THE ROLE OF HOST PLANT CHEMISTRY AND LIGHT-SPECTRAL TRAITS
IN HOST-FINDING AND SELF-DEFENSE BY PARASITIC PLANTS
IN THE GENUS CUSCUTA (CONVOLVULACEAE)

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by
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ABSTRACT

Parasitic plants are notorious for their devastating effects on global food production. They are also gaining recognition for playing keystone roles in ecosystem composition and functioning. Despite their significance in agriculture and nature, many aspects of their basic ecology are not well characterized, including two topics addressed in this dissertation: host-finding and subsequent three-way host-parasite-herbivore interactions.

To address these topics we worked with obligately parasitic vines in the genus *Cuscuta* (Convolvulaceae), also referred to as dodder. Dodder seedlings are extremely limited in their foraging range and will therefore perish if they do not locate a nearby suitable host plant. Prior work revealed that *Cuscuta campestris* seedlings orient toward hosts by responding to at least two classes of cues—host volatiles and host light-spectral cues. It is not known whether *Cuscuta* species that are more host-specific than the cosmopolitan *C. campestris* incorporate these classes of host cues in their foraging behaviors. In a series of biological preference assays with seedlings of *Cuscuta epilinum*, a putative specialist of flax plants, we found no evidence that these parasites use volatile cues to select their preferred host plants (flax). Rather, *C. epilinum* seedlings manifested positive growth responses to light cues common to all chlorophyllous plants, and they were surprisingly more attracted to tomato plants, which are inferior hosts, than to flax plants. In subsequent choice tests we found that seedlings prefer objects (whether plants or model plants) characterized by low red to far-red wavelengths (R:FR) over objects characterized by higher R:FR. R:FR varies as a function of host architecture and of host location. We conclude that seedling responses to R:FR cues represent a strategic foraging strategy to locate the nearest host plants of suitable quality.

Our second line of research examined three-way chemical interactions that occur between host plants, parasitic plants and insect herbivores. Many records in the scientific literature document the fact that secondary metabolites from host plants can translocate into parasitic plants, but little is known concerning the ecological implications of this phenomenon, despite well characterized roles for many secondary metabolites in plant defense. We present the novel finding that glucosinolates, secondary metabolites found in
Brassicas and their relatives, transfer readily into dodder parasites. To examine the importance of this transfer \textit{in vivo}, we grew multiple genotypes of \textit{Arabidopsis thaliana} Col-0 that vary in their ability to produce aliphatic and indole glucosinolates and infested them with the parasite \textit{Cuscuta gronovii}. Here we report our findings that host-derived glucosinolates or related metabolites can protect dodder against \textit{Acrythosiphon pisum} aphids (Hemiptera: Homoptera: Aphidae), which refuse to settle on dodder that contains host glucosinolates. In contrast, \textit{Myzus persicae} aphids are not at all deterred by the presence of glucosinolates in dodder vines, which indicates that protective benefits of glucosinolate uptake are contingent upon the susceptibility of the herbivore species. We similarly investigated the importance of glucosinolate transfer for deterring oviposition by \textit{Lygus rubrosignatus} plant bugs (Hemiptera: Miridae) and find tentative evidence for a deterrent effect of these compounds.

During the course of these studies we observed that dodder growth is negatively correlated with the presence of indole glucosinolates, camalexin and auxin in \textit{Arabidopsis}, which presents the intriguing possibility that well-characterized anti-insect and anti-microbial defense compounds in \textit{Arabidopsis} might also contribute to defense against plant parasites.
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CHAPTER 1

IMPLICATIONS OF BIOACTIVE SOLUTE TRANSFER
FROM HOSTS TO PARASITIC PLANTS

ABSTRACT

Parasitic plants—which make their living by extracting nutrients and other resources from other plants—are important components of many natural ecosystems; and some parasitic species are also devastating agricultural pests. To date, most research on plant parasitism has focused on nutrient transfer from host to parasite and the impacts of parasites on host plants. Far less work has addressed potential “bottom-up” effects of the translocation of bioactive non-nutrient solutes—such as phytohormones, secondary metabolites, RNAs, and proteins—on the development and physiology of parasitic plants and on their subsequent interactions with other organisms such as insect herbivores. A growing number of recent studies document the transfer of such molecules from hosts to parasites and suggest that they may have significant impacts on parasite physiology and ecology. We review this literature and discuss potential implications for management and priorities for future research.

INTRODUCTION

Parasitic plants are important components of many terrestrial ecosystems and also have significant impacts on human agriculture. Pestiferous species, primarily from four genera of parasitic plants (*Striga*, *Orobanche*, *Phelipanche*, and *Cuscuta*), afflict farmers in many parts of the world, posing serious threats to subsistence agriculture in developing regions and causing several billions of dollars in annual economic losses to grain and vegetable production [1,2]. Reduced crop yields—and in some cases complete crop loss—result directly from parasite extraction of water and nutrients from cultivated host plants [2]. Consequently, the mechanisms of resource extraction by parasites and resulting impacts on host plants have been the subject of considerable research efforts [3,4], even though the biology and ecology of parasitic plants remains poorly studied compared to other plant groups [5]. In contrast, relatively little work to date has explored the bottom-up influence of host-derived factors on plant parasites and their subsequent interactions with other organisms.

Most previous research in this area has focused on nutrient transfer [e.g., 6], which in addition to supporting the growth of the parasite can influence the accessibility of resources (e.g., carbon and nitrogen) to other community members—including herbivores, pollinators, decomposers and, ultimately, other plants. Thus, plant parasites can significantly influence ecosystem dynamics via effects on nutrient cycling [4,7]. However, a diverse pool of non-nutrient bioactive solutes are also likely to be translocated from host plants to parasites, including phytohormones, proteins, and RNAs that can have sweeping effects on plant development, and small molecules and proteins involved in host plant defense that might also influence subsequent interactions of the parasite with other organisms. Thus, diverse aspects of host-plant biochemistry are likely to influence the physiology and ecology of plant parasites, with implications for broader community dynamics and potentially for the management of parasitic weeds—though we currently have only a limited understanding of such influences. Here we review recent evidence regarding the transfer of host-derived extra-nutritive solutes into plant parasites and explore the known and potential implications of these exchanges for parasite biology and ecology.
BACKGROUND: MECHANISMS OF SOLUTE TRANSFER FROM HOST TO PARASITE

Understanding the translocation of solutes from host plants to parasites requires a basic understanding of solute transport pathways in plants. Short-range, cell-to-cell movement of solutes occurs via diffusion through spaces outside of cell membranes (i.e., the apoplast), as well as through spaces within cell membranes (i.e., the symplast) that are joined by membrane-lined transcellular tunnels called plasmodesmata [8]. Long-range movement occurs through the vascular tissues phloem and xylem. Phloem tissue comprises living sieve tube element cells with conjoined membranes that transport sugars from photosynthetically-active tissues to sink tissues (e.g., roots, and new leaves). Xylem comprises dead tube-like cells (vessel elements or tracheids) that form continuous apoplastic channels for moving water and minerals from roots to the upper parts of plants [8]. Phloem and xylem additionally facilitate movement of various hormones, proteins and RNAs that function in development and defense processes [9,10].

Parasitic plants can potentially intercept host solutes moving via any of the above pathways by using specialized feeding organs (haustoria) that penetrate host roots or shoots and associate with host vascular tissues [3]. For example, some parasites actively recruit solutes from host apoplast via haustorial parenchymal cells, which account for the largest surface of internal host-parasite contact [11]. This appears to be a primary feeding mechanism for some parasite species [12,13], and in some cases parenchyma adjacent to host vascular tissue possess membrane invaginations that are thought to improve efficiency in solute uptake [3]. Parasites may also feed directly from the host’s xylem stream by forming a conductive bridge that connects the xylem of both plants. The xylem bridge is a widespread haustorial feature among parasitic plant species; however, the nature of the xylem interface varies considerably among genera: while some parasites form cross-species perforations or pits typical of conjoined xylem elements, others penetrate host xylem with open conductive tubes called osculae, and still others do not form visibly open passageways and presumably load solutes from apoplastic spaces outside of the xylem [3] (see Table 1 for specific examples). A less common feeding strategy, documented in members of Cuscuta, Striga and Orobanche, is to form
symplastic connections to host tissues. For example, *Cuscuta* spp. form transient plasmodesmata with adjacent host tissue during the development of their haustoria, and experiments have confirmed the ability of these structures to serve as portals for virus movement proteins [14,15]. Interspecific plasmodesmata were also seen in *Striga gesnerioides* [16]. Additionally, sieve pores connecting parasite and host phloem elements occur in *Orobanche* spp. [17]. Thus, parasites have evolved mechanisms to exploit each of the major solute transportation pathways in plants, although different species exhibit different strategies and rarely utilize all pathways (see Table 1). These contrasts raise the question of how haustorial morphology relates to feeding efficiency and selectivity—a topic with potentially important implications for parasite-host ecology as well as for the development of RNAi-based control strategies (discussed in the next section).

The degree of selectivity in solute uptake by parasites is frequently discussed in the parasitic plant literature [e.g., 6,15,18-20]. Open xylem-xylem or phloem-phloem connections might be expected to allow non-selective bulk flow of materials, whereas transfer pathways that cross cell membranes should result in more selective uptake. Guided by these assumptions, the reputed lack of phloem connectivity for *Cuscuta* spp. (Figure 2) has been called into question by recent reports that phloem-bound green fluorescent protein (GFP) moves freely from tobacco into *Cuscuta reflexa* [21,22] and that various sugars, amino acids, and hormones move non-selectively (i.e., with unaltered ratios) from hosts into *Cuscuta* spp. [15]. In addition to these observations of protein transfer between hosts and parasitic plants, the transfer of other classes of macromolecules has been documented, including mRNAs and large (70kDa) xylem-bound dextrans [23-25]—implying that cross-species portals between some parasites and their hosts may be large enough to accommodate nonselective transfer of smaller solutes including phytohormones and other secondary metabolites. Yet studies of many parasites that form open connections to vascular tissues (including *Cuscuta* spp.) show differences in nutrient levels and ratios between hosts and parasites that suggest the operation of selective uptake processes [e.g., 6,19]. Even early insights into macromolecule transfer into *Cuscuta* spp. indicated upper size limits to protein transfer from host phloem [22], and possibly other mechanisms functioning to prevent the transfer of some phloem-
mobile mRNA species [23]. Thus, our ability to predict solute transfer is currently limited, posing a significant challenge to the development of hypotheses about the transport of native or bioengineered solutes relevant to parasite ecology or weed management. Consequently, further research into the mechanisms of selectivity and their relationship to haustorium morphology is needed. Such work will shed light on whether and how parasites and host plants regulate the transfer of nutrients and other bioactive molecules important in plant development and defense.

**HOST SOLUTE INFLUENCE ON PARASITE DEVELOPMENT**

Plants coordinate their development via a suite of mobile hormones, proteins and RNAs that regulate essential and sensitive aspects of plant life, ranging from intracellular processes such as gene expression to systemic processes such as the onset of flowering [8,9]. Our current knowledge of solute transfer between hosts and parasites (discussed above) suggests it may be possible for any of these important signals to move from hosts into their parasites—and many of these solutes are potentially capable of influencing parasite development. Indeed, the translocation of such signals may underlie the tendency of parasites in the genera *Arceuthobium, Rafflesia*, and others to exhibit synchronization of developmental events (e.g., branching patterns and flowering onset) with their host plants [26]—an effect that could be achieved either by direct action of the host signal (e.g., as in the following discussion on phytohormones) or by a more complex response of the parasite to the information that these signals carry about the host’s developmental state [27]. The later is plausible by analogy to plant immunity cascades, in which xenobiotic molecules from pathogens trigger changes in plant gene expression that are apparently adaptive for the plant [28]. Thus, parasites could conceivably exploit chemical signatures of their hosts’ developmental status—for example, to optimize the timing of flowering onset, to regulate virulence to the host, or to anticipate biotic stress [27]. Whether parasitic plants indeed respond to host cues in this manner remains unknown; however, some observations indicate that it is possible, and sometimes physiologically necessary, for parasites to incorporate host-plant signals.
These cross-species signals include phytohormones (cytokinins, auxin and abscisic acid [ABA]) that, in certain instances, transfer into parasites and may influence parasite development [e.g., 15,29,30]. For example cytokinins, which are transported in xylem and play a role in cell differentiation and growth [31], are acquired from host plants in high concentrations by the parasite *Rhinanthus minor*, which exhibits stunted leaf development in the absence of this resource [30]. Seedlings of *Striga asiatica* may similarly benefit from exogenous cytokinin and auxin [32], although these may not be essential since *S. asiatica* has been grown in media without them [33]. Auxin, which participates in many developmental processes related to cell growth and differentiation [8], can also be transferred into *Cuscuta* spp. when supplied exogenously to the host plant [15], but no effects on the parasite have been studied. *Cuscuta* spp. also take up ABA, a xylem- and phloem-mobile plant hormone involved in responses to drought [34]. This contributes to repeated observations that ABA tends to be higher in *Cuscuta* spp. and other parasites than in host plants [29,35-37]. But *Cuscuta* spp. do not appear to selectively recruit host ABA [15], nor to be dependent on host plant production of this hormone [38], so host-derived ABA is likely not essential to their development. It remains to be determined whether other phytohormones (e.g., gibberellins, ethylene, brassinosteroids) also translocate into and impact parasites.

The significance of macromolecule transfer into parasitic plants is even less well characterized than hormone transfer. Protein movement into parasites has only been documented for non-plant proteins of low molecular weight from transgenic host plants (e.g., GFP and virus movement protein, as discussed above) [15,21,22,25]. Yet, these observations suggest that mobile plant proteins of sufficiently small size can be transferred from the host and therefore possibly play a role in parasite development. For example, the long sought after signal mediating long-distance flowering induction, “florigen”, was recently identified as the FLOWERING LOCUS T protein, or FT [39]. Since FT is smaller than GFP (20 vs 27 kDa) and mobile in the phloem, it might also be expected to translocate from flowering host plants into phloem-feeding *Cuscuta* parasites. Flowering synchrony has been observed in certain host-*Cuscuta* spp. combinations and could be the result of translocated host factors such as flowering hormones [40]—but this has not been established conclusively, and other plausible explanations (e.g., parasite
response to nutrient changes in the host) are discussed in Dawson et al. and Costea and Tardif [40,41]. Now that florigen has been identified, it will be interesting to examine whether the FT protein in fact transfers and plays a role in synchronizing host and parasite flowering.

RNA transfer provides an additional means by which host plants might influence parasites. As mentioned above, mRNAs move from tomato, alfalfa and pumpkin into Cuscuta spp. and remain detectable at significant distances from the site of attachment (at least 20 cm) [23,24]. Nearly half of the translocated mRNAs in these experiments encoded transcription factors and calmodulin proteins that could potentially play regulatory roles in the parasite if translated into protein—but whether this occurs has yet to be verified [27]. It has also been suggested that noncoding RNAs might interfere with translation of homologous sequences within the parasite in a fashion similar to siRNA defenses used by plants to selectively silence expression of virus genes [27,42]. In an evolutionary context, RNA transfer might additionally facilitate horizontal gene transfer which can occur bi-directionally between parasites and their hosts, although this idea remains speculative [43-45, and references therein].

