INFLUENCE OF CULTIVAR, INOCULUM DENSITY AND PLANT AGE ON THE INCIDENCE OF *FUSARIUM* ROOT AND STEM ROT IN COWPEA

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ABSTRACT

This study was undertaken to determine the effect of host genotype, inoculum density and plant age on the severity of *Fusarium* root and stem rot in cowpea (*Vigna unguiculata*). Different levels of *Fusarium solani* inoculum, namely: 8×10^4, 2×10^6 and 4×10^8 conidia/mL were inoculated to 5-, 17- and 22-day old seedlings of TVX 289-4G, VCS 6-1 and CES 42-2 cowpea cultivars. Among the cultivars tested, CES 42-2 was the most resistant since it exhibited a lower degree of infection in contrast to VCS 6-1 and TVX 289-4G. The degree of infection was not significantly affected by the different levels of inoculum used. However, plant age significantly affected the percentage of infected plants. *Fusarium* root and stem rot infection was higher in 22- than in 17-day old plants, and was completely suppressed in 5-day old seedlings. Significant interaction effects of inoculum density, cultivar and plant age as well as their combinations on disease severity were noted.

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KEY WORDS: *Fusarium* root and stem rot. *Fusarium solani*. Inoculum density. Cultivar. Cowpea *(Vigna unguiculata)*.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp] an important source of protein in the tropics, is subject to attack by pathogens throughout its life cycle. One of the most destructive root attacking organisms is *Fusarium solani* (Mort.) Appel and Wr. f. sp. *phaseoli* (Burt) Snyd and Hans. This soil borne pathogen causes severe rotting of the stem and roots of beans in many areas in the world (Snyder, 1959; Hagedorn and Rand, 1974). In the Philippines, most commercial varieties of cowpea are susceptible to root and stem rot disease. The control of the disease is essential to develop and maintain a high level of cowpea production. This could best be attained through the use of resistant cowpea cultivars. Knowledge of the plant age most susceptible to the disease and the effective inoculum density serve as key factors in screening and breeding processes. This study was therefore conducted to determine the effect of varietal differences, inoculum density and plant age on the severity of *Fusarium* root and stem rot disease; and to know the interaction effect of inoculum density, plant age and cultivar on disease severity.

MATERIALS AND METHODS

Preparation of the Soil Medium

The soil medium used was a mixture of two parts coarse sand and one part of moderately fertile garden loam soil. It was sterilized by baking, and allowed to stand for 7 days after sterilization before planting the test plants.

Care and Management of the Test Plants

Seeds of three cowpea cultivars, namely: TVX 289-4G, VCS 6-1 and CES 42-2 were obtained from the Institute of Plant Breeding, U.P. at Los Baños, and planted in plastic trays with sterilized soil. The trays were placed on wooden benches and the soil moisture was kept within the range of 75-100%.

Preparation of the Inoculum

An isolate of *Fusarium* causing bean root rot, which was originally obtained from infected cowpea plants, was grown in potato dextrose agar (PDA) slant cultures. Four-day old culture of the isolate was scraped carefully after addition of sterile distilled water. Using a Microstar microscope and a haemacytometer, the spore concentration of the suspension was determined by counting the total number of spores in two 25 square areas of the haemacytometer just prior to inoculation. The suspension was a mixture of macroconidia and microconidia.

From the stock solution which contains 4.368×10^8 conidia/mL, three concentrations were prepared namely: 8×10^4, 2×10^6 and 4×10^8 conidia/mL. These inoculum densities were inoculated on test plants of the three cowpea cultivars at three seedling stages at the rate of 1 mL per plant.

