How rapid is aphid-induced signal transfer between plants via common mycelial networks?

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Arbuscular mycorrhizal (AM) fungi are important plant mutualists that can connect roots of neighboring plants to form common mycelial networks. A recent study demonstrated that these networks can act as conduits for aphid-induced signals between plants, activating chemical defenses in uninfested neighboring plants so that they become unattractive to aphids but attractive to their enemies (parasitoids). The benefit to the neighboring plants will increase if the signal speed is rapid, enabling them to respond before aphids attack. Here, we determine the speed of aphid-induced signal transfer between plants infested with aphids ("donor") and neighboring aphid-free plants that were either connected or unconnected to the donor via a common mycelial network. Induced changes in plant volatiles from neighbors connected to donors started within 24 h of aphid infestation of donors. This demonstrates a rapid signal, implying potential benefit to plants receiving the signal, and raises intriguing ecological and evolutionary questions.

The mutualistic relationship between arbuscular mycorrhizal (AM) fungi and plants is one of the most functionally important associations on land.[1] Plants usually benefit from increased nutrient uptake delivered by the fungi and in return provide the fungi with large amounts of carbohydrates.[1] For compatibility of this association, AM fungi regulate the signaling of defense-related plant hormones[2] and confer resistance against pathogens,[3] nematodes,[4] and

abiotic stresses.[1] Importantly, AM fungi have the ability to connect roots of neighboring plants to form common mycelial networks[5] (CMNs), which have major roles in recycling of soil nutrients and water and regulating plant community dynamics.[6]

In addition to the role of CMNs in transfer of molecules involved in primary metabolism, CMNs can transfer allelochemicals[7] and pathogenic fungal disease resistance signals.[8] A recent study demonstrated that CMNs can act as conduits for aphid-induced signals between plants, where after four days they activated chemical defenses in uninfested neighboring plants so that they become unattractive to aphids but attractive to their enemies (parasitoids).[9] Major ecological and evolutionary questions have risen from these recent findings. For example, what are the fitness benefits of plants sending and receiving the signal and of the fungi from transmitting the signal? Critical to thinking about such questions is whether the receiving plant can benefit from the signal. One clear benefit would be that the receiving plant could launch its own defenses to prevent aphid attack. Migrating aphids use volatile organic compounds (VOCs) emitted by plants to distinguish between host and non-host individuals.[10] However, many plants have evolved a sophisticated defense mechanism whereby the herbivore triggers the release of VOCs that are no longer attractive to subsequent herbivores, and may even become repellent to them.[11] Reduced attractiveness of plants to aphids can therefore be used as an indication of herbivore-induced plant defense.
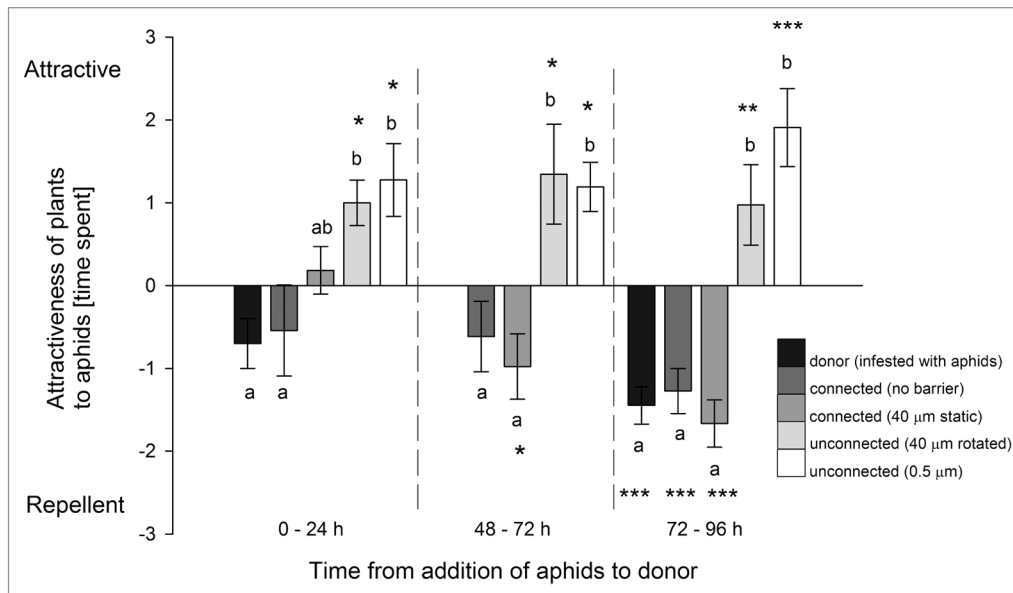
Figure 1. Attractiveness to pea aphids (*Acyrthosiphon pisum*) of headspace samples of broad beans that were either connected or unconnected to an aphid-infested donor plant via a common mycelial network. Samples were collected during 0–24, 48–72, and 72–96 h after aphid placement on donor plants (donor not analyzed 48–72 h). Attractiveness was calculated as the mean time spent (minutes) in olfactometer areas treated with headspace samples minus that with solvent only. Differences in attractiveness among treatments are indicated by letters. Bars sharing a letter are not significantly different (p > 0.05) and each time period is interpreted independently. Within treatments, asterisks indicate that samples were significantly attractive or repellent compared with solvent controls (*p < 0.05; **p < 0.01; ***p < 0.001).