Whether or not host plants leverage RNA transfer as a defense against parasitic plants, the occurrence of this exchange may present a strategic opportunity to manage parasitic weeds using transgenic crop plants. RNA interference, in which siRNA constructs from one organism are used to silence target genes in another, was first demonstrated successfully in parasitic plants by Tomilov et al. [46], who showed that expression of a beta-glucuronidase (GUS) marker gene in a transgenic parasite (Triphysaria versicolor) could be silenced by a hairpin GUS-specific construct from a transgenic host plant (lettuce). Remarkably, this study also showed that siRNA from one host could pass through the parasite and into another host—raising the intriguing idea that parasites might also be capable of influencing their hosts via mobile RNAs. Other research groups have subsequently implemented RNAi strategies targeting native parasite genes and achieved significant deleterious effects on Orobanche aegyptiaca infesting tomato [47], on Cuscuta pentagona infesting tobacco [48], and on Triphysaria versicolor infesting Medicago truncatula [49], but not on Striga asiatica infesting maize [50]—
suggesting that this is a promising technique, but may be challenging to implement in some systems. For an in depth discussion of future prospects in this area of research, readers are referred to recent reviews [51,52].

In summary, it seems likely that parasite development may be significantly hindered or facilitated by the transfer of many small and macro-molecules from host to parasite, although the limited scope of current knowledge on this topic leaves much to be explored.

**HOST SOLUTE INFLUENCE ON PARASITE DEFENSE**

Plant defense responses to herbivores are also regulated and actualized by small and macro-molecules that may be transferred through haustoria into parasitic plants. The translocation and effects of such solutes are discussed below, starting with a brief overview of plant chemical defenses.

In general, healthy plants invest somewhat limited resources into constitutive mechanical and chemical defenses—the later commonly involving solutes that may be relevant to parasitic plants, including toxic proteins and secondary metabolites. When attacked by herbivores, however, chemical cues related to the attack (e.g., lipids from damaged cell walls or components of insect oral secretions) elicit the expression of additional defense responses, which can be specifically targeted against particular antagonists [53]. The signaling cascades that mediate these induced responses frequently involve the phytohormones jasmonic acid (JA), salicylic acid (SA), and ethylene [54]. These hormones (and related signaling molecules) can travel long distances through the plant to initiate resistance even in leaves and stems that are not (yet) under attack [54]. Thus, the chemical signals and toxins involved in these responses present seemingly ripe opportunities for parasitic plants to acquire resistance against herbivores with host-derived solutes.

Whether parasites take up defensive phytohormones remains largely unknown, although we know that parasitic plants can induce changes in concentrations of these hormones in their hosts: Runyon et al. [37] observed sequential elevation of JA and SA in
tomato plants in response to haustoria formation by *Cuscuta campestris*. Notably, neither the transient elevation of JA nor the more persistent elevation of SA in the host plant resulted in elevation of the same hormones in *C. campestris* vines that subsequently sprouted from sites of attachment. This may imply that host signals responsible for triggering systemic JA- and SA-mediated defenses are excluded, metabolized, or simply not recognized by *C. campestris* vines, a notion further supported by the finding that vines connected to caterpillar-damaged tomato plants did not have significantly greater JA levels than control vines on healthy tomato hosts, despite clear differences in host JA levels [55]. Nevertheless, it would be interesting to measure the responses of other parasite species to host defense signals that are variously induced by herbivores and pathogens, since such antagonists may also pose a threat to parasites.

Relative to hormone transfer, more information is available regarding the translocation of host-derived toxins. Among secondary metabolites, a wide range of host-derived alkaloids have been detected in parasitic plants from several genera (*Castilleja, Pedicularis, Orobanche, Loranthus, Tristerix* and *Cuscuta*), and host-derived cardenolides and phenolics have been found in species of *Cuscuta, Santalum* and *Nerium* [56-58, and references therein]. These classes of compounds are well known to have adverse effects on insects [59], and thus it has been suggested that secondary metabolite uptake may enhance parasitic plant defense [e.g., 56,60]. Yet, there have been surprisingly few tests of this hypothesis to date. Several studies have demonstrated that host species identity can impact the performance of insect herbivores on parasitic plants [e.g., 61], but it is difficult to isolate the effects of toxin translocation from other potential effects of feeding on a given plant species. Adler et al. [57] overcame this problem by using near isogenic high- and low-alkaloid-producing lines of *Lupinus albus* as hosts for *Castilleja indivisa*. They found that host alkaloids in the parasite reduced herbivory and indirectly increased parasite fitness. This study represents the strongest evidence to date that host secondary metabolites are important in parasite-herbivore interactions. More recently, alkaloid toxins produced by a fungal endophyte were found to be translocated through a host plant and into the parasite *Rhinanthus serotinus*, where their presence was associated with a marked decrease in aphid performance [62]. These studies strongly suggest that exogenous defensive solutes derived from hosts can alter the interactions of
parasitic plants with their own insect antagonists. This phenomenon may have implications for classical biocontrol approaches to the management of parasitic weeds that utilize insect herbivores, since control organisms would need to tolerate not only the parasite’s natural defenses, but also any toxic solutes that a parasite may acquire from its host plants. It remains unknown whether other classes of secondary metabolites may function in similar ways. Notably, there are currently no documented examples of macromolecules with anti-herbivore properties (e.g., protease inhibitors) being translocated into parasitic plants from hosts, despite the recent observations (discussed above) that proteins and RNAs can be transferred; this would seem an interesting topic for further investigation.

CONCLUSIONS

Past work on parasite-host and parasite-community interactions have focused heavily on nutrient transfer, but a growing body of literature shows that many non-nutrient solutes also translocate into parasites and may influence physiologically and ecologically important processes such as development and plant defense. The implications of such effects of host plant biochemistry on parasitic plants may hold significance for understanding the outcome of parasite interactions with other community members (especially insect herbivores) and also suggest strategic opportunities to manage parasitic weeds, including via the development of transgenic crops. Yet our current limited knowledge regarding these phenomena leaves important questions unanswered. For example, what is the relationship between haustoria morphology and the selectivity of solute uptake? Do parasites translate host mRNA into proteins and, more generally, do host derived proteins or transcripts impact important processes in the parasite? How significant is the contribution that defensive molecules from host plants make to the resistance of parasites against their own antagonists? Addressing these gaps in our current knowledge will not be a trivial task (especially considering the great diversity represented by parasitic plants [45]), but is likely to inform the development of enhanced strategies for managing parasitic weeds, while also providing novel insights into the physiology, ecology and evolution of these fascinating and relatively understudied organisms.
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Figure 1. Illustration of several groups of plant parasites discussed in this review: (1) mistletoes; (2) witchweeds; (3) broomrapes; (4) dodders; (5) corpse flowers; (6) figworts. (Note: Depicted here on a fictive generic host, these parasites utilize different and diverse hosts in nature [see Table 1]). Illustration by Nick Sloff.
A. 

B. 

Figure 2. *Cuscuta campestris* on tomato plants 
*Cuscuta* spp. connect directly to host xylem and phloem, and are the first parasitic plants known to uptake protein and mRNA from host plants [21,23].
Table 1. Life history and haustoria details for parasite genera referenced in this paper and depicted in Figure 1. (Information on host species obtained from [26] and/or the primary references included in the table.)

<table>
<thead>
<tr>
<th>Parasite genus</th>
<th>Family Fig. 1 Icon, Group</th>
<th>Description</th>
<th>Common hosts</th>
<th>Xylem contact</th>
<th>Phloem contact</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arceuthobium</em></td>
<td>Viscaceae 1, dwarf mistletoes</td>
<td>obligate aerial hemiparasites&lt;sup&gt;1&lt;/sup&gt;</td>
<td>pines and cypresses</td>
<td>p.x. have thin walls by h.x. pits; p.par. form half-bordered pits with h.x.</td>
<td>p.phl. absent; some p.par. abut h.phl.</td>
<td>[63, 64]</td>
</tr>
<tr>
<td><em>Castilleja</em></td>
<td>Orobancheaceae 6, figworts</td>
<td>facultative root hemiparasites</td>
<td>non-woody monocots and dicots</td>
<td>p.x. penetrate h.x. with open tubes (osculae)</td>
<td>p.phl. uncommon; digitate p.par. abut h.phl.</td>
<td>[65]</td>
</tr>
<tr>
<td><em>Cuscuta</em></td>
<td>Convolvulaceae 4, dodders</td>
<td>obligate aerial hemiparasites</td>
<td>dicot crops</td>
<td>terminal p.x. cells form hyphae that connect openly to h.x.</td>
<td>terminal p.phl. cells form hyphae that grasp h.phl., no open connections apparent</td>
<td>[66]</td>
</tr>
<tr>
<td><em>Olax</em></td>
<td>Olacaceae (not pictured)</td>
<td>facultative root hemiparasites</td>
<td>woody dicots</td>
<td>p.x. terminate near or touching h.x., no open connections apparent</td>
<td>p.phl. absent</td>
<td>[12]</td>
</tr>
<tr>
<td><em>Orobanche</em></td>
<td>Orobancheaceae 3, broomrapes</td>
<td>obligate root holoparasites&lt;sup&gt;2&lt;/sup&gt;</td>
<td>dicot crops</td>
<td>p.x. connect to h.x. with open pits</td>
<td>p.phl. connect to h.phl. with open sieve pores</td>
<td>[67, 68]</td>
</tr>
<tr>
<td><em>Phelipanche</em></td>
<td>Orobancheaceae 3, broomrapes</td>
<td>obligate root holoparasites</td>
<td>dicot crops</td>
<td>unknown (refer to <em>Orobanche</em>)</td>
<td>unknown (refer to <em>Orobanche</em>)</td>
<td>[25]</td>
</tr>
<tr>
<td><em>Rafflesia</em></td>
<td>Rafflesiaceseae 5, corpse flowers</td>
<td>obligate root holoparasites (lives inside host except for flowers)</td>
<td><em>Tetra-stigma</em> spp.</td>
<td>p.x. absent, endophyte strands contact most h. tissues</td>
<td>p.phl. absent, endophyte strands contact most h. tissues</td>
<td>[26]</td>
</tr>
<tr>
<td><em>Rhinanthus</em></td>
<td>Orobancheaceae 6, figworts</td>
<td>facultative root hemiparasites</td>
<td>monocot crops, legumes</td>
<td>p.x. penetrate h.x. with open tubes (osculae)</td>
<td>p.phl. absent</td>
<td>[69]</td>
</tr>
</tbody>
</table>

Footnotes: 1 Hemiparasite: capable of photosynthesis; 2 Holoparasite: not capable of photosynthesis

Key: h.phl. host phloem sieve element, h.x. host xylem vessel element or tracheid; p.x. parasite xylem vessel element or tracheid; p.par. parasite parenchyma; p.phl. parasite phloem sieve element
Table 1, cont. Life history and haustoria details for parasite genera referenced in this paper and depicted in Figure 1

<table>
<thead>
<tr>
<th>Parasite genus</th>
<th>Family</th>
<th>Fig. 1 Icon, Group</th>
<th>Description</th>
<th>Common hosts</th>
<th>Xylem contact</th>
<th>Phloem contact</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striga</td>
<td>Orobanchaceae</td>
<td>2, witchweeds</td>
<td>obligate root hemiparasites</td>
<td>monocots crops</td>
<td>p.x. penetrate h.x. with open tubes (osculae)</td>
<td>p.phl. absent</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triphysaria</td>
<td>Orobanchaceae</td>
<td>6, figworts</td>
<td>facultative root hemiparasites</td>
<td>non-woody monocots and dicots</td>
<td>p.x. contact h.x. in some haustoria, transfer pits seen in h.x. but not in p.x.</td>
<td>p.phl. absent</td>
<td>[13]</td>
</tr>
</tbody>
</table>

Footnotes: 1 Hemiparasite: capable of photosynthesis; 2 Holoparasite: not capable of photosynthesis
Key: h.phl. host phloem sieve element, h.x. host xylem vessel element or tracheid; p.x. parasite xylem vessel element or tracheid; p.par. parasite parenchyma; p.phl. parasite phloem sieve element
REFERENCES AND RECOMMENDED READING


(special) A differential ability of GFP and a GFP-ubiquitin fusion to translocate from host phloem into *Cuscuta* parasites in this study indicated there is a size-exclusion mechanism regulating protein traffic between these plants.


(outstanding) Provides the first documented occurrence of mRNA transfer between hosts and a parasitic plant. More than a dozen transcripts from pumpkin and tomato moved into *Cuscuta pentagona*, including some that were previously thought to be non-mobile. The notable absence of some mobile host transcripts within the parasite indicated that mRNA transfer may be a selective process.

(special) Contributes additional examples of mRNA translocation into and travel through parasites using *Cuscuta pentagona* on tomato and alfalfa. Their work showed that transfer can occur through cross-species plasmodesmata formed by haustorial parenchyma.


(special) First documentation of protein movement into a parasite in the family Orobanchaceae using transgenic tomato plants expressing GFP in their companion cells. Also demonstrated the potential for macromolecule transfer via xylem connections by tracing xylem-bound dextrans from hosts into parasites.


(outstanding) Demonstrates the feasibility of using transgenic hosts to silence genes in an attacking parasite. Expression of the GUS reporter gene in a transgenic parasite (Triphysaria versicolor) was silenced when the parasite attached to roots of a
transgenic host (lettuce) expressing a hairpin GUS-specific construct. Remarkably, siRNA from one host passed through the parasite and into another host—which revealed that RNA transfer can be bi-directional.


(oustanding) Reports the first implementation of RNA interference that is effective against an attacking parasitic plant. dsRNA in transgenic tomato plants suppressed levels of mannose 6-phosphate reductase in seedling Orobanche aegyptiaca, resulting in lower mannitol levels and increased mortality rates among developing parasites.


(special) Provides a second example of RNA interference that successfully influences an attacking parasite, and demonstrates the utility of RNAi for studying the role of specific parasite genes in parasite-host interactions.


(special) Identifies a new potential target for RNAi in transgenic crops: cytosolic acetyl-CoA carboxylase (ACCase) genes. Silencing ACCase genes in Triphysaria versicolor by transgenic hosts (Medicago truncatula) resulted in up to 80% loss in parasite root viability.


67. Dörr I, Kollmann R: **Structural features of parasitism of Orobanche III. The differentiation of xylem connexion of O. crenata.** *Protoplasma* 1976, **89**:235–249.


69. Cameron DD, Coats AM, Seel WE: **Differential resistance among host and non-host species underlies the variable success of the hemi-parasitic plant Rhinanthus minor.** *Ann Bot* 2006, **98**:1289–1299.