Inoculation and Transplanting

At the time of inoculation, 5-, 17- and 22-day old seedlings of different cowpea varieties were uprooted, washed with tap water to remove the soil particles, rewashed with distilled water, and placed on a piece of tissue paper to drain the excess water. The plants were wounded by cutting the root tips with a pair of sharp scissors sterilized in 90% alcohol. The wounded plants were dipped for 10 minutes in a beaker containing the inoculum and then four plants were transplanted per pot. The excess amount of inoculum was pipetted equally into the pots, i.e. at the base of the hole prepared for transplanting. Check plants were similarly treated with distilled water. The transplanted seedlings were kept in the shade for 2 days and then exposed to sunlight for 2 weeks. The plants were regularly watered with ordinary tap water. The percentage of infected plants was determined at 14 days after inoculation.

Determination of Degree of Infection

The development of root and stem rot diseases in the plants as shown by the external appearance of the plants was observed at 7, 10 and 14 days after inoculation using the following rating scale:

0 = Healthy appearance; no infection
1 = Very slightly infected
2 = Slightly infected
3 = Moderately infected
4 = Severely infected
5 = Very severely infected or dead

The percentage of infected plants was determined at 14 days after inoculation.

Experimental Design

Age and inoculum densities were used as treatments in this 3 x 3 factorial experiment arranged in a completely randomized design (CRD) with two replications for each variety.

RESULTS AND DISCUSSION

Table 1 shows that significant differences in the percentage of infected plants exist among different cultivars. Among the three cultivars tested, VCS 6-1 was highly susceptible followed by TVX 289-4G, while CES 42-2 was resistant.

As far as the amount of inoculum is concerned, high density of inoculum does not always result in high intensity of the disease. The data in Table 1 show no significant differences in the percentage of infected plants with increasing inoculum density. This implies that disease intensity is not dependent only on one factor, e.g. inoculum density, but also on other factors that affect the efficiency of the initial inoculum such as temperature, moisture, soil pH, amount and form of nitrogen, fertilizer and host resistance. If the interaction of the different factors is favorable for the pathogen, then the intensity of

Table 1. Percentage of infected cowpea plants at 14 days after inoculation as influenced by inoculum density and/or variety.

Variety	Percentage of Infected Plants[1]			
	Inoculum Density (conidia/mL)			Mean
	8×10^4	2×10^6	4×10^8	
TVX 289-4G	9.67c	26.33bc	29.17bc	21.72b
VCS 6-1	54.17ab	62.50a	33.33abc	50.00a
CES 42-2	8.33c	16.67c	25.00bc	16.67c
Mean	24.06a	35.17a	29.17a	

[1]In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

infection is higher even at low level of inoculum. It should also be noted that during the conduct of this experiment; frequent rains, low light intensity, low temperature and high relative humidity prevailed. Reports of Griffin (1964) and Kouyeas (1964) indicate that low temperature was one of the limiting factors for the growth of the pathogen.

Analysis of variance revealed significant interaction effects of inoculum density and variety on disease severity (Table 1). The percentage of infected plants was significantly highest in VCS 6-1 at inoculum density of 8×10^4 conidia/mL. This can be attributed to the high susceptibility of this cultivar to *Fusarium* root and stem rot. With high susceptibility, minimum amount of inoculum is required to cause infection. TVX 289-4G and CES 42-2 gave significantly lower percentages of infection even at high inoculum densities.

Plant age significantly affected the incidence and severity of *Fusarium* root and stem rot on cowpea (Table 2). Generally, the percentage of infected plants was higher in the 22-day old plants, followed by the 17-day old plants. However, 5-day old plants were not infected. These observations imply that younger cowpea plants possess considerable resistance to the growth of *Fusarium solani*, but this resistance is lost with age. Findings of Williams (1975) strongly support this contention.

Plant age and cultivar showed significant interaction effects on disease severity (Table 2). Twenty two-day old VCS 6-1 plants obtained the highest percentage of infection. Infection by *F. solani* was completely inhibited in 5-day old seedlings of all cultivars studied. This is attributed to the presence of some unknown resistance mechanisms in the younger cowpea plants.

