

Witches' broom disease of *Theobroma cacao* in Brazil. I. Inoculation of ungerminated seeds

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Abstract

Ungerminated seeds of cacao infiltrated under vacuum with spore suspension of *Crinipellis pernicioso* (Stahel) Singer and then pre-germinated before planting produced hypertrophied hypocotyls and developed hypertrophied axillary buds. This technique of inoculation could be useful in mass-screening of individual plants for their reaction to infection by *C. pernicioso*.

Key words: *Theobroma cacao*, *Crinipellis pernicioso*, inoculation

Vassoura-de-bruxa de *Theobroma cacao* no Brasil. I. Inoculação de sementes

Resumo

Sementes de cacau infiltradas com suspensão de *Crinipellis pernicioso* (Stahel) Singer sob vácuo e pré-germinadas antes do plantio apresentaram hipertrofia do hipocótilo e desenvolveram gemas axilares hipertrofiadas. Esta técnica de inoculação pode ser usada em seleção massal de plantas individuais, pelas suas reações à infecção por *C. pernicioso*.

Palavras-chave: *Theobroma cacao*, *Crinipellis pernicioso*, inoculação

Introduction

Witches' broom disease of cacao, which is caused by the fungus *Crinipellis pernicioso* (Stahel) Singer, was first reported in Surinam in 1895 and is wide spread in several South American countries. Decline of cacao production as a result of infection by *C. pernicioso* had a direct effect on development of cacao industries and as a result, intensive

research on methods of control was initiated. Various methods have been used, such as application of chemotherapeutants and sanitary cultural practices (reduction of inoculum by removal of all infected tissues). However, the best suitable means of control is the use of resistant varieties. In order to identify resistant genotypes and varieties they have to be evaluated for their reaction

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to *C. pernicioso* to determine their value in breeding.

Methods of inoculation of different parts of cacao by *C. pernicioso* as means of studying the infection processes were reported by several authors (Baker and Crowdy, 1943; Baker and McKee, 1943; Briton-Jones and Cheesman, 1931; Cronshaw and Evans, 1978). Holliday (1955) was the first to investigate inoculation of germinated seeds as a means of evaluating resistance in cacao seedlings. In view of the low rates of infection obtained during the early phases of germination, methods of improving the possibility of infection in ungerminated seeds was investigated.

Materials and Methods

Basidiocarps produced by dried brooms were collected and prepared as described by Evans (1978). The basidiospores were rinsed with sterile glass-distilled water (5ml/plate) and brought to suspension with a camel-hair brush. Suspensions of basidiospores were pooled together to give a concentration $> 5 \times 10^5$ /ml.

The seeds used for inoculation consisted of the embryos after removal of testas. The seeds were immersed in the spore suspension in groups of five in a 50ml beaker and were infiltrated in a dessicator using a vacuum pump for 0.5 hr. Control seeds were treated with sterile glass-distilled water. Since germinated spores do not infect, the suspension were examined periodically to make sure that they contained spores with the ability to infect.

After infiltration, the seeds were germinated for 4 days under room temperature in Petri dishes lined with moist filter paper. The seedlings were planted in potting soil in plastic pots and grown in the greenhouse.

Results and Discussion

Infiltrating ungerminated seeds of cacao with spore suspension of *C. pernicioso* did not have any effect on germination. Within 3-4 weeks seedlings produced symptoms which were identical with those described by Holliday (1955) when meristematic tissues of 4-day old seedlings were dipped for a few seconds in spore suspension of *C. pernicioso*. The symptoms consisted of hypertrophy in the hypocotyl and axillary buds at the cotyledonary node (Fig. 1). The development of the cotyledonary buds resulted from the effect on the terminal bud of nutrient imbalance caused by disturbance in the hypocotyl and root system. Three months after the first symptoms, seedlings with severe symptoms started to become necrotic.

Ninety per cent of the plants treated by this method of inoculation were infected. Inoculation of ungerminated cacao seeds by vacuum infiltration could be used as an alternative method in screening large quantities of cacao material against infection by *C. pernicioso*, studies on pathotypes of *C. pernicioso* and the effect(s) of infection on the physiology of the aerial parts of cacao seedlings.

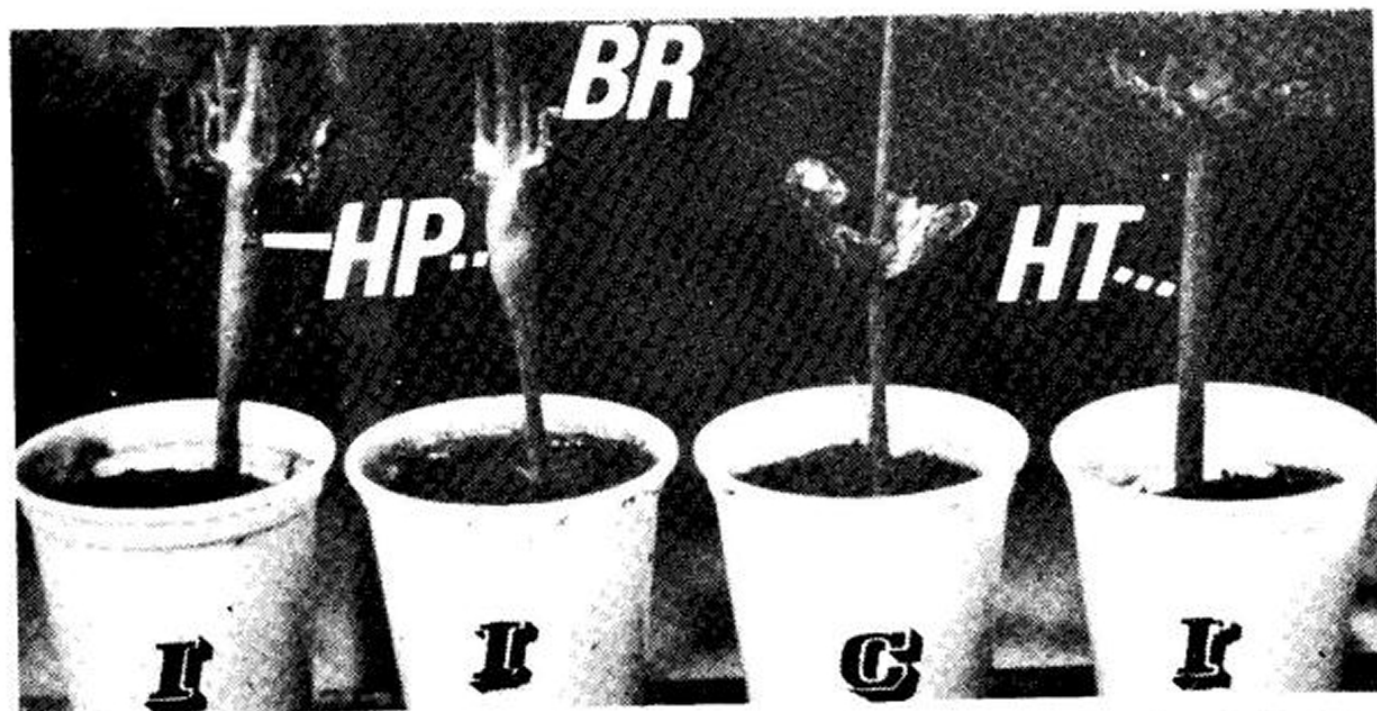


Figure 1 – Seedlings of *T. cacao* 4 weeks after inoculation by infiltration. (I) and Controls (C) and inoculated seeds (I) showing typical symptoms. BR = broom, HP = hyperplasia, HT = hypertrophy.

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