70. Dörr I: **How Striga parasitizes its host: a TEM and SEM study.** *Ann Bot* 1997, **79**:463–472.
CHAPTER 2

PARASITIC PLANTS IMBIBE HOST PLANT TOXINS
THAT AFFECT HERBIVORES IN A SPECIES-DEPENDENT MANNER

ABSTRACT

Secondary metabolites are prominent in plant defense against herbivores and other antagonists. Parasitic plants have been reported to obtain a wide range of secondary metabolites from their host plants, but potential ecological effects of this phenomenon, such as parasite defense enhancement, are largely unexplored. Here we report a novel finding that glucosinolates from brassicaceous host plants transfer readily into parasitic dodder vines (Convolvulaceae: Cuscuta gronovii).

To examine the significance of glucosinolate translocation to dodder defense against insects we grew Cuscuta parasites on lines of Arabidopsis thaliana that vary in their production of glucosinolates and used these vines in bioassays with two aphid species. The settling and survival of Acryothismus pisum aphids was strongly inhibited on dodder with glucosinolate-producing hosts (wildtype and atr1D) relative to dodder with glucosinolate-free hosts (cyp79B2 cyp79B3 myb28 myb29), but this was not true of Myzus persicae aphids, which did not distinguish between treatments. This suggests that host-derived glucosinolates bolster dodder defenses against aphids in a species-specific manner, although we can not exclude the possibility that other host compounds that differed in the mutant genotypes (including auxin and camalexin) might also contribute to the effects we observed.
In a more sensitive assay with *M. persicae* we found these aphids reproduce faster on dodder hosted by glucosinolate-producing Arabidopsis than on dodder hosted by glucosinolate-free Arabdipsis, but this pattern was not observed among aphids confined directly to the Arabidopsis host plants. These performance differences do not appear to correlate with nutritional differences since *cyp79B2 cyp79B3 myb28 myb29* hosts and parasites have more total free amino acids than wildtype and *atr1D* hosts and parasites. *M. persicae* reproduction was negatively correlated with levels of glutamic acid, a known aphid deterrent that may contribute to this performance pattern across treatments. Also, dodder itself was affected by Arabidopsis genotype: parasite vines grew the faster on *cyp79B2 cyp79B3 myb28 myb29* quadruple mutants than on wildtype Arabidopsis; they also grew slower on *atr1D* mutants (which have elevated indole glucosinolates) than on wildtype controls. Dodder growth was enhanced on *cyp79B2 cyp79B3* double mutants but not on *myb28 myb29* double mutants—which raises the intriguing possibility that compounds reduced in *cyp79B2 cyp79B3* but elevated in *atr1D* plants, including indole glucosinolates, camalexin and/or indole acetic acid, could play a role in Arabidopsis resistance to dodder and thereby lead to an indirect positive effect on *M. persicae* aphids.

**INTRODUCTION**

Parasitic plants extract not only nutrients but also a wide range of secondary metabolites from host plants (Smith et al., 2013). These include metabolites that function in plant defense against insects (Roberts, 1998) and frequently move from host plants to parasitic plants (Stermitz, 1998). It is conceivable that parasitic plants may bolster their defenses against insect herbivores by imbibing host compounds in a manner analogous to the well documented phenomenon of metabolite sequestration by insect herbivores (Opitz and Mueller, 2009)—and this defense translocation hypothesis that has been previously raised in scientific literature (Atsatt, 1977; Smith et al., 2013; Stermitz, 1998).

While this idea is consistent with several observations that host plant species identity can alter the performance of insect herbivores on parasitic plants (Harvey, 1966; Marvier, 1998; 1996; Rowntree et al., 2014; Schädler et al., 2005), it has proven difficult to test owing to the conflation of indirect and direct effects of host plant species on
consumers of parasitic plants. For example, host plants may impact parasite-feeding herbivores indirectly via changes in parasite biomass (Rowntree et al., 2014) or nutritional quality (Marvier, 1998), as well as directly via changes in parasite metabolomes (Adler, 2000; Adler et al., 2001; Lehtonen et al., 2005), and still other pathways are conceivable (e.g., by altering the parasite’s endogenous defenses). Consequently, the anticipated effects of translocated toxins on parasite herbivores may be masked by other host-dependent factors that alter herbivore performance (e.g., parasite attachment success and C:N ratios)—and this poses a difficulty to interpreting studies that fail to detect an effect of host secondary metabolite transfer on parasite consumers (Adler, 2002; Marvier, 1996; e.g., Stermitz et al., 1989). Reciprocally, when a negative correlation between host toxin transfer and herbivore performance on the parasite is observed (e.g., Loveys and Tyerman, 2001), host-dependent nutritional effects may pose a competing explanation.

To adequately test the effects of secondary metabolites, methods are required that selectively manipulate secondary metabolite levels without altering other aspects of parasite quality for herbivores. Adler et. al. (2001) approached this problem by using near-isogenic lines of *Lupinus albus* (Fabaceae) that varied in alkaloid production as host plants for the root parasite *Castilleja indivisa* (Scrophulariaceae). Parasites infesting high-alkaloid plants incurred less floral damage from insect herbivores than those infesting low-alkaloid plants. A similar effect was observed in the root parasite *Rhinanthus serotinus*, which gained resistance to aphid herbivores from toxins that originated in the fungal mutualists of the host plant (Lehtonen et al., 2005). Although treatments in each of these studies (Adler, 2003; Lehtonen et al., 2005) resulted in parasite biomass differences that conceivably could contribute to effects on herbivores (high alkaloid parasites were larger than low alkaloid parasites), alkaloid translocation appeared to be the most parsimonious explanation of the reduced herbivore pressure and performance on the parasites. Nevertheless, additional studies that control for nutritional differences while manipulating allelochemical levels are merited to clarify the role of host plant secondary metabolites in ecology of parasitic plant ecology.
Also, it remains unknown whether non-alkaloid classes of secondary metabolites are involved in similar host-parasite-herbivore interactions—despite records of cardenolide, phenolic and iridoid glycoside compounds moving between hosts and parasitic plants (Boonsong and Wright, 1961; Rothe et al., 1999; Srimathi and Sreenivasaya, 1963; Stermitz et al., 1993; Subraman and Nair, 1966)—and this poses a significant frontier in the ecology of parasitic plants (Smith et al., 2013).

This study addresses the defense translocation hypothesis by utilizing *Arabidopsis thaliana* (Brassicaceae) as a model host plant for the aerial stem parasite *Cuscuta gronovii* (Convolvulaceae), known commonly as dodder. *Cuscuta* spp. are highly cosmopolitan in their host preferences and utilize several Brassicaceous host plants (Gaertner, 1950). Their haustoria (invasive attachment organs) connect directly to host xylem and phloem and extract a diverse suite of solutes including amino acids, sugars, hormones, proteins, mRNAs and various other secondary metabolites (Birschwilks et al., 2006; 2007; LeBlanc et al., 2013; Rothe et al., 1999). It not known, however, whether dodder or any other parasitic plant is capable of imbibing glucosinolates—a major class of defense compounds produced by plants in the Brassicaceae and related plant families (order: Cappareles)—but these compounds are good candidates for transport into dodder because they are present in Brassica phloem (Chen et al., 2001). In this chapter we report for the first time the movement of glucosinolates from host plants into a parasitic plant.

Parasite uptake of glucosinolates could have interesting ecology consequences because glucosinolates mediate diverse interactions in insect-plant communities (Halkier and Gershenzon, 2006). Glucosinolate metabolites protect plants against pathogen invasion (Bednarek et al., 2009; Buxdorf et al., 2013; Fan et al., 2011; Pedras et al., 2007; Sanchez-Vallet et al., 2010; Schlaeppi et al., 2010; Tierens et al., 2001) and at least one metabolite functions in a defense signaling pathway (to induce callose production) (Clay et al., 2009). A better known function of glucosinolates is their direct action against herbivores. When plant tissue is disrupted by herbivore feeding, glucosinolates come into contact with myrosinase enzymes that hydrolize the thioglucoside linkage, resulting in aglycone glucosinolates that further break down into isothiocyanates, thiocyanates, nitriles, and other compounds that vary in their toxicity to insects (Halkier and
Volatile isothiocyanates and nitriles serve the additional but indirect defensive function of attracting parasitoids that help to regulate herbivore populations (Blande et al., 2007; Bradburne and Mithen, 2000; Kos et al., 2012; Mumm et al., 2008; Murchie et al., 1997; Pivnick, 1993; Titayavan and Altieri, 1990). Despite the broad effects of glucosinolate defenses, specialist insects that utilize Brassica hosts (e.g., *Pieris rapae*, *Plutella xylostella* [Lepidoptera: Pieridae, Plutellidae], *Brevicoryne brassicae* and *Lipaphis erysimi* [Hemiptera: Aphididae], and *Athalia rosae* [Hymenoptera: Tenthredinidae]) have evolved various ways to metabolically disarm these defenses (e.g., Ratzka et al., 2002; Wittstock et al., 2004), and may further exploit the glucosinolate defense system by utilizing related metabolites as feeding and oviposition cues (De Vos et al., 2008; Gabrys and Tjallingii, 2002; Moon, 1967; R. Müller et al., 2010; Renwick, 2002; Sun et al., 2009) and by incorporating glucosinolates into their own defensive systems against insect predators (Bridges et al., 2002; Dawson et al., 1987; Jones et al., 2001; Kazana et al., 2007; C. Müller et al., 2001; Opitz et al., 2010). Thus a range of ecological effects of glucosinolate transfer into parasitic plants are conceivable.

We hypothesized that dodder, like some specialist herbivores, might gain protection from insect antagonists by imbibing host glucosinolates. The Arabidopsis host system presents tools to test this hypothesis, including well characterized mutants such as the quadruple mutant cyp79B2 cyp79B3 myb28 myb29 which is deficient in indole and aliphatic glucosinolates (Beekwilder et al., 2008; Sun et al., 2009; Sønderby et al., 2007; Zhao et al., 2002), and also the mutant atr1D, which is enriched relative to wildtype plants in indole glucosinolates that are known to deter aphid feeding (Celenza et al., 2005; Kim et al., 2008). We grew *C. gronovii* on these mutants and on wildtype Col-0 plants and confirmed that glucosinolates are present in parasite vines hosted by wildtype and atr1D plants, but not in vines growing on cyp79B2 cyp79B3 myb28 myb29 hosts (hereafter “–GLS”). Next we tested the suitability of the parasite for two aphid species that differ with respect to their tolerance for feeding upon Brassica plants: *Acyrthosiphon pisum* and *Myzus persicae*. In settling assays, *A. pisum* aphids preferred –GLS-hosted vines to those grown on glucosinolate producing hosts (wildtype or atr1D) and their survival was greatly enhanced when given access to a –GLS vine. In contrast, *M. persicae* did not discriminate between vine treatments and survived well in all choice
tests. In a population growth assay, *M. persicae* actually reproduced faster on wildtype- and *atr1D*-hosted vines than on –GLS-hosted vines. This result was unexpected because although *M. persicae* feed on Brassicas in nature, they are negatively affected by indole glucosinolate metabolites in artificial diets and in Arabidopsis (Kim et al., 2008). Analysis of amino acid and sugar levels within the samples revealed that metabolite differences were present between parasites grown on the different Arabidopsis lines that possibly contributed to the treatment effects on *M. persicae* development. In a second aphid performance assay we found that aphid performance was not compromised on the –GLS treatment if feeding directly on Arabidopsis, whereas it was reduced when feeding upon dodder on –GLS host plants. We also noted that *C. gronovii* vine growth differed across the genotypes used in our study, which raises raise the intriguing possibility that glucosinolates or other related metabolites contribute to Arabidopsis defense against parasitic plants; and moreover, their direct effects on dodder may indirectly enhance *M. persicae* performance on the parasite. Results from these experiments are presented in detail below.

**RESULTS**

*Glucosinolates transfer from host plants into vines of the parasite Cuscuta gronovii*

To test the hypothesis that *Cuscuta* parasites imbibe glucosinolate toxins from their hosts, we infested flowering wildtype *Arabidopsis thaliana* (var. Col-0) with *C. gronovii* seedlings. Since it was not previously known whether *Cuscuta* parasites themselves produced glucosinolates (although this is unlikely), we also grew the parasites on a quadruple *cyp79B2 cyp79B3 myb28 myb29* mutant (hereafter ‘-GLS’) Arabidopsis that is greatly reduced in its production of aliphatic and indole glucosinolates (De Vos et al., 2008). All hosts and parasites were assayed for glucosinolates using a desulfo-glucosinolate HPLC analysis protocol (Barth and Jander, 2006). As expected, no glucosinolates could be detected in either the mutant Arabidopsis or their parasitic vines, which confirms that *C. gronovii* does not possess endogenous glucosinolates. In contrast, parasites grown on wildtype Arabidopsis contained each of the eight major glucosinolates that occur in host inflorescence stalks (Figure 1), and most of these were present in levels
higher than in the host. Two notable exceptions include the indole glucosinolates 4MI3M and 1MI3M (Figure 1), which are known to play a role in deterring *Myzus persicae* (Kim et al., 2008; Kim and Jander, 2007). Parasite tissue at the site of attachment generally possessed higher glucosinolate levels than distal tissue, which could indicate dilution and/or metabolism of the glucosinolates. It is nevertheless interesting that these metabolites are delivered to the tips of the vines in levels that are similar to those in the host plants—high enough to conceivably interfere with herbivores that attempt to feed upon the parasite.

*Host Arabidopsis genotype affects dodder palatability for Acyrthosiphon pisum aphids but not for Myzus persicae aphids in settling assay*

The discovery that glucosinolates readily transfer into *Cuscuta* parasites raises the question of whether these molecules bolster *Cuscuta* spp. defenses against insect herbivores. We addressed this question with two aphid species that feed on *Cuscuta* spp.—*Acyrthosiphon pisum* and *Myzus persicae*—by allowing groups of aphids to choose between parasite vines grown on Arabidopsis plants that vary in their production of glucosinolates. One choice set employed wildtype and -GLS mutant hosts to produce vines with and without glucosinolates; a second set employed wildtype and *atr1d* mutants to produce vines with intermediate and high levels of glucosinolates (*atr1d* mutants overproduce a transcription factor in the tryptophan pathway which leads to elevated levels of indole glucosinolates—key aphid-deterring compounds (Kim et al., 2008; Kim and Jander, 2007))—as well as elevated indole acetic acid (IAA auxin) and the phytoalexin camalexin (Celenza et al., 2005)). Over the course of 24 hours, *A. pisum* aphids settled on -GLS vines with greater abundance than on WT vines (Wilcoxon Signed Rank Test, $Z = 45, P = 0.008849$), indicating a significant preference for glucosinolate-free *Cuscuta* tissue (Figure 2). They did not distinguish between wildtype and *atr1D* vines (Paired T-test: $T_{(8)} = -0.5443, P = 0.6011$) and seldom settled on either of these options. Nearly all *A. pisum* aphids in the *atr1D/wildtype* choice replicates died within three days, whereas aphids in the -GLS/wildtype choice arena survived at a much higher rate (post hoc Tukey test: $P < 0.0000$). In contrast to *A. pisum, M. persicae* aphids
were not deterred by glucosinolates in either of the choice tests (Paired T-tests: -GLS/WT $T_{(9)} = 1.108, P = 0.2965$; atr1D/WT $T_{(8)} = 0.0102, P = 0.9921$); indeed, between the one and 24 hour time points they displayed a weak tendency to migrate to vines with greater glucosinolates (Figure 2). Furthermore their survival was high relative to A. pisum aphids and consistent between the two choice tests (Figure 3) (post-hoc Tukey HSD test: $P = 0.9908284$). We also tested for interaction effects between glucosinolate treatments and aphid species on preference and survival, which was possible because choice test experiments were conducted simultaneously. At 24 hours there was significant interaction effect of aphid species by host type in the –GLS/WT choice test (mixed factorial ANOVA: $F_{(1,18)} = 47.72, P = 1.853e-06$), but not in the art1D/WT choice test (mixed factorial ANOVA: $F_{(1,16)} = 2.658e-01, P = 0.6132$). Additionally, there was a significant interaction effect of aphid species by choice test type (-GLS/WT versus atr1D/WT) on the survival of aphids after three days (2-way factorial ranked ANOVA: $F_{(1,34)} = 38.99, P = 4.15E-07$). These analyses confirm that A. pisum and M. persicae are differentially affected by host plant genotype when feeding on dodder.