Table 3 presents the interaction effects of plant age and inoculum

Table 2. Percentage of infected cowpea plants at 14 days after inoculation as influenced by plant age and/or variety.

Variety	Percentage of Infected Plants[1]			
	Plant Age (days)			Mean
	5	17	22	
TVX 289-4G	0f	26.67cde	37.50bcd	21.72b
VCS 6-1	0f	66.67ab	83.33a	50.00a
CES 42-2	0f	8.33ef	41.67bc	16.67c
Mean	0c	34.22b	54.17a	

[1] In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

Table 3. Mean interaction effect between plant age and inoculum density on percentage infected plants.

Plant Age (days)	Percentage of Infected Plants[1]		
	Inoculum Density (conidia/mL)		
	8×10^4	2×10^6	4×10^8
5	0f	0f	0f
17	38.83abc	38.83a-d	25.00cde
22	33.33cde	66.67a	62.50ab

[1] In a column or row, treatment means followed by a common letter are not significantly different at 5% level, DMRT.

density on percentage of infected plants. Percentage infection by *Fusarium solani* was significantly highest in 22-day old plants at inoculum density of 2×10^6 conidia/mL. This might be the optimum amount of inoculum needed to cause the highest infection at this specific age of plant.

The development of *Fusarium* root and stem rot as judged by external appearance is summarized in Table 4. No infection was noted in 5-day old plants in all varieties and inoculum densities studied. For 17-day old cowpea plants, the first symptom of infection was observed 7 days after inoculation in both

Table 4. Evaluation of disease development based on external appearance.

Variety	Inoculum Density (conidia/mL)	Age (days)	Degree of Infection [1]		
			7 days	10 days	14 days
TVX 289-4G	8×10^4	5	0	0	0
	2×10^6	5	0	0	0
	4×10^8	5	0	0	0
	8×10^4	17	1	1	1
	2×10^6	17	3	3	3
	4×10^8	17	3.5	3.5	3.5
	8×10^4	22	2.5	2.5	2.5
	2×10^6	22	0	0	2
	4×10^8	22	3	3	3
VCS 6-1	8×10^4	5	0	0	0
	2×10^6	5	0	0	0
	4×10^8	5	0	0	0
	8×10^4	17	3.5	4.75	5
	2×10^6	17	3.5	3.5	5
	4×10^8	17	0	1	3
	8×10^4	22	0	1.5	3.5
	2×10^6	22	1	2	4
	4×10^8	22	1	2	4
CES 42-2	8×10^4	5	0	0	0
	2×10^6	5	0	0	0
	4×10^8	5	0	0	0
	8×10^4	17	0	0	3
	2×10^6	17	0	0	0
	4×10^8	17	0	0	0
	8×10^4	22	0	0	0
	2×10^6	22	0	0	3
	4×10^8	22	2	2	3

[1] Mean of two individual infected plants based on the following rating scale: 0 = healthy appearance, no infection; 1 = very slightly infected; 2 = slightly infected; 3 = moderately infected; 4 = severely infected; and 5 = very severely infected or dead.

TVX 289-4G and VCS 6-1. In CES 42-2, infection occurred 14 days after inoculation. In TVX 289-4G, the degree of infection remained constant with time while in VCS 6-1, infection increased in severity with time.

In 22-day old plants, symptoms of infection first appeared 7 days after inoculation in both TVX 289-4G and VCS 6-1 at inoculum levels of 8×10^4 and 2×10^6 conidia/mL, respectively. However in CES 42-2, the symptom was visible only at the highest inoculum density of 4×10^8 conidia/mL at 7 days after inoculation.

This result clearly manifests how the different varieties react when infected by a pathogen. A susceptible host like VCS 6-1 reacts in such a way that infection increases in severity with time. On the other hand, resistant varieties such as CES 42-2 and TVX 289-4G allow a certain degree of infection (remarkably less) that remains constant with time. Most of the resistant plants even exhibited signs of recovery after showing the first symptom.

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