NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Revolt of the Fungus People: Mycorrhizae in the Ecosystem

by

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Take One

"I've got it."

"What?"

"The theme for your sci-fi flick. Your requirement for your film class."

"It had better be good. My whole grade depends on it. Just don't give me a bunch of weird stuff. This is supposed to be realistic."

"It'll be great, I'm telling ya. Suppose it's like Avatar, *except it's here on Earth."*

*"*Avatar? *The movie? What do you mean?"*

"Remember that part where Earthlings destroy the HomeTree and then try to do the same to the Tree of Souls, really ticking off the mother goddess, Eywa? And she sends out the word. Then the whole Pandora planet's creatures take revenge."

"So? Are you telling me you want to redo the movie? Get serious."

"No, listen, it would be awesome; it would be here on Earth. Something like that is probably happening right now."

"You've lost it."

"No really. I mean, I just read that right here on Earth there's an underground web connecting the plants and they communicate to fend off predators. Like it's fungus or something that connects everything. And the plants send chemical messages to one another, telling the predators to back off or they'll kill them."

"Think of it. It could be realistic with scientists discovering that a slow creepy takeover of the planet is happening under our feet. Or a fantasy like Oz *with trees throwing apples at the Scarecrow and Dorothy … or like* Lord of the Rings *where TreeBeard led the Ents army on an attack on Saruman's fortress at Isengard, or like a horror film, with giant celery plants stalking the night."*

"Stop. Stop. That's awful. Where do you get stuff like this? But the idea doesn't totally suck, if it's really true."

"I'm telling you, this is real stuff. The prof will eat it up."

Part I – Networking

Almost all land plants are connected together by an underground network of symbiotic fungi called mycorrhizae. There are thousands of different species of these symbiotic soil fungi which entangle plants' roots living between and within their cells. A spider web of tiny fragile fungal cells tunnel through the soil linking all of the plants together. The fungi draw carbohydrates from the plants and the plants in turn collect nutrients and water from the fungi. As much as 80% of a plant's nitrogen and phosphorous plus minerals like copper, iron, potassium, zinc, calcium and sulfur can come from the mycorrhizae. But can plants share their resources with each other via the fungal grid?

Biologists have long suspected so. Scientists in Switzerland have now traced carbon molecules slowly moving by cytoplasmic flow from tree to tree by this underground fungus pipeline (Klein *et al.,* 2016). It has been estimated that up to 4% of the forests' carbohydrate production from photosynthesis passes along this superhighway.

Klein and his coworkers started their experiments by blowing "standardized" CO_2 through a system of thin perforated tubes interwoven among the needles of Norway pine trees. They followed carbon 13 atoms (using the technique of stable isotope labeling) as they were picked up during photosynthesis and passed from CO_2 into sugar and other organic molecules. The labelled CO_2 was not detected in the air of neighboring trees or on the ground so the scientists felt secure that only the test trees were receiving the labels. This meant that when the carbon 13 label was detected in neighboring trees, the scientists could safely conclude that the molecules had been transported between plants by way of the soil network and not by way of the air. Interestingly, some of these adjoining trees participating in the exchange were European Birch and Larch trees, completely different species than the pine (Figure 1). The researchers became satisfied that the fungus was indeed the pipeline when they found that labelled organic molecules were detected in the mushrooms of fungi that were linked to the Norway Pine. These are called ectomycorrhizal mycorrhizae because they live on the surface of roots and between the root cells. They did not find labelled carbon in mushrooms that were not part of the mycorrhizae network but were soil fungi that break down dead organisms (i.e., saprotrophic fungi).

In another important related study, researchers in British Columbia studied the fungal and Douglas fir tree interactions in a 30 × 30 meter plot of ground (Beiler *et al.,* 2010). They collected samples of two mycorrhizal species connecting the various trees. They determined that all of the 67 trees in the plot and 64 trees outside were interconnected in a complex network (Figure 2). Trees of all ages were involved but the oldest trees had the most connections; one 94 year old "hub tree" connected to 47 other trees by way of 8 mycorrhizal individuals of one fungus species and 3 of the other. In principle chemical products could be interchanged throughout the system.