M. persicae population growth rate is positively correlated with host glucosinolates in Cuscuta vines

Although M. persicae did not show a behavioral response to glucosinolates in the settling assay, the literature contains evidence that glucosinolates in Brassicas can reduce the growth rate of M. persicae. In particular the indole gluconolates produced by Arabidopsis and overproduced by atr1D mutants are deterrent to M. persicae (Kim et al., 2008; Kim and Jander, 2007). This raises the question of whether these compounds have this same effect in the novel context of a Cuscuta vine. To address this, we confined single M. persicae nymphs to C. gronovii vines with the same glucosinolate treatments as employed above, and recorded their population growth over time. Glucosinolate treatments were confirmed by HPLC analysis of plant tissues following the experiment. Surprisingly, M. persicae reproduced faster on wildtype and atr1D vines than on –GLS vines (Figure 4) (One-way ANOVA, Tukey post-hoc tests: –GLS v. WT $P =0.0031$; –GLS v. atr1D $P = 0.0146$). There was no indication that the atr1D genotype inhibited
aphid reproduction (atr1D v. WT $P = 0.5776$)—indeed, the data are suggestive of a slight (but non-significant) increase in population growth rate in response to this genotype.

M. persicae *aphids perform well on* –GLS *Arabidopsis but poorly on* –GLS-hosted dodder

In light of these findings it became important to test how *M. persicae* respond to the glucosinolate treatments (genotypes) when they are allowed to feed directly upon Arabidopsis. We conducted a performance assay similar to the above by confining each adult aphid individually to either a *Cuscuta*-free Arabidopsis inflorescence or to a dodder vine and allowing them to reproduce over a period of eight days. Aphid populations grew considerably larger on Arabidopsis plants than on dodder vines, and this effect was consistent across all genotypes (see Tukey HSD tests in Figure 7). In accordance with the prior *M. persicae* population assay, population growth rate was slower on –GLS dodder than on atr1D dodder (–GLS dodder and WT dodder did not separate statistically although the seemingly better performance on –GLS was also consistent with the prior assay). In contrast, performance did not significantly differ across glucosinolate treatments for aphids confined to Arabidopsis (Figure 7). This juxtaposition was evident in treatment interaction plots, which exhibited non-parallel effects across treatments (Figure 7), and in a factorial ANOVA, which showed a significant interaction effect between factors ($F_{(2,81)} = 6.265$, $P = 0.00295$).

**Sugars and free amino acids in hosts and parasites**

To examine whether the observed difference in aphid performance might relate to the nutritional makeup of Arabidopsis and dodder across treatments we quantified the total free amino acids and sugars (fructose, glucose and sucrose) in Arabidopsis stalks and *Cuscuta* vines. Samples were taken from the first *M. persicae* population assay immediately after the aphids were counted. Polar metabolites were derivatized and relatively quantified by GC-MS according to the method published by Lisec et al. (Lisec
et al., 2006). Mean concentrations and statistical analyses for each metabolite are summarized in Table 1. There was no effect of Arabidopsis type upon sugar levels, although sugars concentrations were markedly different between Arabidopsis and *Cuscuta* tissue. In contrast, several amino acids in Arabidopsis and/or dodder showed an effect of Arabidopsis genotype. Treatment effects on each metabolite were assessed with a combination of factorial ANOVAs and post-hoc tests. Only three metabolites had a significant tissue by genotype interaction term: alanine, glutamic acid and leucine (Table 2). Of these three, glutamic acid and leucine were higher in the –GLS dodder than in the other dodder groups (Table 1), but corresponding effects were not evident in the Arabidopsis— which distinguishes them as candidate effectors of poor aphid performance on –GLS dodder.

C. *gronovii* growth is enhanced on cyp79B2 cyp79B3 mutants and inhibited on atr1D mutants relative to wildtype controls

In the course of our experiments we noticed that dodder vines appeared to grow at different rates on the different host lines. To verify this effect, we infested plants of each Arabidopsis genotype with *C. gronovii* seedlings and recorded the parasite length after a period of 11 days. There was a significant effect of Arabidopsis type on vine length (Ranked ANOVA: $F_{(2, 64)} = 42.76, P = 1.6e-12$): –GLS vines were longer than WT vines, and WT vines were longer than *atr1D* vines (Figure 8).

Since the –GLS quadruple mutant is deficient in aliphatic glucosinolates as well as indole glucosinolates, it was not clear whether aliphatic glucosinolates might be contributing to the inhibition of parasite growth; although a role for indole glucosinolates (or camalexin or auxin) was implied by the difference between the *atr1D* and wildtype treatments. To clarify this point, we established vines on each of the double mutants that were previously crossed to create the –GLS quadruple mutant: *cyp79B2 cyp79B3*, which lacks indole glucosinolates, and *myb28 myb29*, which lacks aliphatic glucosinolates. Vines were established on wildtype and *atr1D* plants for reference. Again, we observed a significant effect of Arabidopsis genotype on vine length after 11 days of growth.
(ANOVA: $F_{(3,48)} = 4.92$, $P = 0.0046$). This effect was driven solely by $cyp79B2$ $cyp79B3$ plants, which supported vines that were significantly longer than vines in each of the other treatment groups (Tukey HSD: $P < 0.05$ for each contrast with $cyp79B2$ $cyp79B3$). Vines on $myb28$ $myb29$ did not appreciably differ in length from those on wildtype plants (Figure 8), which suggests that indole glucosinolates or related metabolites but not aliphatic glucosinolates play a role in inhibiting parasite growth.

**Discussion**

Prior to this study it was unknown if parasitic plants, like insect herbivores, are capable of exploiting the glucosinolate metabolites they encounter in crucifer host plants. However, it has been noted that dodder (C. campestris) grown on turnip is less palatable for several aphid species than dodder grown on a range of other host plants (Harvey, 1966), which could result from the transfer of a turnip-specific toxin. One aphid species in this report survived unusually well on turnip-hosted dodder: *Brevicoryne brassicae*. Also known as cabbage aphids, *B. brassicae* are specialists of crucifers and, unlike most aphid species, respond positively to glucosinolates (Cole, 1997). Prior work has shown that *B. brassicae* can be stimulated to feed on non-host plants (e.g., fava bean leaves) or sucrose solutions by infusing them with glucosinolates (Gabrys and Tjallingii, 2002; Klingauf et al., 1972; Moon, 1967; Nault and Styer, 1972; Wensler, 1962). Therefore glucosinolate transfer is a likely explanation for the improved survival of *B. brassicae* and reduced survival of other aphid species on dodder on turnip in Harvey’s study. Our discovery that glucosinolates move readily from Arabidopsis into dodder parasites and alters aphid performance supports this possibility.

Similar to Harvey’s work (Harvey, 1966) we found that host plant effects on parasite-aphid interactions can vary as a function of the aphid species. Whereas *M. persicae* aphids were not deterred by the presence of glucosinolates in dodder vines, *A. pisum* aphids in our study were so averse to feeding on vines with glucosinolates that they perished markedly faster than pea aphids that had access to glucosinolate-free vines (Figure 4). This is consistent with other studies that noted deterrent effects of
glucosinolates on *A. pisum* aphids feeding on sucrose solutions or even on leaves of *Vicia fava*, a preferred host plant (Gabrys and Tjallingii, 2002; Klingauf et al., 1972; Nault and Styer, 1972).

The potent effect of host-derived secondary metabolites against *A. pisum* feeding on dodder represents a new observation of a parasitic plant deriving resistance against an insect herbivore by virtue of toxins they acquire from host plants. This type of host-parasitic plant-herbivore interaction has seldom been observed (Adler et al., 2001; Lehtonen et al., 2005; Stermitz, 1998) despite many known examples of host secondary metabolites occurring in parasite tissues (e.g., Boonsong and Wright, 1961; Rothe et al., 1999; Srimathi and Sreenivasaya, 1963; Stermitz et al., 1993; Subraman and Nair, 1966). Our findings suggest that parasitic plants may derive benefit from imbibing other (non-alkaloid) classes of host secondary metabolites from their hosts as well.

Although glucosinolates are likely responsible for deterring *A. pisum* aphids on dodder, we can not exclude the possibility that other Arabidopsis metabolites that are reduced or absent in *cyp79B2 cyp79B3 myb28 myb29* plants contribute to the deterrent effect associated with dodder on wildtype or *atr1D* host plants. An untargeted LC-MS screening of metabolites in wildtype and *myb28 myb29* double mutants found remarkably high metabolome fidelity between these genotypes with the exception of glucosinolate compounds and four additional unknown compounds (Beekwilder et al., 2008). On the other hand *atr1D* and *cyp79B2 cyp79B3* genotypes, which respectively produce elevated and diminished levels of indole glucosinolates, are altered in their production of camalexin and IAA-auxin, two metabolites important in defense and development. These compounds share a biosynthetic precursor (indole-3-acetaldoxime) with indole glucosinolates that is produced by the enzymatic action of CYP79B2 and CYP79B3, which are impaired in *cyp79B2 cyp79B3* plants and elevated in *atr1D* plants (Celenza et al., 2005; Zhao et al., 2002). Thus camalexin and auxin are positively correlated with indole glucosinolates in our treatments. This raises the question of whether host-derived auxin or camalexin could alter aphid performance on dodder.
Auxin is a phloem-mobile phytohormone that can translocate from host plants into both dodder and aphids (Birschwilks et al., 2006; Maxwell and Painter, 1962). It is likely therefore that aphids encounter Arabidopsis-derived auxin when feeding on dodder. Aphids sequester and excrete auxin in their honeydew (Maxwell and Painter, 1962), but whether auxin can have direct deleterious or beneficial effects on aphids appears to be a matter for further research. We are unaware of any reports showing direct antibiotic or antixenotic effects of auxin on aphids that could lead to the observed pattern of A. pisum aphids preferring dodder on –GLS plants to dodder on wildtype plants. The possibility of indirect effects of auxin on aphids via changes in dodder is addressed below.

Camalexin is a well-studied phytoalexin that contributes to plant defense against fungal and oomycete pathogens (Ferrari et al., 2007; Glazebrook et al., 1997; Rogers et al., 1996; Schlaeppi et al., 2010; Wang et al., 2013). Camalexin is also toxic to M. persicae aphids when added to artificial diet (Kettles et al., 2013). Two studies exploring this effect in vivo, using camalexin-free pad3 Arabidopsis (which is impaired in the enzyme CYP71B15, a cytochrome P450 monoxygenase responsible for the final biosynthetic step in the production of camalexin (Glazebrook and Ausubel, 1994; Schuhegger et al., 2006; Zhou et al., 1999)), found contrasting results. In the first of these, groups of 15 adult aphids confined for two days on pad3 Arabidopsis produced similar numbers of offspring as groups confined to wildtype plants (Pegadaraju et al., 2005). In the second study, nymphs born and reared on pad3 Arabidopsis for a period of 14 days produced more offspring than those on wildtype plants (Kettles et al., 2013). It has been noted that differences in these results may be due to the use of different aphid strains or the amount of time spent on host plants (2 versus 14 days) (Kettles et al., 2013; Louis and Shah, 2013); additionally, it may imply that M. persicae nymphs are more sensitive to camalexin than adults.

The effects of camalexin on A. pisum have not been studied, but it is conceivable that camalexin could transfer into dodder and contribute to the antifeedant effect we observed against A. pisum aphids. It would be worthwhile to assay camalexin levels in dodder on Arabidopsis to see if translocation occurs. If it does, a follow-up study with
pad3 and wildtype Arabidopsis could be used to test whether host-derived camalexin reduces the palatability of dodder for \textit{A. pisum}.

If camalexin does in fact transfer into dodder, this would only heighten the curiosity of \textit{M. persicae} aphids showing no adverse response to dodder on high camalexin/glucosinolate genotypes (Figures 2-6). In nature \textit{M. persicae} feed on plants that produce camalexin and glucosinolates—hence it is not surprising that they are less averse to Arabidopsis factors in dodder than are \textit{A. pisum} aphids, which do not utilize such plants in nature. Yet as noted, \textit{M. persicae} are sensitive to camalexin (Kettles et al., 2013) and to some glucosinolate breakdown products (Kim and Jander, 2007), which leads to the prediction that \textit{M. persicae} should, like \textit{A. pisum}, prefer dodder on –GLS hosts to dodder on wildtyp hosts. But this is not the case; moreover, \textit{M. persicae} individuals caged to dodder on –GLS hosts reproduced at a slower rate than aphids caged to dodder on wildtype and \textit{atr1D} hosts (Figure 5).

Other studies have similarly failed to detect effects of secondary metabolite transfer on parasite-herbivore interactions (Marvier, 1996; e.g., Stermitz et al., 1989). Marvier (1996) proposed that adverse effects of host secondary metabolites could be masked by nutrition-driven effects, since parasite nitrogen content was the best predictor of aphid performance on root parasites across multiple host species in her study. By analogy it is possible that the nutritional quality of dodder vines varies according to host genotype leading to the observed effects on \textit{M. persicae} performance. We noticed that the –GLS quadruple mutants exhibited more rapid onset of rosette senescence than wildtype and \textit{atr1D} during the aphid population assays. Senescence in flowering Arabidopsis serves the purpose of reallocating resources to reproductive tissues and is associated with changes in amino acid and sugar levels (Diaz et al., 2005) which could conceivably lead to changes in the nutritional quality of associated parasites for aphids.

