

Plant defenses against parasitic plants show similarities to those induced by herbivores and pathogens

Justin B. Runyon,[†] Mark C. Mescher and Consuelo M. De Moraes*

Center for Chemical Ecology; Department of Entomology; Pennsylvania State University; University Park, PA USA

[†]Current address: Rocky Mountain Research Station; USDA Forest Service; Bozeman, MT USA

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Herbivores and pathogens come quickly to mind when one thinks of the biotic challenges faced by plants. Important but less appreciated enemies are parasitic plants, which can have important consequences for the fitness and survival of their hosts. Our knowledge of plant perception, signaling and response to herbivores and pathogens has expanded rapidly in recent years, but information is generally lacking for parasitic species. In a recent paper we reported that some of the same defense responses induced by herbivores and pathogens—notably increases in jasmonic acid (JA), salicylic acid (SA), and a hypersensitive-like response (HLR)—also occur in tomato plants upon attack by the parasitic plant *Cuscuta pentagona* (field dodder). Parasitism induced a distinct pattern of JA and SA accumulation, and growth trials using genetically-altered tomato hosts suggested that both JA and SA govern effective defenses against the parasite, though the extent of the response varied with host plant age. Here we discuss similarities between the induced responses we observed in response to *Cuscuta* parasitism to those previously described for herbivores and pathogens and present new data showing that trichomes should be added to the list of plant defenses that act against multiple enemies and across kingdoms.

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Several thousand species of plants are parasitic, stealing water and nutrients from other plants through a specialized feeding structure, the haustorium.¹ Haustoria are thought to be modified roots that grow into tissues and fuse with the vascular system of their photosynthetic hosts.¹ Considering that these parasites include some of the world's most devastating agricultural pests² and are influential, fascinating components of natural communities,^{1,3} surprisingly little is known about host defenses induced by parasitic plants. To address this shortcoming, we used a metabolomics approach to track biochemical changes induced in tomato shoots by invasion of *C. pentagona* haustoria.⁴

We found that parasitism induced large increases in both JA and SA beginning about 24 hr after formation of haustoria began, but that production of JA and SA was largely separated in time. Host production of JA was transitory and reached a maximum at 36 hr, whereas SA peaked 12 hr later and remained elevated 5 d later. We also found that *C. pentagona* grew larger on mutant tomato plants in which the SA (*NahG*) or JA (*jasmonic acid-insensitive1*) pathways were disrupted, suggesting that these hormones can act independently to reduce parasite growth. Taken together, these findings suggest the staggered production of JA and SA may be an adaptive response to parasitism—by sequentially

activating the JA and SA pathways, tomato plants may minimize the potential for cross-talk between these sometimes antagonistic pathways^{5,6} and utilize both signaling molecules.^{6,7} Thus, defenses against *C. pentagona* contain elements characteristic of responses to both herbivores (primarily JA-mediated⁸) and pathogens (primarily SA-mediated⁹)—though it should be noted that some herbivores induce SA¹⁰ and some pathogens JA.¹¹ It is worth noting that parasitism induced predominately *cis*-JA, the same jasmonate isomer induced by herbivore feeding.¹² Host responses to *Cuscuta* seem to most resemble that of known plant responses to some pathogens in which a similar sequence of JA and SA production is required to limit disease.¹³

C. pentagona also triggered a hypersensitive-like response (HLR) localized around the points of parasite attachment. Using a trypan blue staining technique, we verified host cell death in these parasite-induced lesions. The deposition of eggs by some insect herbivores can elicit the formation of necrotic tissue,¹⁴ but localized cell death is most widely associated with the hypersensitive response (HR) of plants to pathogens. This complex early defense response can restrict the growth and spread of viruses, fungi and bacteria.⁹ Our work adds to existing evidence¹⁵ that the *Cuscuta*-induced HLR can play a similar role by preventing or limiting the growth of the parasite.

*Correspondence to: Consuelo M. De Moraes; Email: czd10@psu.edu

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Figure 1. A newly germinated *Cuscuta pentagona* seedling encircles and attaches to the hypocotyl of a 10-day-old tomato seedling; the early development of haustoria are visible as nod-like swellings. The trichomes on hypocotyls of young tomato seedlings are not dense enough to affect *C. pentagona* seedlings, but the increased density of trichomes on 25-day-old plants can act as a physical barrier that blocks parasite seedlings (inset).

