

Evaluation of the Use of Charcoal in the Study of Soil Fungistasis

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ABSTRACT

Neurospora tetrasperma ascospores which were not sensitive to soil fungistasis germinated completely on three sources of nonwashed and six sources of washed charcoals that were tested. However, nutritionally dependent conidia of *Aspergillus fumigatus* and *Penicillium frequentans* failed to germinate on soils with or without amendment with 10% of the charcoals, or on the charcoals only. When nutrients were added, spores of both fungi germinated completely on charcoal-amended soils. Therefore, it is suggested that removal of inhibition by addition of charcoal to soil not be used as a characteristic of soil fungistasis.

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When Dobbs and Hinson reported the phenomenon of widespread fungistasis in soils, they also mentioned that amendment of soil with 10% charcoal completely removed the inhibition (2). Therefore, reversal of inhibition by addition of charcoal to soil has been considered as one of the characteristics of soil fungistasis in discussions and publications (6, 7, 8). Ko and Lockwood (4) reported that 12 of 22 fungi tested required nutrients for germination. Among those nutrient-dependent fungi were *Penicillium frequentans* Westling, *Mucor rammanianus* Möller, and *Trichoderma viride* Fr. which were also used by Dobbs and Hinson (2) in their study. Germination of nutrient-dependent spores on soil amended with charcoal is difficult to reconcile with the finding that natural soil did not contain sufficient nutrients for germination of this type of spores (4). This particular characteristic of soil fungistasis, therefore, was reinvestigated.

MATERIALS AND METHODS.—Ascospores of *Neurospora tetrasperma* Shear and Dodge and conidia of *Aspergillus fumigatus* Fresenius and *P. frequentans* were obtained as previously described (4). The sources of charcoals used were: Grill Time charcoal (Huskey Industries, Atlanta, Georgia); Revelation brand charcoal briquets (Western Auto Supply Co., Kansas City, Missouri); Kingsford charcoal briquets (Kingsford Co., Louisville, Kentucky); Norit A (Matheson, Coleman and Bell Manufacturing Chemists, Norwood, Ohio); Norit 211 (J. T. Baker Chemical Co., Phillipsburg, New Jersey); Norit SG Extra (J. T. Baker Chemical Co.); and Nuchar C-190-N (J. T. Baker Chemical Co.). To wash charcoal, 200 ml of charcoal in 400 ml of 95% ethanol was filtered through a Whatman No. 1 filter paper after 24 hours of standing at room temperature, washed five times with a total of 1,500 ml distilled water, and dried in an oven for 24 hours at 100 C. The soil used was a sandy loam collected from agricultural land. Approximately 25 g of

moist charcoal or soil with or without a 10% (w/w) charcoal amendment were placed in a petri dish, compressed, and the surface smoothed with a spatula. Spores were placed on a sterilized disk of washed, noncoated cellophane laid on the charcoal or soil surface and incubated at 24 C. After incubation for 4 and 12 hours for ascospores and conidia, respectively, percentage germination was determined by counting 200 spores per treatment. Two replicates per treatment were used and the experiment was repeated at least once.

Decolorizing ability was used to determine if charcoals employed in this study were activated. Ten ml of soil extract (3) which was yellow in color was mixed with 1 g of washed or nonwashed Norit SG Extra, Norit 211, or Nuchar C-190-N, and filtered through a Whatman No. 1 filter paper. The filtrate obtained from nontreated soil extract was used as a control.

RESULTS AND DISCUSSION.—The nonamended soil supported complete germination of *N. tetrasperma* ascospores which were not sensitive to fungistasis in most soils, but not that of nutritionally dependent conidia of *A. fumigatus* and *P. frequentans*. Fungistatic effects of the soil were lost when it was autoclaved or amended with 1% glucose-peptone. Therefore, the soil used has the characteristics of widespread soil fungistasis (2, 5).

Nutritionally independent ascospores of *N. tetrasperma* were used to determine whether inhibitors were present in the charcoals tested. Both nonwashed and washed Grill Time charcoal were completely inhibitory to ascospores of *N. tetrasperma* (Table 1). Nonwashed Kingsford charcoal briquets. Revelation brand charcoal briquets and Norit A were also inhibitory. However, their inhibitory effects were removed by washing. Ascospores of *N. tetrasperma* germinated completely on both nonwashed and washed Norit SG Extra, Norit 211, and Nuchar C-190-N. All the washed charcoals except Grill Time charcoal were subsequently used for studying their effects on spore germination. Our results failed to confirm those of Dobbs and Hinson (2). Neither *A. fumigatus* nor *P. frequentans* germinated on soils amended with 10% of the various charcoals tested. Charcoals alone also failed to support germination of both fungi. However, when nutrients (5% glucose-peptone) were added, both fungi germinated completely on charcoal-amended soils. A relatively large amount of nutrients was used because of the absorptive nature of charcoal. Similar results were obtained when soil amended with 30% charcoal or 30%

TABLE 1. Germination of *Neurospora tetrasperma* ascospores on nonwashed and washed charcoals

Source of charcoal	Germination (%) ^a	
	Nonwashed	Washed
Grill Time charcoal	0	11
Kingsford charcoal briquets	0	100
Revelation brand charcoal briquets	0	100
Norit A	56	98
Norit SG Extra	100	100
Norit 211	100	100
Nuchar C-190-N	100	100

^aData are average of two experiments. Two hundred spores were counted in each treatment for each experiment.

charcoal plus 5% nutrients was used. Both fungi also failed to germinate on soils amended with 10% nonwashed Norit SG Extra, Norit 211, and Nuchar C-190-N. Washed and nonwashed charcoals from these three sources changed the color of soil extract from yellow to colorless, indicating that the charcoals had been activated.

These results are in accord with those of Chinn and Ledingham (1) who also showed no germination of *Helminthosporium sativum* spores in charcoal-amended soil. Wills (9) showed that the charcoal he used contained substances stimulatory to sporulation of *Phytophthora parasitica*. Charcoal used by Dobbs and Hinson (2) might have contained nutrients for spore germination. If this is the case, reversal of soil fungistasis by addition of charcoal to soil would be due to the presence of nutrients rather than to charcoal itself. Therefore, we suggest that removal of inhibition by addition of charcoal to soil not be used as a characteristic of soil fungistasis.

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