In addition, herbivore-induced VOCs are highly attractive to natural enemies of aphids (such as parasitoids) thus increasing the efficacy of this defense mechanism in suppressing herbivory.[12]

Aphids proliferate rapidly[13] and so transfer through CMNs of signals that warn plants of aphid attack must occur as quickly as possible for the receiver plants to gain maximum benefit, i.e., before aphids can colonize the neighboring plants. Here, therefore, we assess the speed of herbivore-induced signal transfer between beans (*Vicia faba*) that were infested with pea aphids (*Acyrthosiphon pisum*) and uninfested neighboring beans. All the plants were grown in association with the mycorrhizal fungus *Rhizophagus irregularis* (syn. *Glomus intraradices*) in mesocosms (n = 8) in groups of 5: one aphid-infested plant in the middle acted as a donor of the signal and 4 receiver plants were positioned at equal distances (15 cm) from the donor (see Figure 1 in Babikova et al., 2013),[9] 2 of which were connected to the donor via CMNs and 2 were not connected, as follows. One receiver plant was grown in a 0.5 μm mesh core so that AM fungal hyphae could not grow through and so this plant could never

form a CMN and was thus not connected to the donor (or other receiver plants). A second receiver was grown in 40 μm mesh core so the AM fungal hyphae could penetrate and form a CMN, but the connections were snapped by rotating the core immediately before placing the aphids on the donor plant, rendering it unconnected to the donor at the time of signaling. The 2 further receiver plants were allowed to form CMNs with the donor and remained connected throughout the experiment: one was grown in a static 40 μm mesh core so the hyphae could penetrate and form CMN, but root contact was eliminated; the other was grown with no barrier enabling it to form a CMN and have direct root contact with the donor. All the mesh cores were water permeable so soil solutes were free to move between all plants in the mesocosm.

Collection of plant headspace samples[9] started immediately before addition of 50 adult aphids to donor plants. Polyethylene terephthalate bags placed over the plant shoots prevented any communication between plants via airborne VOCs. We analyzed plant headspace samples from 0–24, 48–72, and 72–96 h after addition of aphids to the

donor, and tested them for attractiveness to aphids in 4-arm olfactometer bioassays.[14] First, to determine if each headspace sample was attractive, repellent or neutral to aphids, we used paired *T*-tests which tested whether aphids responded to each sample differently from solvent controls in the 4-arm olfactometer bioassay. We predict that uninfested plants that are unconnected to the donor should be attractive while the infested donors should, at some point depending on the speed of their response, become repellent to aphids. Once the signal is transferred, we predict that uninfested plants will also become repellent if they are connected to the donor. Second, to test whether treatments differed in attractiveness, we used general linear models with treatment as a fixed factor and mesocosm as a random factor. Between-treatment comparisons were tested by Fisher's least significant difference post hoc test. These analyses were done for each time period separately. Once the signal has been transferred we predict that connected plants would have similar attractiveness to donors, but differ from unconnected plants.

As predicted, uninfested receiver plants that were not connected to the

donors were significantly attractive to aphids throughout the course of the experiment (**Fig. 1**). Donor plants, also as predicted, became highly significantly repellent to aphids 72–96 h after aphids were added and, indeed, were already repellent (although non-significantly so) at 0–24 h after infestation (**Fig. 1**). This indicates the speed of the plant's defense response to the aphids. Receiver plants that were connected to the donor via both CMNs and root contact (due to no mesh barrier) were similarly repellent as donor plants for all time points, suggesting the signal was transferred from donor to receiver within 24 h. Receiver plants connected via CMNs only (due to 40 μm mesh) also behaved similarly to donors, except that there was little evidence that they were repellent or attractive within 24 h: at this point they appeared intermediate between the unconnected receivers and the donor in attractiveness to aphids. Therefore, some signal had been probably transferred within 24 h but the plant had not fully responded yet. Perhaps the presence of the 40 μm mesh reduced the number of fungal connections, or maybe root contact speeds up the transfer. Further experiments would be required to test why the responses differed in these two treatments.

These results reflect the outcomes of analyses testing the difference in attractiveness between treatments for each time period. Both infested donors and connected receiver plants differed significantly in attractiveness from unconnected receiver plants, while connected receiver plants had similar attractiveness to donors, for all time periods (**Fig. 1**); the one exception being, again, that at 0–24 h the receiver plants connected to the donor via CMNs only (due to 40 μm mesh) were not significantly more attractive than either unconnected receivers or donor plants. This also suggests that the signal is transferred within 24 h, but for the plants connected to donors through a 40 μm mesh, their defense response was not complete within 24 h. This finding implies that the signal transfer between plants is rapid relative to the time-scale of aphid parthenogenetic reproduction,[15] and that neighboring plants likely invoke their defenses before aphid infestation, allowing maximum potential benefit to the receiver of the signal.

The implications of such a rapid signal between plants are far-reaching. CMNs have potential to colonize several individual plants at considerable distance from each other, and thus overcome limitations of aerial signaling pathways.[16] However, how far can the signals travel, and does the distance between connected plants affect the speed or the strength of the signal? If aphids are more likely to colonize close rather than distant plants, there may be less benefit to a distant plant receiving the signal. When does the benefit of protection outweigh the cost of producing defense-related VOCs? In natural communities CMNs formed by AM fungi can also connect different genotypes and species of plant that may determine the speed, direction and benefit of the signal. Unlike aerial signaling,[16] which must be influenced by wind and air currents, signals traveling through a CMN can be more directionally focused and targeted, although we do not yet know if fungi control which individual plants they send signals to. A next step is to identify the underground signaling molecules and to unravel this phenomenon from ecological and evolutionary perspective.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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