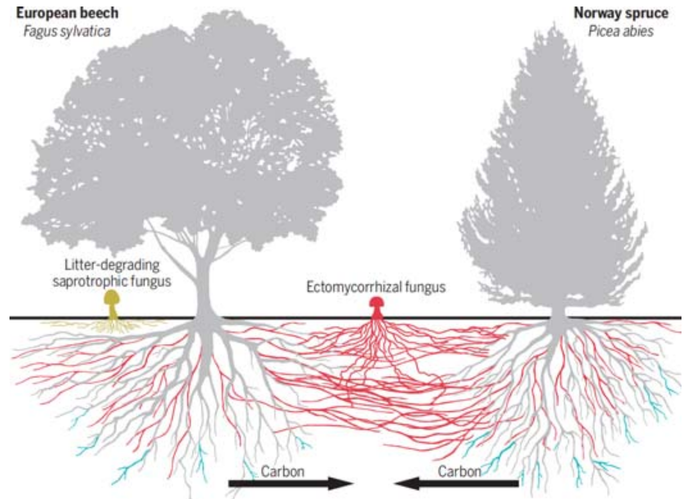

Figure 1. The interconnections of mycorrhizae and trees in a forest. Mycorrhizae are involved but other soil fungi are not. Organic molecules can be exchanged between different species of trees via this route. Carbon and nutrient flow tends to move from trees which are in the sun to trees that are shaded and from older trees to seedlings. *From:* Van der Heijden, M. 2016. Underground networking. *Science* 352: 290–1. Reprinted with permission from AAAS.

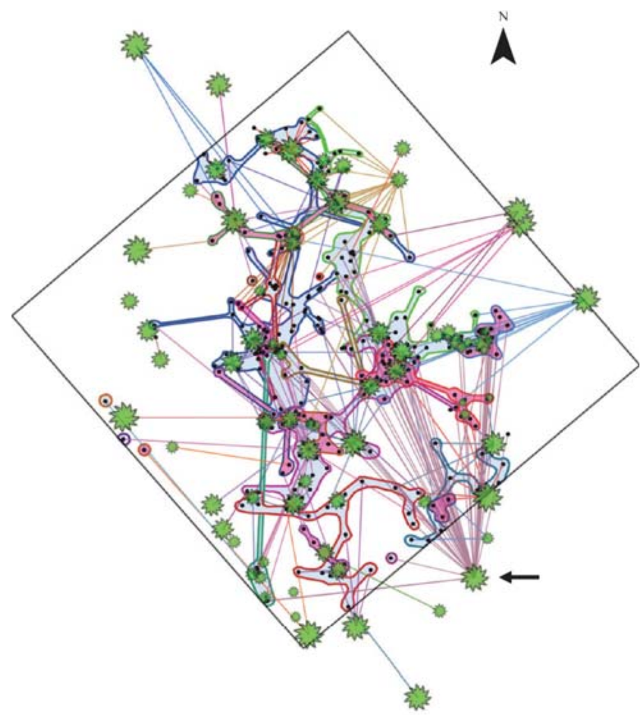

Figure 2. Diagram of a 30 m × 30 m plot in a Douglas fir tree forest of British Columbia. Each green circle is a tree and the various lines depict the connections between individual trees by the mycorrhizae. The tree at the lower right of the diagram (indicated by an arrow) is connected with 47 others in the plot. *From:* Beiler *et al.,* 2010, "Architecture of the wood-wide web," *New Phytologist* 185(2): 543–53, used with permission of John Wiley & Sons.

Questions

1. What propels the movement of chemicals like carbohydrate through the thin hyphal cells of the fungus?

2. How did this sharing of resources evolve? What possible advantage could there be to organisms sharing resources?

3. Can you provide a reasonable sequence of steps for a fungus to establish a symbiotic relationship with plants?

4. There are some plants (e.g., Indian pipe, orchids) that do not have chlorophyll yet they are connected to the mycorrhizal web. What would you suspect their relationship to be?