The ideal approach to testing this hypothesis would be to measure metabolite levels in dodder phloem extracts obtained by aphid stylectomy or ETDA extraction (Dinant et al., 2010; King and Zeevaart, 1974). Stylectomy is a notoriously challenging technique beyond the scope of this study, and EDTA extracts would be suspect of contamination from pressurized laticifers in dodder (Costea and Tardif, 2006; Lyshede,
(although gelling mechanisms of latex glands are not well understood (Pickard, 2008)); therefore we elected to analyze the free amino acids and sugars from total tissue samples. Our results (summarized in Figure 6 and Table 1) indicate that relative nitrogen deficiency is not a characteristic of dodder on –GLS hosts, since these hosts and their parasites have equal or greater amounts of most amino acids as dodder on the other hosts. Unfortunately methionine and cysteine, two phagostimulants for *M. persicae* (Mittler, 1970) were not detected in either host or parasite samples, perhaps due to oxidation of these amino acids in storage or during the analysis. Sucrose, arguably the most important sugar phagostimulant for *M. persicae* (Mittler, 1967a; Mittler et al., 1970), was present in similar levels in each dodder treatment. Although this analysis serves as only a rough indicator of phloem nutrition, it nevertheless suggests that –GLS hosts and dodder may be more nutritious for aphids than the other host/dodder combinations, and thus fails to support the idea that nutritional inadequacies of dodder on –GLS hosts underlie the relatively poor performance of *M. persicae* aphids on this treatment.

Since nutritional effects do not appear to be masking the adverse effects of glucosinolates, it is important to consider whether glucosinolates are capable of adversely affecting *M. persicae* aphids in the novel chemical context of dodder. In general, the scientific literature contains mixed findings concerning the effects of glucosinolates on *M. persicae* aphids. Multiple studies of *M. persicae* gustatory responses to glucosinolates (sometimes only sinigrin) in artificial diets or in plants found that *M. persicae* feeding is either not affected by glucosinolates (Hodgson, 1978; Weber et al., 1986) or is stimulated by them (Klingauf et al., 1972; Nault and Styer, 1972). A more recent study found that *M. persicae* feed less over time when sinigrin is added to artificial diet, but this effect is slight, and interestingly, aphids produced a higher proportion of alate offspring on the sinigrin-added diet than on the control diet (Junde and Lidao, 2011), which could indicate diet-acceptance. A comparative study of *M. persicae* performance across several *Brassica* species found a negative correlation with 2-butenyl glucosinolate but a positive correlation with 3-indoymethyl glucosinolate (Cole, 1997), although 3-indolymethyl glucosinolate can limit aphid reproduction in artificial diet (Kim and Jander, 2007). Thus it is possible that some glucosinolate species (especially sinigrin) are neutral or even favorable to *M. persicae* feeding and/or performance depending upon the context. It is
also apparent in the literature that glucosinolate-related defenses can effectively limit \textit{M. persicae} success: two studies employing Arabidopsis mutants altered in their defensive chemistry found a negative correlation between glucosinolate levels and aphid performance (Kim and Jander, 2007; Mewis et al., 2005). Indole glucosinolates in particular mediate antibiosis toward \textit{M. persicae} by breaking down in aphid guts (in the absence of known myrosinase enzymes) and forming toxic adducts with ascorbigen and cysteine (Kim et al., 2008; Kim and Jander, 2007). In light of these results it is only somewhat surprising that our performance assays failed to detect adverse effects of translocated indole glucosinolates on \textit{M. persicae}. Differences in indole glucosinolate levels and aphid performance between \textit{atr1D} and wildtype plants can vary by plant age—effects on aphids were previously seen in rosette-stage but not flowering-stage plants (Kim and Jander, 2007)—and this might explain why aphids did not discriminate between dodder on flowering \textit{atr1D} and wildtype plants. Yet it is harder to explain why \textit{M. persicae} did not discriminate between dodder on flowering –GLS and wildtype hosts. It is possible that indole glucosinolates failed to form toxic metabolites due to stabilizing factors or missing adducts in the novel chemical context of dodder phloem. It is also possible that translocated aliphatic glucosinolates enhance \textit{M. persicae} acceptance of dodder as a food source, resulting in positive effects on aphid performance that mask negative effects of indole glucosinolates.

Another more probable explanation is that \textit{M. persicae} performance could be affected by an interaction between the parasite and the host genotype that renders dodder on –GLS hosts more resistant to aphids. It appears from the second \textit{M. persicae} population assay, in which aphid performance was evaluated on dodder as well as on Arabidopsis simultaneously, that an interaction of this sort is occurring (Figure 7). In general aphids on Arabidopsis outperformed aphids on dodder. In light of this is it interesting to consider whether the six-fold increase in glucose and sucrose levels in dodder relative to Arabidopsis contributes to this performance gap since \textit{M. persicae} performance is reduced on sucrose solutions that exceed optimum levels (Mittler, 1967a). This would need to be tested in another study since our methods did not allow for absolute quantification of phloem sugar.
The most striking outcome of this experiment is that aphid performance was adversely affected by the –GLS genotype only when aphids were confined to dodder, but not when they were confined to Arabidopsis (Figure 7). We examined our metabolite data for corresponding interactions to identify compounds that might help explain this phenomenon. Only three metabolites had significant plant by genotype interactions (Table 2), and of these only leucine and glutamic acid exhibited contrasting trends in –GLS Cuscuta versus –GLS Arabidopsis (both were uniquely elevated in –GLS dodder). Leucine can stimulate M. persicae feeding when added to sucrose solutions (Mittler, 1967b), so it is not likely to explain the reduction in M. persicae performance on –GLS dodder. In contrast, glutamic acid serves as an antifeedant to M. persicae in the context of sucrose solutions (Mittler, 1967b), and it is also negatively associated with B. brassicae rate of increase across a wide range of brassicas (Cole, 1997). High glutamic acid levels are also implicated in oat and barley resistance to the aphid Rhopalosiphum padi (Weibull, 1988). Thus glutamic acid may contribute to the poor performance of M. persicae on –GLS dodder vines.

In light of these effects on aphid performance it is interesting to note that dodder growth was significantly affected by the Arabidopsis genotype. When measured 11 days after host infestation, vines were longest on –GLS hosts, intermediate on wildtype hosts and shortest on atr1D hosts (Figure 8). In a follow-up assay involving the two double mutants that were crossed to form the –GLS genotype, cyp79B2 cyp79B3 had an effect on vine length but myb28 myb29 did not. Although we did not measure parasite biomass (because plants were needed for insect assays), length can be regarded as a conservative proxy for parasite biomass of the parasite because longer vines were generally thicker than shorter vines. This suggests that IAA-auxin, camalexin and/or indole glucosinolates but not aliphatic glucosinolates may play a role in the defense of Arabidopsis against dodder parasites.

It is unknown whether auxin mediates dodder-host interactions, although this has been observed for the root parasite Triphysaria, which may utilize host auxin to induce xylem formation in its developing haustoria (Goldwasser et al., 2002). Dodder almost certainly encounters host auxin since it can imbibe auxin that is applied exogenously to
Moreover auxin can mediate developmental processes in dodder such as apical tip dominance (Maheshwari and Sreekrishna, 1982). It also can modulate the initiation of haustoria: when applied exogenously to dodder seedlings *in vitro*, IAA can inhibit the formation of pre-haustorial outgrowths that are otherwise induced by exogenous zeatin or far-red light, although the mechanism of this interaction is not clear (Haidar et al., 1998). This auxin-sensitive developmental switch occurs before parasite seedlings would encounter host IAA, so it does not imply that host auxin can inhibit haustoria formation in the parasite. During attachment, there is a transient flux of auxin at the parasite-host interface that appears to come from dodder and alters cell properties in the hosts (Löffler et al., 1999); but effects on host cells do not appear to be critical for attachment since dodders attach efficiently to auxin-insensitive Arabidopsis mutants (Birschwilks et al., 2007). Since no important auxin-mediated dodder-host interactions have been identified, there is little basis to speculate on the relationship between the relatively high auxin levels in wildtype and *art1D* Arabidopsis and the reduction in dodder growth. This poses an area for further research.

Camalexin, another candidate for inhibiting dodder, is toxic to bacteria, fungi and even Arabidopsis plant cells cultured *in vitro* (Rogers et al., 1996) but has not been studied in regard to parasitic plant attack. It also inhibits fungal enzymes that detoxify certain phytoalexins (Pedras and Minic, 2014). It should be possible to verify whether camalexin also affects dodder attachment and growth using *pad3* Arabidopsis mutants.

Indole glucosinolates are also interesting candidates because they mediate defenses against herbivores and pathogens (discussed above) although a role against parasitic plants has not been previously identified. If camalexin is not found to contribute to parasite defense, the role of indole glucosinolates in dodder resistance could be further tested with the mutant *myb51*, which has decreased indole glucosinolates but elevated camalexin (Gigolashvili et al., 2007; Schlaeppi et al., 2010). A complimentary approach with less risk of altering auxin and camalexin would be to manipulate glucosinolate levels by varying sulfur nutrition for host plants (Falk et al., 2007).
In addition to the mechanism of Arabidopsis resistance to dodder, it is also not clear why dodder on –GLS genotype is more resistant to aphids. It is possible that, in addition to potential explanations discussed above (involving glucosinolates and glutamic acid), vigorous parasite vines on –GLS hosts have stronger endogenous defenses against aphids than vines on wildtype and atr1D hosts. Dodder defenses against insects are not characterized, but it hast been observed that relatively few insects feed on dodder in nature (Costea and Tardif, 2006). Dodder’s clear, pressurized latex might play a role in deterring herbivores but this has not been verified experimentally (Riviere et al., 2013). Dodders are also rich in phenolic compounds that constitute potential agents of antibiosis or antixenosis against insects (Loffler et al., 1997; 1995). The contrast in dodder’s ability to resist \textit{M. persicae} across Arabidopsis genotypes employed in this study presents an ideal opportunity to characterize dodder’s endogenous defenses using a comparative approach.

\textbf{Conclusions}

The ecological consequences of secondary metabolite transfer from hosts into parasitic plants is a largely overlooked area of research (Smith et al., 2013). We have presented new evidence that allelochemicals from hosts can confer defensive benefits to parasitic plants. But our findings indicate that host secondary metabolites do not always benefit parasitic plants. Defense enhancement by metabolite translocation can vary widely as a function of herbivore species, which we noted in the different responses of \textit{A. pisum} and \textit{M. persicae} to glucosinolates in dodder. Moreover, host allelochemicals may pose direct threats to parasitic plants themselves. Enhanced dodder growth on \textit{cyp79B2 cyp79B3} raises the intriguing possibility that well-characterized Arabidopsis metabolites that function in defense against herbivores and pathogens might also play a role in resistance to parasitic plants.
METHODS

Cuscuta gronovii seeds and growing conditions

*Cuscuta gronovii* seeds were collected from a wild population along Spruce Creek, in Huntingdon County, PA (40°37'17" N, 78°07'25" W). To initiate germination, *C. gronovii* seeds were soaked in concentrated sulfuric acid for one hour (to scarify the hard seed coat), rinsed in running de-ionized water for two minutes, cleaned on paper towels, and then sprinkled onto moistened filter paper in 9 cm petri dishes at a density of about 50 seeds per dish. Dishes were covered, sealed with Parafilm and incubated in a growth chamber (25°C) for five days. Seedlings that shed their seed coats were placed at the base of flowering Arabidopsis plants (radicles were slightly submerged in the soil to anchor the seedlings). To facilitate attachment, florescent growth chamber light was enriched in far-red wavelengths by adding approximately 180 Watts of incandescent light per flat of host plants and, in some experiments, by reducing florescent light to one third of original strength during the three day period of attachment. After this period, light conditions were returned to their original settings so that parasites did not make subsequent attachments.

Arabidopsis seeds and growing conditions

Seeds for Arabidopsis lines were kindly supplied by Dr. Georg Jander (Boyce Thompson Institute). Seeds were vernalized on moistened potting media (Pro-Mix BX, Premier Horticulture Inc.) for two or three days in the dark at 4°C, and afterward germinated in an insect-free walk-in growth chambers set to 50% relative humidity with a 16-hr photoperiod and a 23°/21° light/dark temperature cycle. At the two-leaf stage (about 10 days after vernalization) seedlings were transplanted into plastic pots (200mL) with approximately 35 g potting media mixed with 1g 14-14-14 Osmocote and 0.3g Micromax Micronutrients (Scotts, Griffin Greenhouse Supplies, Morgantown, PA). Light was provided from a rack of cool white florescent light fixtures and modified as described above during attachment of the parasites.
**Insects**

*M. persicae* aphids were obtained from Dr. Georg Jander (Boyce Thompson Institute) and reared in a lab colony on pepper (*Capsicum anuum*) plants. *A. pisum* aphids were field-collected in 2013 (Centre County, PA) and maintained in a lab colony on fava bean (*Vicia fava*) plants.

**Aphid settling and survival assay (*M. persicae* and *A. pisum*)**

To observe the effect of glucosinolates on dodder-aphid interactions, groups of *M. persicae* and *A. pisum* were allowed to choose between 8 cm-long apices of *C. gronovii* vines grown on Arabidopsis plants that differed in their glucosinolate production. There were two choice tests conducted in parallel: WT versus –GLS and WT versus *atr1D*. Numbers of aphids on each vine were counted after one and 24 hours. Aphid survival was also recorded after three days. Foraging arenas consisted of 9 cm petri dish bases inverted on top of filter paper disks supported by up-side-down petri lids (which provide a better seal than right-side up lids). Twelve days after infesting Arabidopsis hosts, parasite vine tips were placed into the arena through a 1x1.5cm U-shaped hole that we earlier cut into the side of each petri dish using a hot metal file. At the point of entry, vines were wrapped in cotton to prevent aphid escape. Aphids were collected from fava bean plants (*A. pisum*, 20 individuals, instars 2-4) and from wilting pepper leaves (*M. persicae*, 30 individuals, mixed ages) and held temporarily in 0.2 mL polypropylene tubes on ice. *A. pisum* aphids were poured into the foraging arenas, whereas *M. persicae* were allowed to walk out of uncapped tubes directly on to the vines (because they have less mobility than *A. pisum* aphids). The two aphid species and two glucosinolate choice experiments produced four combinations that were nearly equally represented among 38 replicates split between two consecutive weeks.

Aphid distributions at the one and 24-hour time points were analyzed by two-way analysis of variance (ANOVA) with two factors, Arabidopsis (with three levels: WT, -
GLS, *atr1D*) and aphid species (with two levels: *A. pisum* and *M. persicae*), as well as a blocking factor for the experiment week. Separate ANOVAs were conducted for each choice test. The survival of the aphids was also analyzed by factorial ANOVA with two factors: aphid species and choice set.

