Endophytes influence protection and growth of an invasive plant

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We investigated the symbiotic activities of fungal endophytes isolated from spotted knapweed, Centaurea stoebe. Previously, an analysis of community similarity had demonstrated differences in the endophyte communities of C. stoebe in its native and invaded ranges. Here, we found that specific endophytes can exert positive effects on their host, whereas others exert negative effects. Endophytes produced metabolites that inhibited germination of a competitor of C. stoebe. Endophytes also repelled a specialist insect herbivore, perhaps by producing biologically active volatiles. Yet other endophytes acted as cryptic pathogens of C. stoebe, suppressing its germination, reducing its growth, increasing the abundance of a generalist insect herbivore, and delaying or suppressing its flowering. Since, as reported here, endophytes are not functionally interchangeable, previously reported community differences could be contributing to the invasiveness of C. stoebe.

Recently we reported significant diversity in endophytic fungi in an invasive plant, Centaurea stoebe, or spotted knapweed.1 Communities in the invaded and native ranges differed according to an analysis of similarity. Preliminary experiments to investigate functional activities of endophytes suggest that differences in the presence or absence of key endophytes could affect the invasiveness of this plant that is native to Eurasia and invasive in North America and elsewhere.

Positive Effects

Culture filtrates of 12 endophytes (Experiments 1–3, Table 1) suppressed germination of Festuca idahoensis, a plant that competes with C. stoebe in its invaded range in western North America.2 This result demonstrated that specific endophytes produce allelopathic effects that might aid C. stoebe in competition with other plants.

Symbionts can also have positive or mutualistic effects on their hosts by protecting them. Two endophytes, Alternaria CID62 and Epicoccum CID66 (CID = Cultivation Identification Number, or endophyte isolate number. A complete list of our CIDs is here), appeared to protect C. stoebe from Larinus minutus, a seed-feeding weevil from the native range of C. stoebe, that was deliberately released in North America for biological control.3 In dual-choice laboratory bioassays (Experiments 4–9), mated Larinus minutus females spent more time on uninoculated, control flowerheads than on those inoculated with either Alternaria CID62 or Epicoccum CID66, and preferred flowerheads inoculated with Epicoccum CID66 to those inoculated with Alternaria CID62 (Fig. 1). A similar pattern occurred when the isolated fungi were applied to cottonflower mimics, except that the difference in preference for Epicoccum CID66 over Alternaria CID62 was not significant (Fig. 1).

The effects we have detected thus far are potentially mediated by chemical factors. We sampled each of 16 endophytes for their capacity to release volatile organic compounds (VOC) in pure culture (i.e., Experiment 10), following methods similar to those that have been used to detect biologically active VOC produced by an endophytic fungus.4 Fourteen of these isolates in pure liquid culture produced volatile sesquiterpenes. Total production of sesquiterpenes ranged from zero to 236.8 ng/0.5 h/20 ml sample of culture. Volatile sesquiterpenes are implicated in many interorganismal interactions.5

Negative Effects on Flowering

Although the endophytes reported thus far1 are not overt pathogens they could be cryptic pathogens.6 In Experiment 11, knapweed seedlings inoculated with Alternaria isolate ‘CID62’ produced fewer flowering heads than seedlings inoculated with Epicoccum CID66, Fusarium CID107, and an uninoculated, E- (i.e., endophyte-free) control (ANOVA F1,38 = 5.276, p = 0.03). In Experiment 12, seedlings inoculated with Alternaria CID123 and Fusarium CID124 flowered significantly later than E- controls (ANOVA F2,46 = 17.173, p < 0.001).

Negative Effects on Seed Germination

We also performed knapweed germination assays following inoculation with endophyte cultures (Experiments 13–15), or following treatment with liquid culture filtrates (Experiments 11–13); germination was 100% suppressed by Botrytis CID360, Alternaria CID120...
Endophytes influence protection and growth of an invasive plant

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Negative Effects on Growth of *C. stoebe*

Some seedlings survived if they were first germinated and then inoculated with *Fusarium* CID107 (Experiments 14–16), but survivors had fewer and shorter leaves (ANOVA F1,52 = 8.987, p = 0.004 for number of leaves and ANOVA F1,52 = 7.307, p = 0.009 for length of maximal leaves) during a forty-day period of growth, and fewer mature, dissected leaves (χ² test for independence, χ² = 4.103, p = 0.043) than E- controls. Final, aboveground biomass was lower for *Fusarium* CID107-inoculated plants (ANOVA F 1,50 = 11.292, p = 0.001) than E- controls.

Negative Effects on Protection of *C. stoebe*

*Fusarium* CID107 also attracted a generalist herbivore, the aphid, *Myzus persicae*, to plants it had infected. In Experiment 17, abundance of aphid infestations differed on E+ and E- knapweed seedlings (ANOVA F3,35 = 5.023, p = 0.005). *Fusarium* CID107-inoculated seedlings hosted aphid populations 6.3 times higher than plants inoculated with *Alternaria* CID62, *Epicoccum* CID66, or controls, although this difference eventually disappeared when aphid populations became very large on all treatments (ANOVA F3,36 = 0.951, p = 0.426).

Balance of Positive and Negative Effects

With both negative and positive effects on characters associated with fitness (Fig. 2), it seems likely that endophytes strongly influence the ecology and invasiveness of *C. stoebe*. The effects of endophytes were seen in all growth stages of *C. stoebe*, from germination to flowering. Increases in aboveground biomass due to endophytes have been observed in other plants, although not yet
Endophytes influence protection and growth of an invasive plant

in *C. stoebe* (Fig. 2). We expect that with further experimentation, we will discover many additional, biotic interactions mediated by endophytes in *C. stoebe*.

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