5. What do you anticipate the consequences are to the plants when the earth is plowed up or strip mined?

6. What impact might a logging practice of cutting the largest trees in the forest have on the health of the ecosystem?

Tree Talk

"No BS. The whole earth is crawling with these fungi. They're like a Facebook network connecting friends everywhere."

"Okay, okay, you've convinced me that trees share food with fungi. I'll never eat a Portobello sandwich again without thinking I'm munching on the whole forest. But what's this news you were giving me about trees warning each other about parasites and predators and impending doom?"

"It's real. Plants talk to each other. They send gas messages through the air. It's kinda like that kid book Buried Onions *where Gary Soto talks about vapors coming from a giant onion buried under Fresno. 'The remarkable bulb of sadness.' "*

"What the devil are you talking about? Do you mean plants are spewing out hallucinogens? Droppin' acid?"

"Be serious. When plants are attacked by insects, their leaves give off vapors or fumes that let their neighbors know there's trouble and the neighbors start making chemical defenses that will ward off the attack."

"But wha…?"

"Hang on. There's more. Even better. Plants can send these warnings by the underground network. Like tiny TV cables. Fungi with connections everywhere."

"So what am I supposed to do with all of this botany? I'm shooting a film and I've got to put together a trailer this week."

"Like I told you. These plants talk to each other and let's say they want to take over the world. Humans are making a mess of everything. We've screwed up the air. We've screwed up the water. We've screwed up everything we touch—ruining the land with cities and parking lots—rippin' up the network. Killing all of the animals in the forest and land. Overfishing and murdering whales. The whole planet is in deep doodoo. Extinction is everywhere. The world is a mess and the plants know it. So do the animals. It's revenge time. That's when the fungi rebel. That's your theme in a nutshell. Now go and make your trailer. Remember the remarkable bulb of sadness."

Part II – Airborne Alert

Plants aren't helpless; and yet when you look at them just standing there with limbs outstretched to the world, they seem to be welcoming all insults. Cows, zebras, snails, grasshoppers, and rabbits graze upon them. Deer, giraffes, koalas, and goats browse on them. Bees, hummingbirds, moths, and butterflies suck upon them. Elephants rip them from their moorings and gorge upon them and even cute pandas munch on their shoots. It is the same under water. Hordes of minute algae are being gobbled up almost the moment they are born by tiny zooplankton and a thousand other wiggly invertebrates. Everything depends on plants, at least indirectly. They are the ones who capture the sun's energy and convert it to food, for themselves and all of the freeloaders of the land. It is almost surprising there are plants at all. How do they survive this onslaught? Why don't the animals eat up all of the plants? Why indeed?

Plants have an arsenal of defensive weapons, many lethal, that discourages or wards off potential herbivores who would like to feast upon them. The obvious ones include cactus spines, thorny bushes, and poison ivy that repel all but the most dedicated visitors. But the most subtle are chemical defenses. Virtually all plants produce a deadly cocktail of chemicals. Tobacco plants produce nicotine that discourages most consumers. Coffee plants produce caffeine that does the same. Milkweed plants produce toxins that taste terrible or kill those animals who chew on them. But all plants, even those with chemical weapons, have predators, herbivores which have evolved countermeasures. They are specialists which have developed antidotes to the plant poisons and thus have the food source all to themselves. The tobacco hornworm does just fine eating tobacco plants. The monarch butterfly caterpillar happily preys on milkweeds, and humans brew, filter, and savor their morning coffee.