**M. persicae aphid population growth assays**

A population assay was conducted to test the effects of glucosinolates on the development of *M. persicae* aphids confined to *C. gronovii* grown on WT, -GLS and *atr1D* Arabidopsis. Age-standardized third-instar aphids raised on pepper plants were caged individually to vines. Cages for the first experiment (Figure 5) were constructed from the translucent plastic bulbs of disposable transfer pipets (with foam seals to prevent aphid escape). Cages for the second experiment (Figure 7) were constructed from petri dishes with holes cut in their sides to allow vertically-oriented vines or flower stalks to enter and exit. Bases were secured to lids with revered orientations to create an aphid-proof seal. A 1-inch circular hole was cut through the enter of the lid and covered with breathable 3M micropore tape to enhance air diffusion for the cages. Aphid escape as prevented by cotton wrappings. Cages were positioned within 10 cm of the point of connection between parasite. Aphids were allowed to grow and reproduce for a period of 9 days (experiment 1) or 8 days (experiment 2), at which time they were frozen and counted. Host and parasite tissue were also sampled for chemical analysis.

*Amino acid / sugar analysis (for plants in M. persicae population assay)*

Tissue samples were stored at -80°C temporarily until they could be freeze-dried, weighed and subsequently stored at -20°C. Sugars and amino acids were prepared for quantification by gas chromatography by a method described by Lisec et al (2006) in which polar metabolites were extracted from finely ground samples through the sequential addition of methanol, chloroform and water (in a 2:1:2 ratio) and derivatized with methoxyamine hydrochloride (to protect ketones and aldehydes) followed by N-
methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) (to silyate organic acids, amines and alcohols). The total extraction volume was adjusted according to sample mass to maintain a volume to sample mass ratio of 140mL:1±0.3mg. Ribitol (12 mg in 0.2mg/mL aqueous solution) was added at the beginning of the extraction to control for mass loss during transfers and for use in relative quantification.

Compounds were separated on an Agilent 6890 gas chromatograph (GC) fitted with a DB-5MS column (60m length, 0.25mm internal diameter, 0.25µm film). Inlet temperature was set to 230°C. Run temperature was held at 70°C for five minutes, ramped at 5°/minute to 315°C, and held again for five minutes. Eluting compounds were transferred to an Agilent 5973 Network Mass Selective Detector (MS) through a transfer line set to 250°C. The MS was run in electron ionization mode at 200°C and recorded fragments ranging from 50-600 m/z (mass-to-charge ratio) at a rate of two scans per second. Solvent delay was set to 12.5 minutes. GC and MS programs were controlled with Chemstation (2003) software.

Metabolite identification was facilitated by comparison of compound mass spectra to entries in a reference libraries (National Institute of Standards and Technology 2008) and by comparing retention times to chemical standards (Amino acids standards 1/2/3 from Phenomenex and from Sigma Aldrich [#A9906]; and glucose, sucrose and fructose standards from Sigma Aldrich) that were derivatized as above. Asparagine and glutamine represent tentative identifications because they were not detected in the standard runs. Compound structures, retention times and CAS numbers are reported in Table 3. Relative quantification of each compound was achieved by dividing the area of the most intense diagnostic ion peak by the area of the 217 m/z peak from the internal standard ribitol using a deconvolution and quantification method developed in MassHunter software (Agilent, 2013). Results were also normalize by dry mass. For compounds with two derivatized forms (e.g., valine-TMS and valine-2(TMS)) the areas from both peaks were combined to produce the values presented in Table 1.
Glucosinolate analysis

Glucosinolates were quantified as desulfoglucosinolates with an HPLC-DAD after extracting finely ground samples in 80% MeOH, filtering extracts with an anion exchange resin (DEAE Sephadex A-25 (Amersham, Buckinghamshire, UK)) and enzymatically eluting desulfo-glucosinolates with aryl sulfatase (Sigma, St. Louis, MO). These analyses were conducted in accordance to the methods described in Kim et al. (2004) with the kind assistance of Dr. Georg Jander (Boyce Thompson Institute).
Figure 1. Dodder (*Cuscuta gronovii*) imbibes glucosinolates from wildtype Arabidopsis in levels that are equal to or greater than their levels in host plant tissue. Plots show relative concentration ± standard error of eight major glucosinolates in infested Arabidopsis flower stalks and their parasites at base (adjacent to connection) and at tip (apical section of 30cm vine). Abbreviations: 3MSP = 3-methylsulfinylpropyl-GLS; 4MSB = 4-methylsulfinylbutyl-GLS; 5MSP = 5-methylsulfinylpropyl-GLS; 4MTB = 4-methylthiobutyl-GLS; 8MSO = 8-methylsulfinyloctyl-GLS; I3M = indol-3-ylmethyl-GLS; 4MI3M = 4-methoxyindol-3-ylmethyl-GLS; 1MI3M = 1-methoxyindol-3-ylmethyl-GLS
Figure 2. *A. pismum* aphids discriminate between dodder vines grown on low and wildtype glucosinolate Arabidopsis hosts, but *M. persicae* aphids do not. Aphids were confined in groups of 20 (*A. pismum*) or 30 (*M. persicae*) individuals to growing tips of parasite vines grown on Arabidopsis hosts that vary in their production of glucosinolates. **A.** Distribution of aphids after one and 24 hours. **B.** Numbers of aphids settling on vines after one and 24 hours. Treatment effects were significant for *A. pismum* but not *M. persicae* at an $\alpha = 0.05$ confidence level as tested by Paired T-tests (for normal data) and Wilcoxon Signed Rank tests (for non-normal data) ($N = 9$ or 10 per set).
Figure 3. Aphids (*A. pisum* and *M. persicae*) do not choose between dodder vines grown on wildtype and high glucosinolate (*atr1D*) Arabidopsis lines. **A.** Distribution of aphids after one and 24 hours. **B.** Numbers of aphids settling on vines after one and 24 hours. Paired T-tests confirmed that treatment effects were not significant at $\alpha = 0.05$ confidence level ($N=9$ or 10 per combination).
Figure 4. Differential effects of host-derived glucosinolates on survival of *M. persicae* and *A. pisum* on dodder. Following the choice tests described in Figure 2, *A. pisum* survival was contingent upon the presence of glucosinolate-free vines. This effect was not present for *M. persicae* aphids. The interaction effect of aphid species by host type was significant (Two-way factorial ranked ANOVA $F_{(1,34)} = 38.99$, $P = 4.15E-07$). Arabidopsis treatment effects within each aphid species were also tested with ad hoc Tukey HSD Tests that showed a significant treatment effect on *A. pisum* ($P < 0.0000$) but not on *M. persicae* ($P = 0.9908$).
Figure 5. *M. persicae* aphids produce fewer offspring on dodder on –GLS Arabidopsis than on dodder on *atr1D* and wildtype Arabidopsis. Columns represent aphid colony sizes after nine days of feeding on vines. Colonies were started by caging 3rd instar aphids individually to dodder vines near to the point of attachment. Means from 11-13 replicates of each treatment are shown. Lowercase letters indicate statistical groupings (Tukey HSD Comparisons, $\alpha = 0.05$).
Figure 6. Total amino acids and sugars in Arabidopsis hosts and Cuscuta parasites by host genotype. Parasite vines and host flower stalks were sampled immediately after harvesting aphids (Figure 5). Metabolites were relatively quantified by GC-MS (Lisec et al, 2006). Individual sugar and amino acid concentrations are presented in Table 1.
A significant interaction between Arabidopsis lines and C. gronovii underlies the poor performance of M. persicae aphids on –GLS dodder. [A] Aphid population size after eight days of growth on dodder or non-infested Arabidopsis crossed with three Arabidopsis lines. Means from 13-17 replicates of each group are shown. Each colony was founded from a single adult aphid confined to either an Arabidopsis inflorescence or a dodder vine. Matching lowercase letters above bars indicate groups that were not significantly different (Tukey HSD $P < 0.05$). [B] Interaction plots showing the interaction between Arabidopsis type and aphid feeding location. The Arabidopsis*location interaction was significant (ANOVA: $F_{(2,81)} = 6.265$, $P = 0.00295$).
Figure 8. Dodder growth is enhanced on –GLS mutants and –IG mutants but inhibited atr1D mutants. [A, B] Dodder vine lengths measured eleven days after the initial coiling of parasites around Arabidopsis flower stalks. Matching lowercase letters above columns indicate group means that are not significantly different (Tukey HSD $P \geq 0.05$). Numbers of replicates in A (left to right) are 18, 12, 33; and in B, 11, 11, 10, 26.

Genotype Key:
- AG = myb28 myb29 (deficient in aliphatic glucosinolates);
- IG = cyp79B2 cyp79B3 (deficient in indole glucosinolates);
- GLS = cyp79B2 cyp79B3 myb28 myb29 (deficient in indole and aliphatic glucosinolates).
Table 1. Polar metabolites in Arabidopsis and Cuscuta gronovii.

Table presents relative concentrations (mean ± standard deviation of 8 replicates per group) as well as results from factorial ANOVA and post hoc Tukey HSD tests for each compound. ANOVA factors were Plant (two levels: Arabidopsis and Cuscuta) and Arabidopsis (three levels: WT, -GLS and atr1D). Samples were collected from parasites and hosts immediately following the 9-day aphid population experiment reported in Figure 5. Polar metabolites were extracted, derivatized and analyzed by GC-MS.

Significance test categories: ns P ≥ 0.10; ~ P < 0.10; * P < 0.05; ** P < 0.01; *** P < 0.001

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Arabidopsis</th>
<th>Cuscuta</th>
<th>ANOVA F-tests</th>
<th>Tukey HSD Contrasts</th>
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Table 2. Tukey HSD test results for compounds with significant plant*mutant interactions.

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<td>-GLS v atr1D</td>
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<td>~  ns</td>
<td>** ns</td>
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<tr>
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<td>* ns</td>
</tr>
<tr>
<td>leucine</td>
<td>ns  ns</td>
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Significance test categories:

- **P ≥ 0.10**
- * P < 0.10
- ** P < 0.05
- *** P < 0.001
Table 3. Ion list for polar metabolite quantification by GC-MS
Each compound was quantified as the area of its designated quantification ion relative to the area of the ribitol 217.1 m/z ion.

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<th>CAS #</th>
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*Serine TMS assignment is ambiguous. NIST 2008 library describes O,O-TMS but references the CAS # for N,O-TMS serine.
REFERENCES


Gaertner, E.E., 1950. Studies of seed germination, seed identification, and host relationships in dodders, Cuscuta spp. Cornell University Agricultural Experiment Station 294, 56.


CHAPTER 3

EFFECTS OF GLUCOSINOLATES ON OVIPOSITION AND DEVELOPMENT OF THE PLANT BUG *LYGUS RUBROSIGNATUS*

**ABSTRACT**

Brassicaceous plants are defended against herbivores by a well studied class of secondary metabolites known as glucosinolates. When Brassicaceous plants are infested with the parasitic plant dodder (*Cuscuta* spp.), glucosinolates transfer into the parasite and bolster parasite defenses against some aphid species. Another group of herbivores that may be affected by translocated glucosinolates are the *Lygus* bugs (Hemiptera: Miridae). Yet it is not clear in the scientific literature whether glucosinolates actually deter *Lygus* bugs, which feed readily on Brassicas and dodder. Therefore we studied *Lygus* responses (oviposition and nymph development) to wildtype and mutant Arabidopsis lines that vary in their glucosinolate production, including plants impaired in the production of aliphatic glucosinolates (*myb28 myb29*), in the production of indole glucosinolates (*cyp79B2 cyp79B3*), or in the production of both classes of foliar glucosinolates (*cyp79B2 cyp79B3 myb28 myb29*), as well as plants with elevated indole glucosinolate levels (*atr1D*). In choice tests, oviposition was increased on *cyp79B2 cyp79B3* and *cyp79B2 cyp79B3 myb28 myb29* plants but reduced on *atr1D* plants relative to wildtype plants. In no-choice assays Lygus nymphs grew slower on *atr1D* plants than on wildtype controls. Other genotypes did not have statistically significant effects. These results suggest that aliphatic glucosinolates do not alter *Lygus* preference or development, but indole glucosinolates or other biosynthetically related compounds (including camalexin and auxin) do affect plant-*Lygus* interactions.
INTRODUCTION

Glucosinolates are plant defense compounds produced by members of the Capparales (including economically important *Brassica* and *Sinapa* species), that pose resistance to plant antagonists ranging from vertebrate to invertebrate herbivores and from fungal to bacterial pathogens (Halkier and Gershenzon, 2006). The ecological effects of these compounds are well characterized with regard to many insect herbivores, which respond to glucosinolates in divergent ways depending upon the degree to which each species is adapted to consuming Capparales: specialists insects (e.g., Homoptera: *Brevicoryne brassicae*, Lepidoptera: *Pieris rapae*) are stimulated by glucosinolates to oviposit and/or feed, whereas generalists (e.g. *Trichoplusia ni*) are deterred by glucosinolates (Hopkins et al., 2009). Insect sensitivity likewise varies by glucosinolate species, therefore different classes and modifications of glucosinolates are necessary to defend against different insect species (R. Müller et al., 2010).

In addition to their prominent roles in the defense Brassicaceous plants, glucosinolates can also contribute to the protection of some organisms that feed on crucifers. For example, specialist insects such as the aphids *Brevicoryne brassicae* and *Lipaphis erysimis* sequester glucosinolates from Brassica hosts and co-opt them for their own protection against insect predators (Bridges et al., 2002; Kazana et al., 2007), and this defense strategy that has been documented in other brassica specialists as well (Aliabadi et al., 2002; C. Müller et al., 2001; Opitz et al., 2010)). By analogy, it is conceivable that parasitic plants—which, like insects, obtain nutrition by feeding on plants—also might also enhance their defenses by stealing glucosinolates from Brassicaceous host plants (Smith et al., 2013).

Until recently it was unknown whether glucosinolates transfer into parasitic plants. Research reported in this dissertation (Smith, Chapter 2) shows that parasitic plants in the genus *Cuscuta* (also known as dodder) imbibe glucosinolates when they feed upon Brassicaceous hosts (*Arabidopsis thaliana*). The ecological implications of this phenomenon remain largely unexplored, but it is likely to hold significance to insect herbivores that utilize dodders as food sources. Two such herbivores are aphid spp. (Homoptera: Aphidae) and *Lygus* bugs (Hemiptera: Miridae) (Harvey, 1966 personal
observation). We found that some but not all aphids are sensitive to host-derived glucosinolates in dodder: glucosinolates decreased the palatability of *Cuscuta gronovii* for pea aphids (*Acyrthosiphon pisum*) but not for green peach aphids (*Myzus persicae*) (Smith, chapter 2). This contrast underscores the importance of herbivore species identity in determining the ecological significance of glucosinolate transfer into *Cuscuta*. Thus it is difficult to predict the effects of translated host glucosinolates on other herbivores such as *Lygus* bugs.

