ECOLOGY

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 magenta boxes containing sand, and after 1 month, plants were either mock-inoculated or inoculated with *Curvularia* sp. by pipetting 10^5 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. S1), 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.

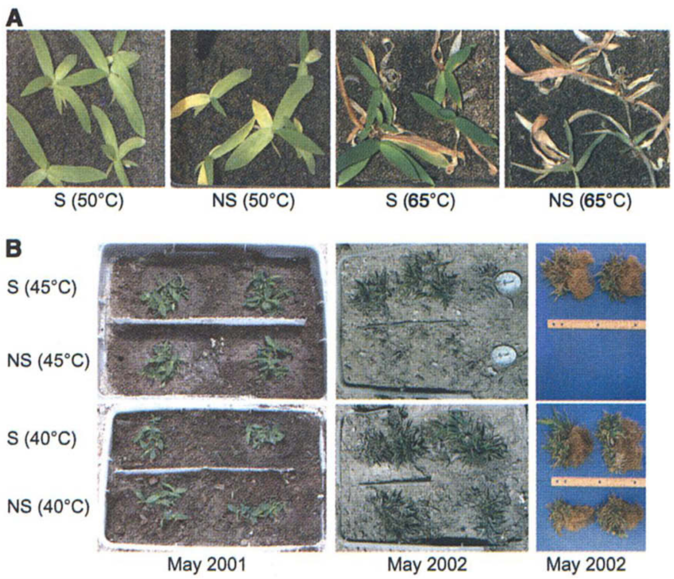

Fig. 1. Representative symbiotic (with *Curvularia* sp.) and nonsymbiotic *D. lanuginosum* plants with rhizosphere temperatures of 50°C for 3 days or 65°C for 8 hours/day for 10 days under laboratory conditions (**A**) and in 40° or 45°C soil under field conditions (**B**).

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 (Table S2) than those of nonsymbiotic plants in soils ≤40°C. In soils above 40°C, nonsymbiotic plants did not survive while symbiotic plants thrived. The beneficial effect of fungal symbiosis increased with soil temperatures, demonstrating that *Curvularia* sp. provided thermal protection for *D. lanuginosum*. We reisolated *Curvularia* sp. from *D. lanuginosum* roots at 45°C field soil temperatures, indicating that thermal protection was also provided to the fungus, which corroborated our laboratory experiments.

In addition to thermotolerance, the basis of mutualism in this system may involve other benefits (e.g., nutrient acquisition by the fungus). Several possible symbiotic mechanisms could confer thermotolerance. In planta, the fungal endophyte produces cell wall melanin (Fig. S3) that may dissipate heat along the hyphae and/or complex with oxygen radicals generated during heat stress (*9*). Alternatively, the endophyte may act as a "biological trigger" allowing symbiotic plants to activate stress-response systems more rapidly and strongly than nonsymbiotic plants (*10*).

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/298/5598/1581/DC1
Materials and Methods
Figs. S1 and S3
Table S2

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