In spite of our knowledge about chemical defenses, scientists were still surprised to learn that plants can communicate with each other. And as a result, when one is attacked it releases chemical signals into the air and alerts plants nearby; the latter rapidly start building defensive chemicals themselves. One of the first experiments to tell us this news involved experiments with poplar tree and sugar maple seedlings growing in a laboratory at Dartmouth University

(Baldwin & Schultz, 1983). In a typical experiment scientists placed 15 poplar tree seedlings into gas-tight Plexiglas containers in a growth chamber. These were called the “true control” group. In another similar chamber together they placed two more groups of 15, one of which was called the “experimental” group and the other “communication control;” they were exposed to a common airflow. Two leaves on the experimental plants were damaged by tearing two leaves in half. This was intended to mimic the action of caterpillars chewing on leaves.

The researchers found that the damaged plants rapidly increased production of defensive chemicals in their leaves (phenol and tannins), which are known to discourage herbivores. But surprisingly so did the neighboring plants in the same chamber that were not themselves damaged. This anti-pest resistance happened within 52 hours. This did not occur in the “true controls” in another isolated chamber.

Questions

1. The scientists speculated that ethylene gas was the distress signal released from the damaged leaves and that this discouraged further herbivory in the plants nearby. How might this hypothesis be tested?

2. What would be the possible selective advantage for a plant to releasing distress chemicals alerting neighboring plants or is this just a byproduct of the injury process?

3. How do you think plants detect airborne chemicals? Interestingly, Goldenrod plants can detect fruit fly odors and build chemical defenses to discourage them from laying their eggs in the plant stem.

4. Why don’t plants have full-strength defensive chemicals always ready rather than only building them when danger is at hand?

Part III – Notes from the Underground

Plants aren't always nice to each other. They compete for sunlight, water and nutrients when they are in short supply. Some of them, like marigolds, produce chemicals that inhibit the growth of plants living nearby. They do so by transporting these chemicals via mycorrhizae networks in the soil. And there is evidence that tomato plants infected with the fungal disease "leaf early blight" can pass chemicals along to neighboring healthy tomato plants by way of mycorrhizae, which leads to improved disease resistance. In other words, not only do nutrients pass between plants by way of the mycorrhizal highway, but the avenue is also used to pass positive and negative signals between neighbors.

Recently, scientists in Scotland wondered if warning chemicals could be passed along this underground highway about potential herbivorous insects and their enemies (Babikova *et al.,* 2013). They decided to study bean plants and aphids. These insects suck the sap from stems and leaves, seriously damaging their productivity. The scientists knew that bean plants normally release odors (called VOCs, volatile organic compounds) that aphids could "smell" and thus find their food source. They also knew that when bean plants are attacked by aphids the composition of the VOCs changes and becomes repellant to the aphids and becomes attractive to a tiny wasp species that is a parasitoid. The odor trail leads wasps to the bean plant where they lay eggs in the aphids. The eggs hatch into larvae and start eating the aphid from the inside out. In a few days the larvae mature into wasps and burst out of the aphid "mummies" and go on to complete their life cycle. A pretty picture.

So bean plants produce airborne distress signals; but do they also release signals underground to be carried along the mycorrhizal highway? That is the question the Scottish researchers wanted to examine. Could you design an experiment to test this hypothesis?

Here is the Scottish experimental design: scientists set up eight small containers called *mesocosms* with soil and mycorrhizae; these were essentially pots 30 cm in diameter. In each mesocosm they planted five young bean seedlings, which were allowed to grow for five weeks to establish fungal connections. The center plant they called the "donor" plant. This was surrounded by four "receiver" plants. All of these plants normally would be connected together by a mycorrhizal network, except in this experiment two of the receiver plants were not permitted to have mycorrhizal connections with the donor. Their roots were either isolated by fine mesh or their mycorrhizal connections were deliberately broken. The other two plants were connected to the donor by roots and/or mycorrhizae.

After five weeks of growth, the receiver plants were all covered by transparent polyester impermeable bags to prevent airflow from entering or escaping. Hoses were connected so that filtered air could be passed though the bags and collected for testing. Then, the donor plant was infested with 50 aphids and it too was covered with a bag to prevent any VOC from escaping. Gases were analyzed by gas chromatography.

