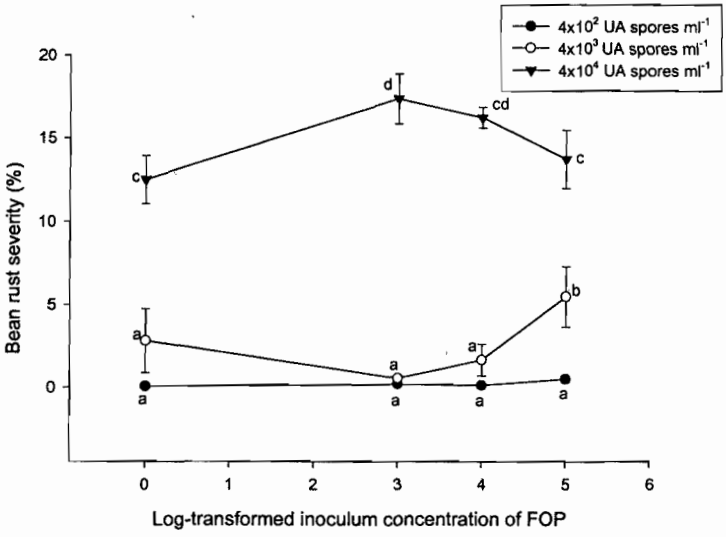


Fig. 6. Effect of post-inoculation of beans with *F. oxysporum* f. sp. *phaseoli* (7 days after *U. appendiculatus*) on rust severity, assessed 31 days after *U. appendiculatus* inoculation. Points followed by the same letter are not significantly different at p=0.05.

Pustule number

High *U. appendiculatus* concentrations resulted in high pustule numbers (Fig. 7). Inoculation of beans with *F. oxysporum* f. sp. *phaseoli* one week after *U. appendiculatus* inoculations did not affect the number of pustules that emerged.

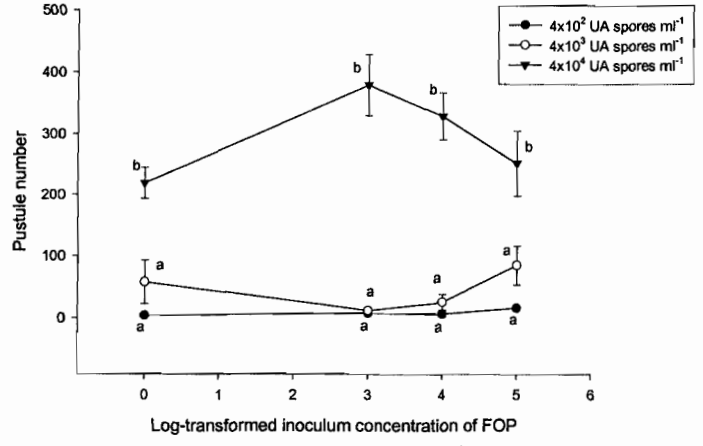


Fig. 7. Effect of post-inoculation of beans with *F. oxysporum* f. sp. *phaseoli* (7 days after *U. appendiculatus*) on number of rust pustules on 9 cm² leaf area, assessed 17 days after *U. appendiculatus* inoculation. Points followed by the same letter are not significantly different at p=0.05.

Wilt severity

The initial wilt symptoms began two weeks after *F. oxysporum* f. sp. *phaseoli* inoculation, which was one week later compared to the *F. oxysporum* f. sp. *phaseoli* pre-inoculation experiment. *Fusarium* wilt severities on plants inoculated with 10^4 and 10^5 spores ml^{-1} were intermediate (≤5) 27 days after *F. oxysporum* f. sp. *phaseoli* inoculation. The severity reached higher levels (>5) 32 days after inoculation (Fig. 8). Similar to the *F. oxysporum* f. sp. *phaseoli* pre-inoculation experiment, *U. appendiculatus* concentrations did not affect wilt disease development. Generally, wilt progression was slower when bean plants were inoculated at later developmental stages rather than earlier ones.

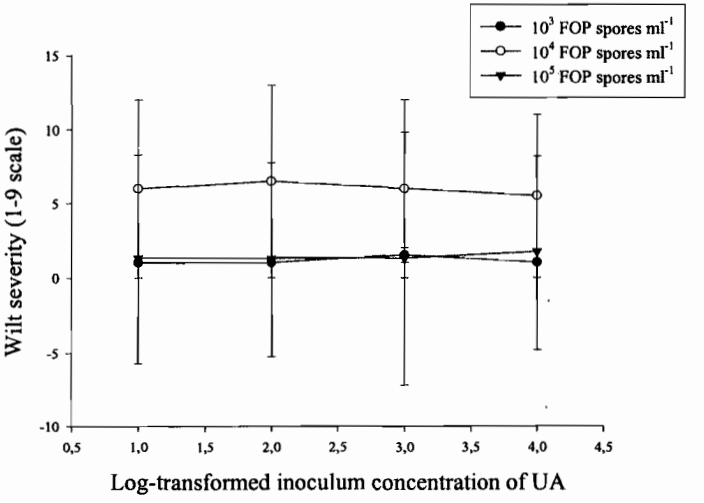


Fig. 8. Effect of pre-inoculation of beans with *U. appendiculatus* (11 days before *F. oxysporum* f. sp. *phaseoli* inoculation) on wilt severity, assessed 32 days after *F. oxysporum* f. sp. *phaseoli* inoculation.