In general the effects of glucosinolates on *Lygus* bugs are poorly characterized (Otani and Cárcamo, 2011). *Lygus* spp. are generalist herbivores that commonly utilize brassicaceous hosts, including wild species such as London Rocket (*Sisymbrium irio*) as well as cultivated rapeseed and canola oil crops (*Brassica* and *Sinapis* spp.) (Dosdall and Mason, 2010; Gavloski et al., 2011; Jackson, 2003). In such crops periodic outbreak of *Lygus* pests cause economic losses (such as a $9 million CAD loss in Canada in 1998 (Braun et al., 2001)). Consequently there is interested in identifying mechanisms of resistance to *Lygus* in Brassicaceous plants (Otani and Cárcamo, 2011). Yet multiple studies comparing the resistance of high and low seed-glucosinolate cultivars for *Lygus* bug feeding and oviposition failed to identify differences between them (e.g., Butts and Lamb, 1990; Gerber, 1998; 1997). The absence of effects on *Lygus* bugs could indicate a general immunity of the bugs to these defenses; alternatively it could imply that the flowering plants used in these studies did not truly differ in their glucosinolate profiles as was assumed based upon their seed profiles (Mithen, 1992). High and low glucosinolate cultivars of canola and rapeseed do not necessarily differ in the glucosinolate content of their foliage even though they differ in glucosinolate content of seeds and seedlings, and thus flower parts employed in the above studies might have been similar in their defenses against *Lygus* bugs (Fieldsend and Milford, 1994; Inglis et al., 1992; Porter et al., 1991). In contrast, *Lygus lineolaris* presented with a choice between seed pods from high and low glucosinolate *Sinapis alba* cultivars damaged the seeds from the low glucosinolate cultivar more than seeds from the high glucosinolate cultivar (Bodnaryk, 1996). This outcome suggests that sinalbin, the major glucosinolate constituent in *Sinapis* seeds, may have a deterrent effect upon *Lygus* bug feeding. It remains unclear if glucosinolates inhibit nymph development and/or deter female oviposition (Otani and Cárcamo, 2011).
Further research is needed to answer these questions and also to elucidate whether translocated glucosinolates can enhance dodder defense against *Lygus* bugs.

To address these questions we conducted experiments with *Arabidopsis thaliana* Col-0 (hereafter “Arabidopsis”) as a model Brassicaceous host plant for dodder and for *Lygus* bugs. This system availed us of mutant lines that are altered in their production of foliar (as well as seed) glucosinolates, thus presenting an opportunity to test the effects of glucosinolates on *Lygus* behavior and development when presented both within their native plant (i.e., in Arabidopsis) and in the novel context of a parasitic plant (*Cuscuta gronovii*). We selected mutants lines that have previously been used to examine the relative importance of aliphatic and indole glucosinolates in plant-insect interactions (e.g., Beekwilder et al., 2008; De Vos et al., 2008; R. Müller et al., 2010), including *myb28 myb29* mutants, which are impaired in the production of aliphatic glucosinolates (Sønderby et al., 2007), *cyp79B2 cyp79B3* mutants, which are reduced in indole glucosinolates (Zhao et al., 2002), *cyp79B2 cyp79B3 myb28 myb29* quadruple mutants, which are nearly devoid of both glucosinolate classes (Sun et al., 2009), and *atr1D* mutants, which have elevated levels of indole glucosinolates (Celenza et al., 2005). These were used in a series of oviposition choice tests and nymph no-choice development assays to determine the relative contributions of these two classes of glucosinolates in plant defense against *Lygus* bugs. We selected the species *Lygus rubrosignatus* for these experiments because they readily feed on dodder as well as Brassicaceous hosts; and moreover, they are easily reared in the lab (Halloran et al., 2013; Schwartz and Foottit, 1992), which enabled us to test nymph performance as well as adult preference in relation to glucosinolates.

Results from these *in vivo* tests are presented below and suggest that indole glucosinolates may be involved in the deterrence of oviposition by female *Lygus* bugs. They may also reduce the development of nymphs when present in sufficiently high levels. It was less clear whether glucosinolates that transfer into the parasite *C. gronovii* (also known as dodder) enhance the parasite’s resistance to *Lygus* bugs, but we observed a trend for more eggs to be deposited in vines grown on the *cyp79B2 cyp79B3* mutants than on vines hosted by wildtype Arabidopsis, which matches the egg laying pattern
observed for oviposition directly upon Arabidopsis. Since the mutant lines employed in our study differ not only in glucosinolate content but also in nutritional chemistry and defensive chemistry (discussed in Chapter 2), these results should not necessarily be considered conclusive apart from further studies that use complimentary means to manipulate glucosinolate levels such as by altering soil nutrition and/or by artificial diet manipulations. Nevertheless they provide new evidence supporting the idea that glucosinolates contribute to plant defense against Lygus bugs.

**RESULTS**

*Oviposition tests with Arabidopsis lines that vary in glucosinolate content*

In order to assay the importance of indole and aliphatic glucosinolates on Lygus oviposition behavior, we presented Lygus bugs with a choice between inflorescence stalks from Arabidopsis plants that varied in their glucosinolate profiles. Over a period of two days Lygus rubrosignatus bugs laid equal numbers of eggs in wildtype stalks as they did in stalks from myb28 myb29 mutants, indicating that aliphatic glucosinolates do not pose a significant deterrent to ovipositing females (Paired T-test: $T_{(16)} = -0.033$, $P = 0.9741$) (Figure 1). In contrast, they laid significantly more eggs in cyp79B2 cyp79B3 plants than in wildtype plants (T-test: $T_{(22)} = -2.1909$, $P = 0.03935$). Bugs also laid more eggs on the quadruple mutant cyp79B2 cyp79B3 myb28 myb29 than on corresponding wildtype plants (Paired T-test: $T_{(14)} = -2.603$, $P = 0.02086$) (Figure 1).

In a subsequent choice assay between wildtype and atr1D mutants, females laid more eggs in wildtype plants than in atr1D plants (Figure 1), which corroborates with the earlier choice tests by indicating a role for indole glucosinolates in resistance to Lygus bugs (T-test: $T_{(30)} = -2.0536$, $P = 0.04882$).
**Nymph Performance tests on Arabidopsis**

Since females distinguished among the various genotypes for oviposition, we sought to learn whether *Lygus* nymph performance is also affected by Arabidopsis genotype. We confined batches of twenty neonate nymphs to intact flowering Arabidopsis plants representing each of the genotypes employed in the oviposition assays. Nymphs survived equally for a period of six days across all plant types (Figure 3). They also accumulated biomass equally on all genotypes except for *atr1D*, which retarded *Lygus* growth (Ranked ANOVA – Arab: $F_{(2,41)} = 9.4726, P = 0.0004151$; Tukey post-hoc tests: WT v -IG-AG: $P = 0.3299$; WT v +IG: $P < 0.001$; -IG-AG v +IG: $P = 0.0174$) (Figure 4).

**Oviposition tests on C. gronovii**

In previous work we discovered that glucosinolates readily transfer from Arabidopsis into parasitic plants (Chapter 2). Since glucosinolates may explain *Lygus* bug oviposition choices between Arabidopsis genotypes, we hypothesized that glucosinolates may also alter *Lygus* choice among dodder vines with and without glucosinolates. To test this we conducted oviposition choice tests similar to those conducted earlier, this time by caging bugs to dodder vines. Two bugs per cage were given access to two dodder vines with wildtype or mutant hosts. Females did not make statistically significant distinctions between wildtype-hosted vines and vines grown on each of the mutant genotypes (Figure 2). There was a nonsignificant preference for vines grown on *cyp79B2 cyp79B3* Arabidopsis that matched the preference of bugs for this genotype when confined directly to Arabidopsis.

**DISCUSSION**

Glucosinolates are known to function in plant defense against a wide range of insect herbivores, but it has not been clear whether they also deter plant bugs genus *Lygus*, which include multiple major agricultural pest species (Braun et al., 2001; Halkier and Gershenzon, 2006; Otani and Cárcamo, 2011). As mentioned above, the previous studies
on this topic are unclear and only one study has linked glucosinolate content to feeding deterrence, which was noted in the context of high- and low-sinalbin seed pods (Bodnaryk, 1996).

This present paper is the first to address this question by making use of mutant Arabidopsis genotypes that allow for manipulation of foliar glucosinolates (as opposed to seed glucosinolates). Arabidopsis foliar glucosinolates can be grouped into two classes, aliphatic or indole, based upon the side chain group (Brown et al., 2003). When stabilizing glucose moieties are cleaved from glucosinolates (which occurs when tissue damage causes glucosinolates to mix with plant myrosinase enzymes), glucosinolates break down into a range of compounds that vary in their toxicity to insects (Halkier and Gershenzon, 2006). Aglycone aliphatic glucosinolates tend to produce stable isothiocyanates, which are potent repellents to many herbivores. In contrast, isothiocyanates from indole glucosinolate are not stable and quickly decompose to indole-3-carbinol and other compounds with biological activity (Agerbirk et al., 2009). Another difference between these groups is that indole glucosinolates can be harmful to aphids in the absence of myrosinase, which indicates that spontaneous decomposition into toxic metabolites occurs (Agerbirk et al., 2009; Kim et al., 2008). These chemical differences likely underlie the observation that some insect species are sensitive to only one or the other glucosinolate class (R. Müller et al., 2010). In light of these findings we selected genotypes that would allow us to examine the effects of each compound class separately on Lygus bugs.

In our assays we found no evidence that aliphatic glucosinolates affect Lygus bugs. Lygus oviposition distributions and nymph growth rates were not different between the myb28 myb29 mutant and wildtype Arabidopsis. This is not altogether surprising in light of a prior study showing that the aliphatic glucosinolate sinigrin and its corresponding isothiocyanate are only weakly deterrent to Lygus lineolaris relative to a range of other secondary metabolites in vitro (Hatfield et al., 1982). It also raises the question of whether isothiocyanates from aliphatic glucosinolates are produced when Lygus bugs feed on Arabidopsis. Plant bugs use a combination of mechanical and enzymatic maceration to transform host tissue into a slurry that can be ingested through
their straw-like stylets (Cohen, 2000; Wheeler, 2001). Plant defenses against this feeding strategy are less understood than their barriers to chewing or piercing/sucking herbivory (Halloran et al., 2013). It could be informative to quantify isothiocyanate production in response to *Lygus* damage, and also to test bug performance on an Arabidopsis line that lacks foliar glucosinolates (e.g., the double mutant *tgg1 tgg2* (Barth and Jander, 2006)).

Two other insects that do not appear to be harmed by aliphatic glucosinolates include the crucifer specialists *Plutella xylostella* and *Pieris rapa* (Lepidoptera) (R. Müller et al., 2010), both of which have enzymes that alter the breakdown pathway of glucosinolates to produce less toxic metabolites (Ratzka et al., 2002; Wittstock et al., 2004). Likewise *Lygus* bugs could have factors in their saliva that prevent or alter myrosinase-catalyzed degradation of aliphatic glucosinolates.

On the other hand, *Lygus* bugs might be sensitive to indole glucosinolates. Females preferred to oviposit in Arabidopsis genotypes that lacked indole glucosinolates (*cyp79B2 cyp79B3* and *cyp79B2 cyp79B3 myb28 myb29*). They also exhibited marginal discrimination against *atr1D* plants which produce elevated levels of indole glucosinolates. This led us to predict that *Lygus* nymphs should perform better on the genotypes that received more eggs. But this prediction was not altogether correct: nymph performance was not enhanced on *cyp79B2 cyp79B3* or *cyp79B2 cyp79B3 myb28 myb29* Arabidopsis. In accordance with our prediction, however, nymph performance was reduced on the *atr1D* plants relative to wildtype plants, which supports the notion that indole glucosinolates—or a related chemical factor—may play a role in defense against *Lygus* bugs.

It is not clear why nymphs that respond negatively to the *atr1D* genotype do not respond positively to the *cyp79B2 cyp79B3* genotype. It is possible that potential benefits to nymphs of reducing indole glucosinolates are non-apparent because development is already constrained by another plant factor (e.g., growth-limiting nutrients or an unidentified defense trait). This scenario would not preclude the possibility of seeing a reduction in nymph performance when indole glucosinolates are high—which occurred on the *atr1D* mutant. It is noteworthy that in our pilot study nearly all of the nymphs died between the time period of 7 and 13 days—and this effect was consistent across all of the
Arabidopsis genotypes. This implies the existence of a glucosinolate-independent factor that plays a large role in nymph performance on Arabidopsis and theoretically could serve to mask the benefits of reducing indole glucosinolates on nymph performance. Alternatively, there could be an interaction between the atr1D genotype and this factor that results in the poor nymph performance on atr1D plants.

In general, a caveat to using genetic mutants to study phenotypic effects lies in the potential for pleiotropic genotype effects that alter phenotypes other than the phenotype intended for study. As discussed in Chapter 2, this occurs in the mutant genotypes employed in our studies. The atr1D mutants have elevated levels of the phytohormone indole acetic acid (auxin) as well as the antifungal phytoalexin camalexin compared to wildtype plants, whereas cyp79B2 cyp79B3 mutants have reduced levels of these metabolites (Celenza et al., 2005; Zhao et al., 2002). Thus it is possible that camalexin or auxin-mediated changes factored in to preference and performance differences noted in this study. In this respect it is noteworthy that camalexin is toxic to aphids (Kettles et al., 2013) although its effects on Lygus bugs are not known. The myb28 myb29 mutants and wildtype plants exhibit remarkably similar (non-glucosinolate) metabolite profiles to wildtype plants and differ only in the levels of a few unidentified compounds (Beekwilder et al., 2008). In order to resolve the effects of glucosinolate phenotypes from the background of pleiotropic effects, some studies employ in vitro assays with herbivores and glucosinolates (e.g., Kim et al., 2008). Likewise, in vitro studies of the effects of camalexin and indole glucosinolate on Lygus bugs could help considerably in interpreting the data presented in this chapter. Additionally, total glucosinolate levels could be manipulated in vivo by varying sulfur nutrition (although this can lead to asymmetric effects on indole and aliphatic glucosinolates since only the later is constructed from a sulfur-bearing amino acid [methionine]) (Falk et al., 2007).

Whereas most previous studies using mutant Arabidopsis to understanding glucosinolate ecology have made use of rosette-stage (pre-flowering) plants (Beekwilder et al., 2008; Kim et al., 2008), our assays were conducted using flowering-stage Arabidopsis plants. This design was selected because Lygus bugs generally prefer to feed and oviposit on plant inflorescences, flower parts and meristems (Wheeler, 2001;
Williams et al., 2011). In agricultural settings Lygus bugs do not invade mustard crops in significant numbers until the crops begin to flower; subsequently nymphs feed on developing seed pods (Boyd and Lentz, 1999; Braun et al., 2001; Butts and Lamb, 1991). One potential drawback of using plants in the flowering stage for our experiment is the possibility that extra-glucosinolate differences between the various Arabidopsis genotypes employed in our study may be more pronounced in the reproductive stage than they are the rosette stage. It was clear, for example that subtle differences exist in the amount of time required to bolt between wildtype, myb28 myb29, cyp79B2 cyp79B3, and cyp79B2 cyp79B3 myb28 myb29 plants; and atr1D mutants require a full six extra days to begin flowering relative to wildtype plants. We compensated for these differences by adjusting planting dates, but the question stands whether other chemical differences inflated or masked the effects of glucosinolates in our experiments. A stage-related difference was encountered in another study in which aphid performance was reduced on rosette-stage atr1D plants relative to wildtype controls, but not on flowering-stage atr1D plants relative to wildtype controls (Kim et al., 2008). The authors noted that flowering atr1D plants have less elevated indole glucosinolates than rosette-stage plants; but they also proposed that nutritional differences could mask the effects of the glucosinolate treatments.