Then several tests were done. First, the scientists wanted to find out how the aphids and wasps acted when they were exposed to the VOC samples. They

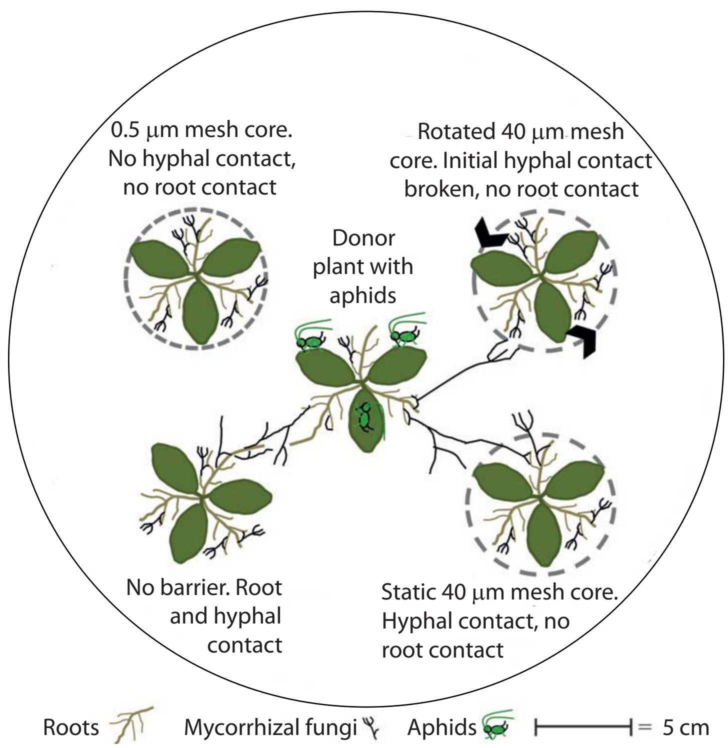

Figure 3. Diagram of the pot arrangement with five bean plants. The center plant ("donor plant) had aphids introduced and they began sucking on sugar fluid in the phloem. Two surrounding plants were connected to the donor by root and or fungal contact. The other two plants shown at the top of the diagram had no root or fungal contact. *From:* Babikova, Z., *et al.* 2013. Underground signals carried through common mycelial networks warn neighbouring plants of aphid attack. *Ecology Letters* 16(7): 835–43. Used with permission of John Wiley & Sons.

used an olfactometer to test this. This device allows odors to be piped into a small chamber via different directions and the insects inside can be watched to determine if they are attracted or repelled. As expected, the aphids were attracted to the initial scent of the bean plant but repelled later when the chemical composition of the VOC changed. On the other hand, the wasps were not attracted to uninfected bean plant VOC in the beginning but soon became attracted to the scent when aphids began their assault. Again, this suggested that the composition of the VOC changed to become attractive to the wasps.

How did the receiver plants respond? The plants that had mycorrhizal connections started producing defensive VOCs in a few hours after the donor plant was infested. In those cases where mycorrhizae were absent no such effect was observed. The evidence clearly indicates that warning chemicals appear to be passing via the mycorrhizae network and that these simulate the production of VOCs.

The active ingredient in the airborne VOCs was identified as methyl salicylate. When it was tested on aphids in the olfactometer it repelled the insects, but it attracted wasps.

Questions

1. Why do plants have natural odors?

2. Freshly cut grass gives off a very strong odor; could this serve a possible beneficial function for the plant?

3. Figures 4 and 5 are based on the Scottish paper. Some of the data are missing; your task is to fill in the missing data using the guidance provided in *(a)* and *(b)* on the following page. On the far left of the graph we present the real data from the experiment to serve as a reference. This is the situation showing that the aphids are initially attracted to the smell of bean plants without aphids. The wasps tend to be repelled.

