Pathogenicity of *Phomopsis ganjae* on *Cannabis sativa* and the fungistatic effect of cannabinoids produced by the host

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**Abstract**

The chronology of *Phomopsis ganjae* conidia germination and infection of *Cannabis sativa* leaves was observed with the scanning electron microscope. A-conidia germination approached 100% after 24 h, appresoria initiation began after 36 h; B-conidia germinated by 52 h but were not infective. Four-week-old *C. sativa* seedlings were more susceptible than 16-week-old plants, males more than females. THC and CBD, extracted and separated via TLC, inhibited *P. ganjae* conidia germination and hyphal growth.

**Introduction**

The genus *Phomopsis* Saccardo (Sphaeropsidales) characteristically produces A- and B-phialoconidia (14); only the A-conidia have been reported to germinate (10). *P. ganjae* has attracted researchers in India as a potential mycoherbicide for control of *Cannabis sativa* (1), although no work has been done concerning the epidemiology and pathogenicity of the fungus. *P. ganjae* was recently isolated from *C. sativa* in the United States (13), where it could serve as a desirable alternative to chemical control of *C. sativa* with paraquat, whose medical hazards have been documented (17).

The ecological utility of cannabinoids, a group of C₂₁ terpenophenolics unique to *C. sativa*, has been debated. The presumed function of cannabinoid resins preventing dessication of leaves and calyxes is a popular but erroneous misconception (4). Hammond & Malburg (11) suggest cannabinoids have served *C. sativa* as a dispersal mechanism by their attraction to both early and modern man as a medicament. Tetrahydrocannabinol (THC) and cannabidiol (CBD) have displayed an antibacterial action on gram-positive bacteria (7). Their effect on fungi has not been evaluated, although some plant pathologists have observed that the flowering tops of *C. sativa*, where cannabinoid concentration is highest (5), are less susceptible to fungal pathogens (2, 12).

**Materials and methods**

**Inoculation procedure.** *P. ganjae*, ATCC 52587, was plated on Difco potato dextrose agar (PDA) supplemented with 20 g dextrose/l, to encourage B-conidia production (14). Fourteen-day-old colonies (in 90 × 15 mm petri plates, 20 ml medium) were blended in 50 ml sterile distilled water and filtered with sterile cheesecloth. Seedlings from a naturalized stand of *C. saliva* were transferred to pots and watered daily for 2 weeks before inoculation. Eighteen 4-week-old seedlings in 1-l pots and eighteen 16-week-old plants (9 males, 9 females) in 5.7-l pots served as either control or test plants. Test plants were sprayed until runoff with $8 \times 10^4$ conidia/ml, controls were sprayed with sterile distilled water. All plants were kept in a growth chamber for 52 h (24 °C, 95 ± 5% RH, complete darkness) before returning them to the field.

**Pathogenicity studies.** Inoculated 4- and 16-week-old leaves were picked 12, 24, 36, and 52 h after...
inoculation, fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 8–10 h at 4 °C, dehydrated in ethanol, and critical point dried. They were examined with either a JEOL JSM-U3 or ISI DS-130 scanning electron microscope (SEM) operating between 10 and 20 KeV accelerating voltage. After 16 days, all plants were rated for disease severity: 1 = no symptoms, 2 = slight to moderate symptoms confined to lower leaves, 3 = moderate to severe symptoms with all foliage affected, 4 = dead. After 21 days, the percentage of dead plants was measured.

Bioassay studies. Cannabinoids were separated from calyx and leaf extracts of *C. sativa* plants collected in Illinois (12) by thin-layer chromatography (TLC) on glass plates (20 × 20 cm) coated with silica gel G, 250 nm thick. Methanolic extracts were developed with chloroform:dioxane:ethyl acetate:ammonium hydroxide (25:60:10:5) (3). Petroleum ether extracts were separated in reverse phase on TLC plates impregnated in dimethylformamide: carbon tetrachloride (60:40) and developed three times with cyclohexane (8). Fast Blue B salt was used for visualizing separated cannabinoids and determining their Rf values. Bands dyed with Fast Blue B were scraped from their plates, eluted with glacial acetic acid:methanol (1:1), and the cannabinoid-azo dye complex absorption maxima were determined on a Beckman Model 25 UV/Vis Spectrophotometer (9). Other plates, run parallel with the above but undyed, were sprayed with [*P. ganjae* conidia suspended in a nutrient solution and incubated in a moisture chamber for 72 h (24 °C, 95 ± 5% RH, darkness). These plates were then coated with graphite or exposed to iodine crystals to visualize areas of germination inhibition and fungistatic activity (15).

Results

After 12 h, 50% of the A-conidia observed on the surface of *C. sativa* had germinated. A-conidia germination approached 100% after 24 h, each conidium producing one or two germ tubes (Fig. 1). After 36 h, greater than 50% of the B-conidia germinated, forming singular lateral protuberances (Fig. 1–2). By 52 h no protuberances exceeded 2 μm, and none formed appressoria. In comparison, 75% of the A-conidia germ tubes had terminated in appressoria by 52 h (Fig. 1–3). No fungal penetration through stomatal openings was observed; on the contrary, germ tubes grew around or away from encountered stomates (Fig. 1–4).

*P. ganjae* was highly pathogenic to *C. sativa* at an inoculum level of 8 × 10⁴ conidia/ml. All inoculated plants developed moderate to severe symptoms of chlorosis and necrosis or were killed (Fig. 1–5). All control plants were symptomless (Fig. 1–5). After 16 days, 4-week-old seedlings exhibited greater susceptibility than 16-week-old plants; after 21 days a greater percentage of the younger plants were killed (Table 1). Male plants exhibited greater disease severity than female plants, and a greater percentage were killed (Table 1).

Petroleum ether extracts of *C. sativa* chromatographed on reverse phase TLC plates produced three separation bands at Rf values (0.20, 0.41, 0.68) nearly identical with Rf values measured for purified CBD, cannabinol (CBN), and THC (8). Treatment with Fast Blue B turned the three bands orange, purple, and scarlet, which agrees with previous results utilizing purified CBD, CBN, and THC, respectively (8). The methanolic extracts, using a different solvent system, produced bands at Rf = 0.20, 0.46, and 0.95, which agree with published results using the same system (3). Ultraviolet absorption maxima of two colored bands (orange = 450 nm, scarlet = 508) were identical with maxima from azo dye-CBD and azo dye-THC compounds (9); the purple band produced no significant peaks.

The TLC bioassays, utilizing the reverse phase system, demonstrated three areas of *P. ganjae* germination inhibition, at Rf = 0.00, 0.20, and 0.68 (Fig. 1–6). Neither of the visualization aids (graphite or iodine crystals) were very effective.

| Table 1. *Cannabis sativa* inoculated with *Phomopsis ganjae.* |
|------------------|------------------|------------------|------------------|
| Age (weeks) | Test group | Disease severitya | Percentage killedb |
| 16 | all plants | 3.4 | 67 |
| 16 | female only | 3.2 | 50 |
| 16 | male only | 3.6 | 80 |
| 4 | all plants | 3.8 | 89 |

a Average ratings 16 days after inoculation: 1 = no symptoms, 2 = slight to moderate symptoms confined to lower leaves, 3 = moderate to severe with all foliage affected, 4 = dead.

b Measured 21 days after inoculation.