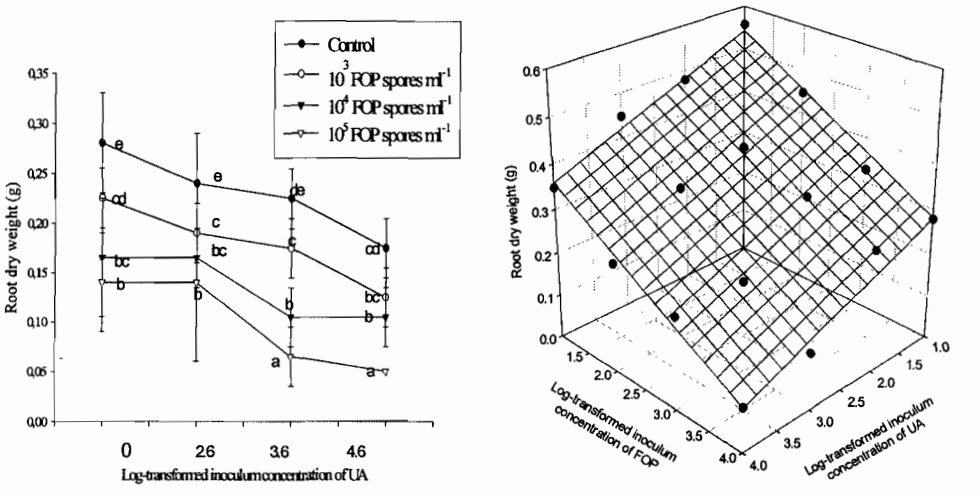
Root dry weight

U. appendiculatus and *F. oxysporum* f. sp. *phaseoli* inoculations both reduced root dry weight (RDW) (Fig. 9a). The fitting of equation (1) to the RDW data resulted in highly significant coefficients for IC_{UA} and IC_{FOP}, but a non-significant coefficient of the interaction term. Therefore, RDW can be predicted with the following equation (Fig. 9b), reflecting that the two diseases had independent effects on root weight:

$$RDW = 0.5846 - 0.0406\ IC_{UA} - 0.0502\ IC_{FOP}$$

The values of the two regression coefficients are similar, meaning that the inoculum concentrations of both pathogens affect RDW in the same extent.

In this experiment it was observed that the roots of bean plants inoculated with both pathogens had a less extensive lateral root system and the number of root hairs was less than that of non-inoculated plants.



(a) (b)

Fig. 9. (a) Effect of post-inoculation of beans with *F. oxysporum* f. sp. *phaseoli* (7 days after *U. appendiculatus*) on root dry weight, measured 58 das. Points followed by the same letter are not significantly different at p=0.05; (b) Response surface of RDW in relation to the inoculum concentrations: $RDW = 0.5846 - 0.0406\ IC_{UA} - 0.0502\ IC_{FOP}$, $R^2 = 91\%$, $n = 16$.

DISCUSSION

In this paper, the effects of co-inoculations with *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* in different sequences on disease expression and host parameters were investigated in a controlled environment. For the analyses, we used a logarithmic transformation for inoculum concentration, although van der Plank (1975) made some critical remarks on the use of the log-transformed amount of inoculum in disease/inoculum relations. Here the logarithmic transformation was used to compress the inoculum concentration data covering a wide range of concentrations.

As generally expected, the severity of each disease was stronger with higher inoculum concentration of the respective pathogen. However, the relationships between the amount of inoculum (log-transformed) and the severity of disease were in most cases non-linear, but curved to the right. For common bean rust, this could be explained by the competition for infection sites (stomata) on the leaf (van der Plank, 1975). Similarly, the

smaller pustule size at high *U. appendiculatus* concentrations presumably resulted from crowding and competition of pustules for space and nutrients (de Paula, 2002).

