Effect of inoculum composition on infection of French bean leaves by conidia of Botrytis cinerea

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Accepted 28 January 1981

Abstract

Inoculation of leaves of French bean (Phaseolus vulgaris) with sprays or small drops of a suspension of conidia of Botrytis cinerea gave rise to spreading lesions, lesions remaining restricted in size or to no visible necrosis. The type of reaction depended on the composition of the inoculum.

In studies with drop inoculations with buffered inocula some of the factors involved were analyzed. The formation of spreading lesions depended on pH, type and molarity of the buffer, presence of glucose, and concentration of conidia in the inoculum. If the phosphate buffer used in most of the inocula was replaced by monobasic phosphate, similar results were obtained. The reactions were not influenced by the proportion of K⁺ or Na⁺ ions in the phosphate buffer.

Inoculations with conidia suspended in a solution of 0.067 M phosphate buffer (pH 5.0) or monobasic phosphate and 0.11 M glucose always evoked a susceptible reaction, i.e. the formation of spreading lesions.

Additional keywords: Phaseolus vulgaris, phosphate buffer, inorganic phosphate, glucose, spreading lesions.

Introduction

Infection of many host plants by Botrytis cinerea Pers. ex Nocca & Balbis starts from moribund tissues colonized by this pathogen (Jarvis, 1977; Verhoeff, 1980). Only a few reports describe briefly how B. cinerea infects (dwarf) French bean plants (Phaseolus vulgaris L.). in the field under natural conditions (Campbell, 1949; Hubbeling, 1955; Zaumeyer and Thomas, 1957). Infection usually occurs where an old blossom has fallen on a leaf or other plant part or has been retained at the tip of the pod, or where discarded cotyledons remain in contact with the hypocotyl. Under moist conditions conidia of B. cinerea can germinate and mycelium can develop on those withered or dead plant parts. Such colonized tissues are used by the fungus as a saprophytic base from where infection in the underlying living tissues can start. Infection is favoured on plant parts damaged by certain viral, bacterial or fungal pathogens, by frost or hail, or by some other causes.

Infection by mycelium growing from a dead plant substrate could be mimicked by inoculation with agar disks supporting mycelial growth or with mycelium in nutrient solution (Polach and Abawi, 1975; Van den Heuvel, 1976; Van den Heuvel and
Grootveld, 1978; Wasfy et al., 1978; Garcia-Arenal and Sagasta, 1980). Spreading lesions in unwounded healthy bean tissue were also obtained after inoculation with conidia suspended in 1% orange juice or in Czapek broth, but not with conidia in distilled water without added nutrients (Dixon and Doodson, 1975; Garcia-Arenal and Sagasta, 1980). Apparently, successful infections are dependent on factors that stimulate conidial germination and further development of B. cinerea directly or via interaction with co-occurring micro-organisms, or that, alternatively, predispose plant tissue to infection.

The present paper describes some factors which determine a susceptible reaction, i.e. the formation of spreading lesions, in unwounded bean leaves after inoculation with conidia of B. cinerea. A preliminary report has already appeared (Van den Heuvel, 1980).

Materials and methods

Growth of B. cinerea and bean plants. Sporulating cultures of B. cinerea (isolate BC-1) (Van den Heuvel, 1976) were obtained after growing the fungus on 25-mm-wide PDA slants at about 23 °C under continuous white fluorescent light (1750 lx). Twelve-to-sixteen-day-old cultures were flooded with distilled water and the conidia were scraped from the cultures. The suspension was filtered through a thin layer of glass wool and the filtrate was centrifuged (1400 g, 10 min). The conidia in the pellet were resuspended in one of several buffer and nutrient solutions to be tested. The final concentration was, unless stated otherwise, 2 × 10⁶ conidia.ml⁻¹.

French bean (cv. Dubbele Witte zonder draad) plants were grown in the glasshouse at 22 °C to 27 °C and used for inoculation when 10 to 13 days old.

Spray inoculations. Plants were sprayed with conidial suspensions until drops started to run off from leaves. Inoculated plants were incubated in the glasshouse at 17 °C to 20 °C under conditions of high relative humidity (in humidity chambers lined with moistened filter paper).

Drop inoculations. Detached primary leaves were placed on perforated plastic grids on a layer of wet cotton-wool in transparent plastic trays. The grids kept the leaf blades separated from the cotton-wool, but care was taken that the cut end of each petiole made contact with the cotton-wool. Six 5-µl drops of conidial suspension were placed on the adaxial side of each of five leaves per treatment. Trays containing ten inoculated leaves were enclosed in transparent plastic bags and incubated at 19 °C and a 16 h photoperiod (fluorescent light, 1200 lx). At various times after inoculation numbers of spreading lesions were determined.

All experiments were repeated at least once. From each set of duplicate experiments with similar results, only results of one experiment are given.

Results

Symptoms after spray and drop inoculations. On most primary and a few trifoliate leaves of glasshouse-grown bean plants sprayed with B. cinerea conidia (2 × 10⁴ or 2 × 10⁶ conidia.ml⁻¹) in a modified Richards' solution, with 0.11 M glucose as the only carbon source, several small, necrotic lesions were formed that remained