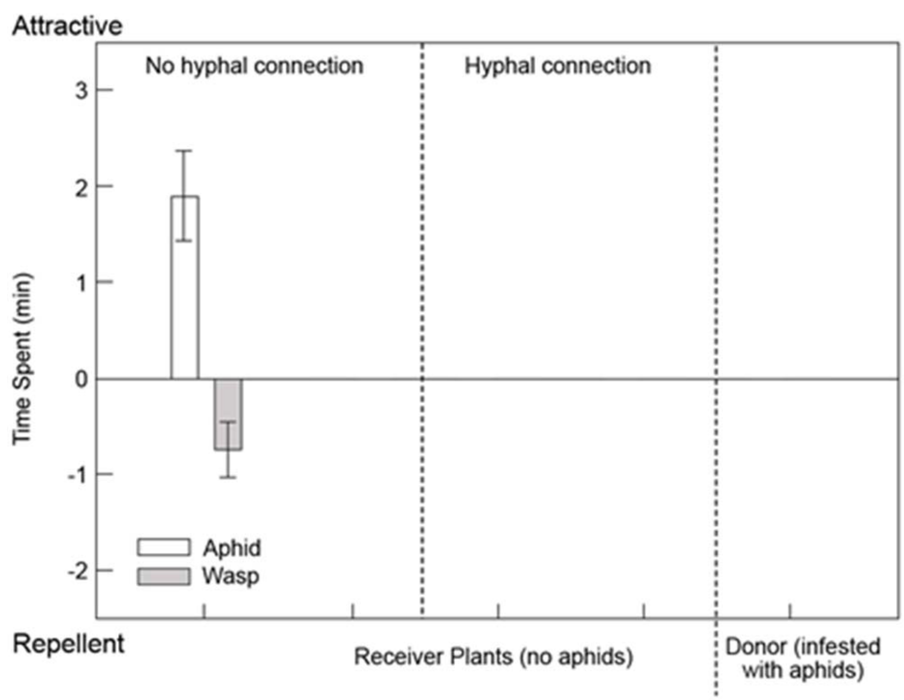

Figure 4. The graph shows how the aphids and wasps act in the olfactometer when they are exposed to the vapors (VOCs) from the various plants. If the bar graph is above the zero line, it means the insects were attracted to the odor. If it is below the zero line, it means the insects were repelled by the odor.

(a) In the middle of the figure the data are missing. Here the receiver plants cannot "smell" odors from a donor plant infested with aphids (remember no air connection), but they are connected to that plant by way of mycorrhizae. If air were now drawn from that receiver plant, what do you think the reaction of aphids and wasps in an odometer would be? Plot your predictions of the data.

(b) On the far right of the figure the data are missing. If air were directly drawn from an aphid infested donor plant chamber and tested on insects in the odometer, what would the data look like?

4. The graph below is laid out to show how the aphids and wasps reacted to the same odor. Please plot the general trend that you think the graph might display given the results we described from the paper. (If an insect was positively attracted by an odor, the data would show how many minutes it spent in a part of the odometer near the source of the smell. If it rejected the odor and went away from the source, it would be scored as a negative value.)