The pre-inoculation with increasing *F. oxysporum* f. sp. *phaseoli* concentration led to a progressive decrease in rust severity (as gauged by a decrease in pustule number and size), which was indicative of the antagonistic effect of *F. oxysporum* f. sp. *phaseoli* on rust development. Host mediated antagonism is thought to impair the development of rust on plants infected by *F. oxysporum* f. sp. *phaseoli*. The underlying mechanism according to Lucas (1998) is through the impairment of flow of water and mineral salts through the xylem, and that affects the water economy of the host. Consequently, it decreases the photosynthetic process, and deprives rust pustules that require sugar, the necessary nutrient for their normal development. Cohen and Rotem (1970) confirmed that in obligate parasites, such as downy mildew and rust, the inhibition of photosynthesis resulted in a most striking decrease in sporulation. In line with these explanations, in our experiments, the initial white rust flecks were seen to be dying before developing into uredinia, thus the reduced number of pustules was evident. In addition, the flecks that developed to uredinia did not achieve their full size on wilted leaves. The combined effects of these phenomena on wilted leaves were presumed to be the main causes for the low level of rust severity on plants infected with *Fusarium* wilt. The antagonistic interactions observed in these experiments between *Fusarium* wilt and common rust appear similar to those between *Rhizoctonia* root rot and rust on beans (de Paula, 2002) and that of nematodes and rust on beans (Bookbinder and Bloom, 1980). Another possible explanation for low level of rust development on plants pre-inoculated with *F. oxysporum* f. sp. *phaseoli* could be initiation of induced resistance by *F. oxysporum* f. sp. *phaseoli* as explained by Nelson (2005).

In the *U. appendiculatus* pre-inoculation experiment, the rust pustules had already emerged with their full sporulation capacity by the time initial wilt symptoms appeared on primary leaves. Hence, *F. oxysporum* f. sp. *phaseoli* had no effect on rust severity and pustule number. Even though we did not observe any significant effect of *F. oxysporum* f. sp. *phaseoli* inoculation on rust severity, it might still have a negative impact on the dynamics of rust as it had caused defoliation and killed the plant before its normal maturity time, thereby reducing the quantity of spores during later reproduction and total dissemination.

Inoculation of plants with *F. oxysporum* f. sp. *phaseoli* at a late age was assumed to be the reason for low wilt severity. This was also true for celery, where two and four week old plants were killed when grown in artificially infested soils; while similarly treated six and eight week old plants were severely affected, but not killed (Hart and Endo, 1981). The low wilt severity and time lag in causing infection might play an important role for the insignificant influence of *F. oxysporum* f. sp. *phaseoli* on rust development in the *U. appendiculatus* pre-inoculation experiment.

The damage caused by biotrophic pathogens is to some extent due to their ability to redirect host nutrients for their own use, i.e., the fungal colonies act as 'metabolic sinks'. In this way, photosynthate originally destined for developing host tissues, such as new shoots and roots, is instead utilized by the pathogen (Lucas, 1998). Under such circumstances, it is also assumed that the roots are weakened and exposed to easy invasion by root pathogens like *Fusarium spp.* The photosynthetic stress-translocation balance concept of Dodd (1980) also supports the above-mentioned idea. The second assumption is that rust reduces the growth of bean roots, thereby minimizing the probability of roots coming into contact with *Fusarium* inoculum. Hence, it decreases the incidence of *Fusarium* wilt. Such an assumption, of course, could not be verified by the root dip inoculation method, which brings the roots into direct contact with the inoculum. However, our experiments contradicted the above-mentioned general and specific statements of protective or activating effects of biotrophic pathogens against or to infection of other pathogens. Rather it was evident that rust disease did not have any effect on the *Fusarium* wilt development. This might have happened due to the presence of leaves that were not inoculated with *U. appendiculatus* to support the whole plant.

The combined effect of both diseases on shoot dry weight was not uniform in either experiment. In experiment 1, both diseases had only weak effects, which were only significant for rust, but not for wilt. This could be attributed to the fact that in this experiment the wilt reached only a medium level (only one wilt score out of 20 was above 7.7). In the second experiment with higher wilt disease (5 wilt scores out of 16 were above 8.5), shoot dry weight consistently decreased with increasing *F. oxysporum* f. sp. *phaseoli* concentrations, while no consistent trend with respect to rust concentration could be detected. This phenomenon could be explained by defoliation, which was promoted more by wilt than by rust. Defoliation, in addition to its effect on reducing the leaf number and thereby the shoot weight, also reduced the photosynthesizing area, and the plant could not

produce enough assimilates to promote and/or maintain its growth.

The negative regression coefficients for IC_{UA} and IC_{FOP} in the equation for RDW are indicative of the reducing effects of *U. appendiculatus* and *F. oxysporum* f. sp. *phaseoli* inoculations on bean root weight. As the values of both coefficients are similar, the reducing impact of both inoculations on RDW was similar.

The results of this experiment may not be extrapolated to the field situation, but it has produced ample evidence that interactions involving the two diseases may frequently occur in many production systems, thereby influencing disease assessment and/or management practices. Hence, it is strongly recommended to undertake verification tests under natural epidemic situation in the field.

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