An interesting discovery was that the first attachment by *C. pentagona* elicited almost no response from young 10-day-old hosts, whereas a subsequent attachment after 10 days induced the wholesale changes discussed above (we also found changes in abscisic acid and free fatty acids). Trials in which we varied the age of the host and parasite indicated that host age, rather than a priming effect on defenses, determined the magnitude of response. We have previously observed that *Cuscuta* spp. in natural populations germinate very early in the growing season, and hypothesized that this tactic promotes successful parasitism by ensuring the presence of young hosts; recent field work seems to corroborate this.¹⁶ As with the response to *Cuscuta* parasitism, levels of host plant defenses against insects¹⁷ and pathogens¹⁸ are known to vary with host age.

In an earlier paper we reported that tomato plants parasitized by *C. pentagona* released greater amounts of volatiles than did unparasitized control plants.¹⁹ The production and release of volatiles is a hallmark of plant responses to feeding by herbivores.²⁰ Herbivore-induced volatiles serve as an indirect plant defense by attracting herbivores' natural enemies,²¹ repelling herbivores,²² or acting as intra-plant signals that prime systemic responses.²³ Although less well documented, pathogen attack can also induce emissions of volatile compounds,²⁴ some of

which are antimicrobial and may serve as a direct defense against infection.²⁵ The same volatile compounds induced by *Cuscuta* (e.g., 2-carene, α -pinene, limonene, β -phellandrene) were also induced by caterpillar feeding and application of JA.¹⁹ Like herbivores, the JA induced by *C. pentagona* may regulate the emissions of plant volatiles. Whether or how parasitic plant-induced volatiles might function in defense is unknown, but they presumably could affect host plant choice by *Cuscuta* seedlings, which use plant volatiles to locate and select hosts.²⁶

Following on from our previous studies we examined the potential role of host trichomes in resistance to parasitism by *C. pentagona*. Plant trichomes have been long appreciated as the first line of defense against insect herbivores^{27,28} and more recently pathogens.²⁹ We hypothesized that trichomes could also defend against parasitic plants based on our observations that (1) tomato trichomes become denser with age (Fig. 1), notably on hypocotyls which is the first area contacted by *Cuscuta* seedlings, and (2) these trichomes can act as a physical barrier to *C. pentagona* seedlings. To test this hypothesis we allowed seedlings of *C. pentagona* to attach to 25-day-old tomato plants (*Solanum lycopersicum* 'Halley 3155') in a climate controlled growth chamber. Of 20 trials conducted, in six (30%) the parasite seedling was completely blocked by trichomes and was unable to reach the host stem—the parasite perished in each of these. Type I glandular trichomes, which are several millimeters long with a glandular tip,³⁰ were primarily responsible for the blocking effect. Thus, trichomes can defend against parasitic plants in a manner analogous to herbivores by physically obstructing their movement. Interestingly, the effectiveness of trichomes is also dependent on age of the host since those on younger tomato plants (<20 days old) are too sparse to impede *Cuscuta* seedlings (Fig. 1).

Considering that the majority of plant defenses are mediated by only a small number of master regulators (e.g., JA, SA, ethylene),⁷ it is not surprising that plant responses to parasitic plants share commonalities with those induced by herbivores and pathogens. These few molecules mediate complex, interacting signaling networks that can be variously activated and modified by plants to tune defenses against a seemingly endless variety of attackers.⁷ Our finding that JA and SA act to defend plants from attack by other plants, further support these phytohormones as 'global' defense signals. It is also apparent that constitutive defenses, such as trichomes, can be effective against diverse antagonists (e.g., herbivores and parasitic plants). These new insights into host defenses against parasitic plants suggest many avenues of needed research including the molecular events induced by parasitic plant attack, the parasite-derived cues that elicit responses, and the ways in which JA and SA act to reduce parasite growth. Finally, our findings suggest it might be possible to manipulate induced responses or host plant age by varying planting date to control parasitic plants in agriculture.

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