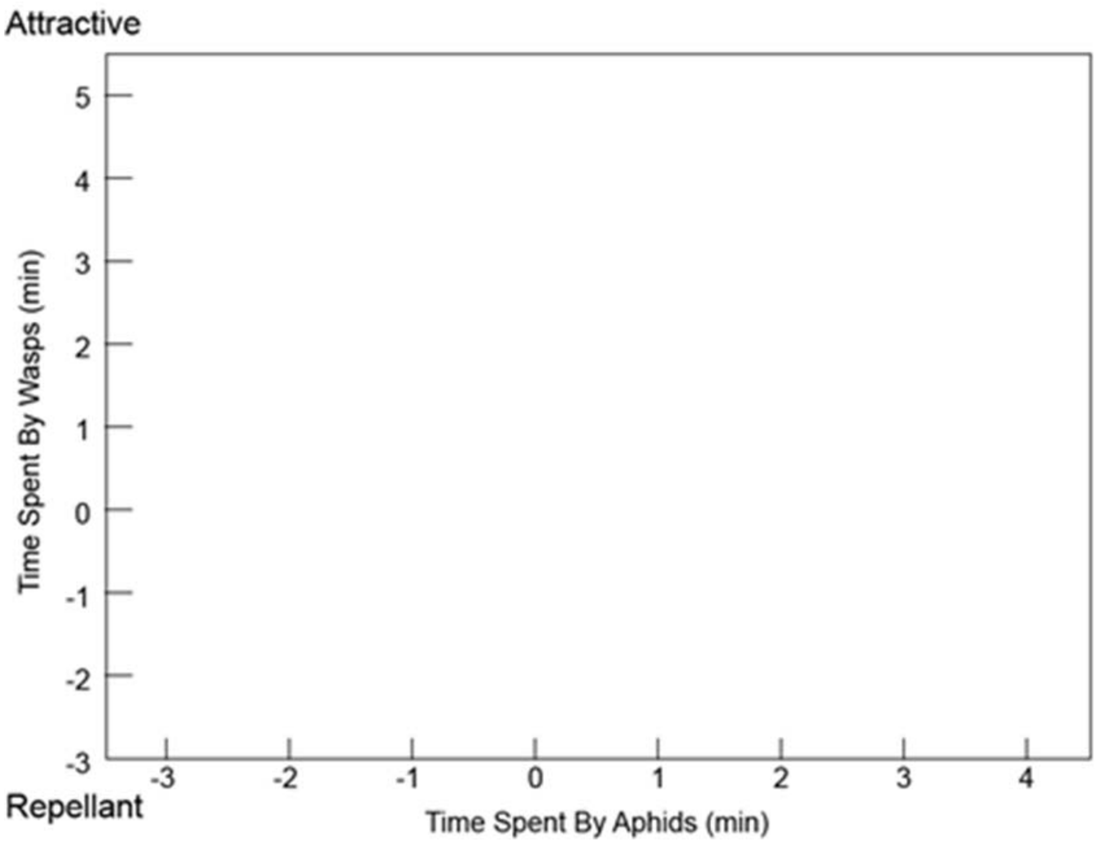

Figure 5. How aphids and wasps reacted to the same odor.

Fungus Rising

"Come on. You've got to start story-boarding this right now."

"Don't have time. I've got to get this together by Friday. Got to check out the camera and lenses. Get the scene stuff together. No Hitchcock junk."

"Well at least let's get this story line together. Seems that you should just set up some establishing shots showing that the world is going to Hell. Later you can get into the theme showing the plants are rebelling and that's when a giant fungus arises from the ground and starts terrorizing the countryside—kind of like the Attack of the 50 Foot Woman.*"*

"Listen. I'm never going to be able to get that together in time."

"Telling you. Forget scenery. This is simple. Fungi are nothing but a bunch of tiny threads. We just have to wrap up John with a bunch of yarn, like in the mummy movies, and set up camera angles from low to make him look gigantic. He rises from the ground throwing a lot of dust and talcum around and starts strangling people with the yarn that he wraps around stuff. He starts small, say killing a dog, then he works up to cows and sheep, and then a local homeless person and…"

"Wait—I thought you said this was a rebellion of fungi against humans. We can't have John killing animals…"

"Hell yeah, we can. Fungus Monster's got to have practice. Start small. Fungi aren't rational. They're on hallucinogens. Anyway, it's easy to film dogs, cats, and barn animals before we get to children. Then we head for the Mayor of the town as the monster throws off spores and they all start popping up and killing everything."

"Okay, okay, I get it. We do it all at night or at dusk with zither music. Death and stench is everywhere and other fungi join in destroying the friggin' world. But how do we end this thing? Just wondering—a blackout at the end?"

"Nada. Nunca. No. How about if a beautiful scientist just leans out the window and sprays a herbicide or fungicide and everything goes quiet. Or better—lot better. How about if the Mayor is crazy about Portobello sandwiches, and sees a great chance to set up a franchise?"

Fade out...