

EFFECT OF MIXED SPORE INOCULA OF *BOTRYTIS*
SPP. ON LESION DEVELOPMENT IN THE
LEAVES OF BEAN (*VICIA FABA* L.)

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There is evidence in the literature that the tissue of a host plant becomes unsuitable for the normal growth of a virulent fungal strain if it is inoculated simultaneously or previously by an avirulent strain of a fungus species (Muller, 1959). It has been reported by Muller and Borger (1940) that even virulent races of *Phytophthora infestans* cannot grow normally on cut surfaces of potato tubers if the same regions are previously inoculated by an avirulent race of the said fungus. The information obtained from these studies also indicates that the growth inhibition of the pathogen depends on the relative spore concentrations of the test strains as well as on the time interval between the successive inoculations. The above authors postulated that the inhibition of growth of the invading fungi is due to a nonspecific principle called "phytoalexin" which is formed post-infectionally in a hypersensitive tissue. Recently, it has been found in case of the "chocolate spot" disease of beans that the two species of *Botrytis* namely, *B. fabae* and *B. cinerea* produce lesions on the leaves but only lesions caused by *B. fabae* spread under high humid conditions although those caused by *B. cinerea* do not (Purkayastha and Deverall, 1965). This observation creates an interest and hence it has been decided to investigate whether the mixed spores of the two *Botrytis* spp. have any effect on the normal development of lesions produced by the spores of *B. fabae* in mixed inocula and also to find out whether different proportions of spores of the two species in the inocula have any role in the spread of lesions.

In the present study, three experiments were designed to determine the effect of mixed spores on lesion development. The proportion of spores of *B. fabae* (virulent species) in the inocula was 50, 67 and 33 per cent respectively (Table 1). Controls were maintained to compare the development of lesions caused by *B. fabae* alone. No separate control, however, was kept for *B. cinerea* as the lesions developed due to the said species do not spread. The methods followed for the experiments have been described below.

Spore suspensions of the test fungi were prepared separately from 11-day old cultures as described (Deverall and Wood, 1961). Second leaves of twelve plants (22-day old) were washed, dried and detached; four leaves were placed on moist filter paper in each transparent polystyrene box (14×4×10 cm.) with their petioles kept immersed in water

in watch glasses. Ten standard drops ($1\mu\text{l}$) of spore suspension containing a known number of *B. cinerea* spores were placed on the left leaflet of each leaf from a hypodermic needle fitted to an 'Agla' micrometer syringe. Immediately after the first inoculation the same spots were reinoculated with the spores of *B. fabae* as shown in Table 1. The leaves were incubated at a temperature of $17-18^{\circ}\text{C}$. and 250 f.c. light intensity of the glass cabinet. Replicates of four leaflets were employed for each treatment. The number of small and big lesions were counted and recorded as percentage of inoculum drops which had given rise to lesions after 24 hours and the lesions spread after seven days as the percentage of leaf areas which had become blackened as a result of infection. The results are shown in Table 1.

Table 1. *Effect of mixed spore inocula of Botrytis spp. on lesion development in detached leaves of bean*

No. of Expt.	Fungus	No. of spores/inoculum	No. of small lesions <1 mm. (%)	No. of big lesions >1 mm. (%)	Total No. of lesions (%)	*Spreading lesions (%)
I	<i>B. cinerea</i>	250	43	45	88	20
	<i>B. fabae</i>	250				
	<i>B. fabae</i>	250	35	50	85	24
II	<i>B. cinerea</i>	250	40	48	88	17
	<i>B. fabae</i>	250				
	<i>B. fabae</i>	500	35	55	90	53
III	<i>B. cinerea</i>	250	35	63	98	33
	<i>B. fabae</i>	500				
	<i>B. cinerea</i>	500	40	43	83	0
	<i>B. fabae</i>	250				

It appears from the results that there is no significant difference in the percentage of spreading lesions produced either by 250 spores of *B. fabae* alone or by the mixed spore inocula containing equal number (250+250) of spores of *B. fabae* and *B. cinerea*. This indicates that apparently the growth of *B. fabae* is not much affected by *B. cinerea* when the host tissue is inoculated almost simultaneously with an equal number of spores of both the species. The little difference might be either due to the different concentrations of spores in the inocula or due to the reduction of germ tube growth of the virulent species on the lesion areas. The result also suggests that the large proportion of spores of *B. cinerea* (avirulent species) in the mixed inocula shows the progress of spreading lesions caused by the spores of *B. fabae*. This inhibition of growth may be due to

* leaf area diseased due to spread of lesions.

the production of antifungal substances in the lesion areas as suggested earlier by Purkayastha and Deverall (1964). However, it is not possible at this stage to state whether the antifungal substances responsible for the growth inhibition of the virulent species are formed as a result of interaction between the avirulent species and the host cells or are formed by one of the two invading fungi in the lesion areas.

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