Thermotolerance Generated by Plant/Fungal Symbiosis

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All plants studied in natural ecosystems are symbiotic with fungi (1), which obtain nutrients while either positively, negatively, or neutrally affecting host fitness (2). Plant adaptation to selective pressures is considered to be regulated by the plant genome (3). To test whether mutualistic fungi contribute to plant adaptation, we collected 200 Dichanthelium lanuginosum plants from geothermal soils at 10 sites in Lassen Volcanic (LVNP) and Yellowstone (YNP) National Parks. These soils have annual temperature fluctuations ranging from about 20° to 50°C (4).

Plants and their roots were removed and assessed for fungal colonization (5). A fungal endophyte was isolated from the roots, crowns, leaves, and seed coats of all plants collected. Cultures established from single spores were analyzed by morphological (6) and rDNA sequence analyses (7) that suggested that the endophyte may be a new species of Curvularia (5). Soils from the base of 30 plants in YNP were devoid of the Curvularia sp., although other fungi were abundant (4). Moreover, axenically cultured Curvularia sp. was incapable of mycelial growth, spore germination, or survival at ≥40°C (5). Because geothermal soils were above 40°C all summer (4) and devoid of the fungus, we conclude that this Curvularia sp., like all known Curvularia species, is exclusively associated with plants.

To assess the effect of the endophyte on the thermotolerance of D. lanuginosum, we removed seed coats and surface sterilized seeds (8) to generate endophyte-free plants. Treated seeds were planted in sterile mangenta boxes containing sand, and after 1 month, plants were either mock-inoculated or inoculated with Curvularia sp. by pipetting 10⁵ spores between the crown and first leaf. In the absence of thermal stress, endophyte-colonized (symbiotic) and endophyte-free (nonsymbiotic) plants showed no measurable growth or developmental differences. When root zones were heated with thermal tape (Fig. 51), nonsymbiotic plants (45/45) became shriveled and chlorotic at 50°C (Fig. 1A). In contrast, symbiotic plants (45/45) tolerated constant 50°C soil temperature for 3 days and intermittent soil temperatures as high as 65°C for 10 days. All nonsymbiotic plants (45/45) died during the 65°C heat regime, whereas symbiotic plants (45/45) survived. The endophyte was reisolated from surface sterilized roots and leaves of all surviving plants, indicating that both the fungus and the host were protected from thermal stress.

We also field-tested symbiotic and nonsymbiotic seedlings in pasteurized geothermal soil collected and returned to Amphitheater Springs (YNP) in May 2001 (Fig. 1B). By May 2002, symbiotic plants were greener with greater root and leaf masses (Ta-