

Some Factors Influencing Asexual Sporulation in a Strain of *Glomerella cingulata* Pathogenic to Camellias

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Dieback and canker of camellias is caused by a strain of *Glomerella cingulata* (Ston.) Spauld. & Schrenk (2). The fungus is easily isolated from diseased stem tissue on a variety of media. Typically, few if any setae are produced in culture, and therefore the form genus may be identified as either *Colletotrichum* or *Gloeosporium*. Many pathogenic isolates of *Gloeosporium* and *Colletotrichum* from various plants sporulate readily under laboratory conditions, but those from camellia often sporulate erratically. In an effort to uniformly obtain large numbers of spores for inoculation purposes, we studied some of the factors influencing asexual sporulation in this strain. A portion of this work has been reported (1, 3).

A single conidiospore isolate, originally obtained from a canker on the camellia hybrid Donation and whose pathogenicity to camellias had been demonstrated, was used. Cultures were maintained, unless otherwise noted, under continuous fluorescent light (cool-white bulbs located about 10 inches above the culture surface) on carrot juice agar (360 ml Eveready carrot juice, 640 ml water, 20 g agar) at 22 to 24 C.

A Levy Ultra Plane counting chamber was used to obtain an estimate of the quantity of spores produced. The surface of each culture was scraped and washed with 100 ml of water, and the washings were transferred to an Erlenmeyer flask. The washings were allowed to stand for a few minutes with occasional agitation before necessary dilutions were made. Each treatment consisted of six replicates; six spore counts were made from each replicate. Thus, values obtained for a given treatment represented the average of 36 counts.

As with many fungi (4), the method of inoculating (seeding) plates affected sporulation. Of eight seeding methods tested, the most abundant sporulation occurred when 1 ml of a heavy spore suspension (approximately 100,000 spores/ml) was added to each petri dish and distributed evenly over the surface of the medium. Spore counts made from such cultures (2-21 days old, inclusive) showed that maximum sporulation occurred 4 days after seeding. All subsequent spore counts were made using 4-day-old cultures, although age of the inoculum from 2-21 days did not appreciably influence sporulation. However, cultures more than 14 days old should not be used for seeding purposes because of the

occurrence of somatic mutations and, hence, of genetic variants in subsequent cultures. In this study, 4-day-old cultures were used to obtain spores for seeding purposes.

Light appreciably influences sporulation. Cultures grown in light sporulated abundantly (2,670 million spores/plate = MSPP), while those grown in darkness under otherwise comparable conditions sporulated sparsely (7 MSPP). It was determined that for maximum sporulation, cultures must be exposed to 12 or more hr of continuous light/day; this is especially important during the 2nd and 3rd days of the 4-day growth period. Various light intensities tested in the range of 50 to 300 ft-c did not affect sporulation. Some effects of light quality upon sporulation were determined by covering the cultures with either a white (No. 1), blue (No. 36), green (No. 48), or red (No. 67) gelatine filter (Brigham Gelatine Co., Randolph, Vermont). Spore counts from these cultures were 4,750, 250, 5,000, and 200 MSPP, respectively.

Very few spores, 1.8 and 1.0 MSPP, respectively, were produced when cultures were incubated under constant temperatures of 10 and 32 C. Sporulation was abundant at constant temperatures between 20 and 29 C, with an optimum around 25 C. More recent studies indicate that alternating temperatures may enhance conidial production.

Of 18 agar media tested, including Czapek's, oatmeal, potato-dextrose, and V-8 juice agars, sporulation was most abundant on carrot juice agar (CJA). On green bean agar and lima bean agar, few spores (less than 1.0 MSPP) were produced by cultures incubated in darkness, while on oatmeal agar, much larger numbers of spores (55 MSPP) were produced in darkness. However, compared to cultures grown on any of the media in light, this number of spores is small. On CJA, sporulation increased when the quantity of media in the petri dish was increased and when the concentration of carrot juice was increased. Approximately 1,600 MSPP were obtained with a carrot juice concentration of 125 ml/liter, and 5,700 MSPP with a carrot juice concentration of 750 ml/liter.

Citrate and phosphate buffers were used to alter the pH of CJA on which the fungus was cultured. In general, sporulation decreased as the pH of the medium was raised above pH 6.0 or lowered below pH 5.0. Approximately 5,000 MSPP were produced on CJA with a pH of 5.1 to 5.5, whereas only 250 MSPP were produced on that with a pH of 7.1 to 7.5.

Abundant conidium production in this strain of *Glomerella cingulata* can be obtained when CJA (500 ml/liter at a pH of approximately 5.5) is seeded by flooding with 1 ml of a spore suspension containing approximately 100,000 spores/ml and incubated under at least 12 hr of light/day at a temperature alternating every 12 hr between 15 and 25 C. The petri dishes should contain approximately 30 ml of medium. In practice it is not necessary to have fluctuating light and

temperature; a two-bulb fluorescent lamp set inside an incubator at 20 C will suffice.

LITERATURE CITED

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