This later possibility seems plausible in light of the amino acid differences among genotypes that we documented in Chapter 2 (Smith), although those measurements represent plants infested with dodder. Prior in vitro studies have shown that sucrose and some amino acids (especially phenylalanine) are important feeding stimulants for Lygus hesperus and L. lineolaris (Hatfield et al., 1982; Strong and Kruitwagen, 1970), and thus nymph performance could be affected by amino acid and sugar levels. The chemical cues that stimulate Lygus oviposition have not been identified (Conti et al., 2011; Wheeler, 2001). Thus we can not exclude the possibility that nutritional plant factors are contributing to the net genotypic effects we observed on Lygus bug oviposition and development.

Pleiotropy also presents potential complications to interpreting Lygus choice among dodder vines grown on Arabidopsis. We did not see a significant effect of host
genotype on rates of *Lygus* oviposition in dodder. However there was a trend for females to favor dodder vines grown on *cyp79B2 cyp79B3* hosts (T-test: $P = 0.091$), which is consistent with their preference for this genotype when confined directly to Arabidopsis. This could reflect a response to translocated glucosinolates, but we can not yet exclude the possibility that other primary or secondary metabolites that vary by host genotype could lead to differences in the relative attractiveness of dodder vines. We noted in Chapter 2 that dodder vine growth is faster on *cyp79B2 cyp79B3* than on wildtype plants for reasons yet unknown. Differences in overall vine vigor could present an additional source of variation in vine attractiveness to *Lygus* bugs. It might be possible to parse the effects of these different factors in choice tests that use equivalent dodder vines cuttings placed in solutions that differ in their amino acid, glucosinolate or camalexin content.

**Conclusions**

The relationship between glucosinolates and *Lygus* bugs is an enigmatic topic in the scientific literature. Only one previous study observed a deterrent effect of glucosinolates on *Lygus* bugs. Our data are consistent with this finding, but they add the insight that aliphatic glucosinolates are not likely contributing to this effect. Indole glucosinolates are implicated in bug deterrence given the observed responses to *cyp79B2 cyp79B3* and *atr1D* Arabidopsis and to dodder on *cyp79B2 cyp79B3* host plants. Yet the possibility remains that phenotypic traits other than glucosinolate production are responsible for deterring *Lygus* bugs. Further studies are needed to clarify these effects.

**Methods**

*Insect Rearing*

*Lygus rubrosignatus* overlaps in host plant range with the better known tarnished plant bug (*Lygus lineolaris*) but is more amenable to rearing in the lab than the later (Halloran et al., 2013; Schwartz and Foottit, 1992). Both species feed readily on Brassicaceous hosts (Gavloski et al., 2011; Schwartz and Foottit, 1992) as well as on dodder (personal
observation; *Lygus* spp. are some of the few insect groups that feed on dodder in nature (Tóth et al., 2008)); therefore *L. rubrosignatus* were obtained from The Phillip Alampi Beneficial Insect Laboratory (West Trenton, NJ, USA) and reared for the purpose of these experiments in plastic tubs on a laboratory shelf with lighting programmed to a 14:10 hr day/night photoperiod. Bugs were provisioned with a meridian, wheat germ- and lima bean-based diet from Bio-Serve (*Lygus Hesperus* Diet # F9644B) that we distributed in pouches constructed from petri dishes and Parafilm membranes (Pechiney Plastic Packaging, Menasha, WI, USA). Fresh beans and/or bell peppers were occasionally added to nymph cages as supplemental food. For oviposition, adult bugs were supplied with Parafilm packets filled with 2% Carrageenan Gelcarin GP812 (PhytoTechnology Laboratories, Shawnee Mission, KS, USA). Egg packets were removed after about three days and used to start new colonies and/or to supply neonates for experiments.

*Plant cultivation*

Seeds for *Arabidopsis thaliana* Col-0 wildtype and mutants were kindly supplied by Dr. Georg Jander (Boyce Thompson Institute). Prior to planting, seeds were sterilized in 70% ethanol for 5 minutes and then vernalized on moist potting media (Pro-Mix BX, Premier Horticulture Inc.) in the dark at 4°C for two to three days before being transferred to a growth chamber to germinate. To ensure synchronized onset of flowering in plants used for choice tests, *atr1D* seeds were germinated and transplanted six days prior to the wildtype seeds. The other mutant lines initiated flowering at similar times as the wildtype plants, although subtle differences were noted. Natural variation in the flowering dates of the wildtype group allowed for pairing of plants with synchronized flowering for the first choice test experiments. In later experiments, planting dates were adjusted to increase the rate of synchronous flowering between wildtype and mutant lines (two days earlier for *myb28 myb29*; one day earlier for *cyp79B2 cyp79B3 myb28 myb29*; one day later for *cyp79B2 cyp79B* relative to wildtype plants). Growth chamber conditions were set to 50% relative humidity with a light/dark cycle of 16:10 hours and 23/21°C. Seedlings in the two-leaf stage (about 10 days post-vernalization) were transplanted into 200 mL plastic pots with about 35g (dry weight) potting media supplemented with 1g of 14-14-14.
osmocote and 0.3g micronutrients (Scotts, Griffin Greenhouse Supplies, Morgantown, PA). Seedlings were kept under clear plastic humidity domes until one week after transplanting. Light was provided exclusively from cool white fluorescent grow lights except during the three day period of parasite attachment (see below).

*Cuscuta gronovii* seeds were field collected from a site along a nearby stream (Spruce Creek, Huntington County, PA, USA 40°37'17" N, 78°07'25" W) and stored dry at 4°C. To initiate germination, seeds were soaked in concentrated sulfuric acid for 60 minutes, rinsed in flowing de-ionized water for two minutes, cleaned with paper towel and then sprinkled onto moistened 9cm filter paper disks in 9cm petri dishes at a density of about 50 seeds per dish. Dishes were closed, sealed with Parafilm to ensure moisture retention, and incubated at 25-30°C for four days in the dark followed by one day in a 25°C growth chamber (16:8 hr light/dark cycle). In some experiments parasite growth was suspended by cooling dishes to 4°C until host plants were ready to be infested. Seedlings that fully shed their seed coats and reached an approximate length of three centimeters were used to infest host plants. Seedlings were planted at the base of each flowering Arabidopsis where they could easily twine around the primary inflorescence. Each host received one or two seedlings (depending upon the experiment) but only one seedling was allowed to attach to each host plant after the first day. During the initial three days of attachment, host plants were illuminated with light from a combination of incandescent and fluorescent bulbs to ensure that far-red light cues were adequate to promote twining and haustorial development (Haidar et al., 1997).

**Oviposition assays**

Reproductive females (4-5 weeks old) from lab colonies of *Lygus rubrosignatus* were caged to plants with fine-mesh nylon sleeves approximately 20 cm long. Plant stems for oviposition were inserted into the cages from either end and wrapped with cotton at the points of entry. Cages were cinched shut around the cotton with yarn strings to prevent bugs from escaping. To test oviposition preference on Arabidopsis, the tip of each plants’ primary inflorescence was inserted into the cage. This included 12 cm of the tallest
branch as well as a portion of the next-tallest branch from plants that had started to flower about 2.5 weeks prior. Flowering plants were used for this experiment because Lygus bugs prefer to oviposit in reproductive plant tissues (Williams et al., 2011; Wilson and Olson, 1992) and because in agricultural ecosystems Lygus bugs migrate into oilseed rape fields whenever these crops begin to flower (Boyd and Lentz, 1999; Braun et al., 2001; Butts and Lamb, 1991). Plant pairs were selected based upon overall similarity in appearance as well as synchrony in flowering onset. Three female bugs were placed into each cage and allowed to feed and oviposit for a period of two days, after which bugs were removed and plants were placed in cold storage (4°C) until eggs could be counted. Each choice combination was replicated 12 to 17 times. Plants were destructively sampled so that eggs could be counted under a binocular stereo microscope.

Preference tests with Cuscuta vines were performed in the same manner as those with Arabidopsis, only it was necessary to thread apices of Cuscuta vines into the cages from the same direction. Two adult female bugs were placed into each cage for a period of two days.

All experiments were conducted in a walk-in growth chamber (Conviron) with 55-60% relative humidity, a 16:10 light/dark cycle and a 23/21°C temperature cycle.

**Neonate performance assays**

To assay nymph survivorship and development in relation to glucosinolate levels, we caged nymphs to Arabidopsis plants that had been flowering for about two weeks. This developmental stage was selected because Lygus nymph densities in rapeseed oil fields peak as seed pods are maturing (Boyd and Lentz, 1999; Braun et al., 2001; Butts and Lamb, 1991). Cages consisted of fine mesh, nylon, 18-inch sleeves that we secured around pots with rubber bands and that gave nymphs access to Arabidopsis rosettes as well as inflorescences. Cages were supported from the inside with autoclaved hardwood stakes. Neonates were 24 hours old less at the start of each experiment. To collect age-standardized nymphs, egg packs from the *L. rubrosignatus* colonies were cleaned of all bugs and placed overnight in plastic containers provisioned with an artificial diet packets
to feed emerging bugs. Nymphs were transferred into the cages within 24 hours of birth. Groups of twenty nymphs at a time were collected in an aspirator (constructed from laboratory tubing, a piece of nylon mesh and a disposable pipet tip) and deposited into cages with gentle taps on the tip of the aspirator. This method proved to be an efficient but gentle way to transfer fragile neonates. Plants with nymphs were kept in a walk-in growth chamber (Conviron) with a 16:10-hour light/dark cycle at 60% relative humidity and with a 23/21°C temperature cycle. Treatments were systematically intermixed to control for any effects of microclimate variation within the growth chamber. After six days, nymphs—which had reached 3rd and 4th instars—were collected from each plant, frozen, counted, weighed fresh, dried under house vacuum for two days, and weighed dry.

Statistics

Oviposition data were analyzed by one-way ANOVA or equivalent T-tests. ANOVA was used to evaluate the significance of replicate pair as a nested blocking factor (with genotype nested within pair). Where the Pair factor was significant we report Paired T-tests; otherwise two sample T-tests are reported. Data were transformed as needed and met assumptions of normality and equal variances between groups.

Nymph performance data was analyzed by ANOVA (with nymph cohort as a blocking factor for experiments set up over consecutive days) after verifying that data met assumptions of normal distribution and equal variances between groups (data were log-transformed or rank transformed to improve normality where needed).

All statistical tests were conducted using the free software R (version 3.0).
Figure 1. Female *Lygus* bug oviposition in Arabidopsis flower stalks shows preference for genotypes with lower indole glucosinolate levels. Columns represent egg accumulation in Arabidopsis inflorescences over a period of two days by three reproductive female *Lygus rubrosignatus* individuals per cage. Each pair was replicated 12 to 16 times. Asterisks designate statistical comparisons (T-tests) with *P* < 0.05.

Genotype legend:

-AG* myb28 myb29, -IG* cyp79B2 cyp79B3,  
-IG -AG* cyp79B2 cyp79B3 myb28 myb29,  
+IG* atr1D, WT wildtype
Figure 2. Female *Lygus* bugs oviposition in dodder vines does not show discrimination between dodder vines grown on various Arabidopsis genotypes. Columns represent egg deposition in apical segments of dodder vines over a period of two days by two reproductive female *Lygus rubrosignatus* individuals per cage. Each pair was replicated 12 or 13 times.

Genotype legend:
-AG myb28 myb29,
-IG cyp79B2 cyp79B3,
+IG atr1D,
WT wildtype
Figure 3. Survival of *L. rubrosignatus* nymphs on wildtype and mutant lines of *Arabidopsis thaliana* that vary in glucosinolate production. Neonate nymphs were caged to flowering plants in cohorts of 20 individuals. Survival was measured after six days. Growth rates are reported in Figure 4. **Statistics:** Treatment effects were tested for significance by ANOVA found to be unsignificant at the $\alpha = 0.05$ level ([A] $F_{(2,41)} = 0.4968$, $P = 0.6121$); [B] $F_{(2,41)} = 0.7530$, $P = 0.477521$).

Genotype legend:
-AG *myb28 myb29*, -IG *cyp79B2 cyp79B3*,
-IG -AG *cyp79B2 cyp79B3 myb28 myb29*,
+IG *atr1D*, **WT** wildtype
Figure 4. Growth of *L. rubrosignatus* nymphs on wildtype and mutant lines of *Arabidopsis thaliana* that vary in glucosinolate production. Neonate nymphs were caged to flowering plants in cohorts of 20 individuals. Biomass accumulation after six days is summarized here. Survival is reported in Figure 3. **Statistics:** [A] Ranked ANOVA: $F_{(2,41)} = 9.4726, P = 0.0004151$; Tukey post-hoc tests: WT v -IG-AG: $P = 0.3299$; WT v +IG: $P < 0.001$; -IG-AG v +IG: $P = 0.0174$). Different lowercase letters above bars indicate statistically different means ($P < 0.05$). [B] ANOVA $F_{(2,40)} = 1.2773, P = 0.28993$.

**Genotype legend:**
-AG *myb28 myb29*, -IG *cyp79B2 cyp79B3*,
-IG -AG *cyp79B2 cyp79B3 myb28 myb29*,
+IG *atr1D*, WT wildtype
REFERENCES


CHAPTER 4

MECHANISMS OF HOST-LOCATION BY FLAX DODDER (CONVOLVULACEAE: CUSCUTA EPILINUM), A PUTATIVE SPECIALIST PARASITE OF FLAX (LINACEAE: LINUM USITATISSIMUM)

ABSTRACT

Host-finding is a critical task for obligate parasites. Parasitic plants in the genus Cuscuta (also known as dodder) face this challenge under the constraint of limited mobility. Seedlings of small-seeded dodders like Cuscuta campestris and Cuscuta epilinum must encounter hosts within about five or fewer centimeters or else they exhaust their resources and perish. Previous studies have shown that C. campestris seedlings utilize light and volatile cues from host plants to locate suitable hosts, but it is unknown whether C. epilinum or other dodder species respond in a similar fashion to host light and/or volatile cues. C. epilinum, also known as ‘flax dodder’, exhibits greater host specificity than the more cosmopolitan C. campestris, and it has been suggested that C. epilinum seedlings require flax plants as their primary hosts. We hypothesized that flax dodder seedlings would respond preferentially to the volatile and/or light cues associated with flax plants, and tested this hypothesis with a series of choice and no-choice assays. Flax dodder seedlings were significantly attracted to flax plants in the absence of other plant options, however in choice tests with flax and tomato plants of equal size the seedlings grew preferentially toward tomato plants, which are inferior hosts. Tomato and flax plants differ both in their volatile profiles and in their light-spectral profiles. In follow-up assays we were unable to clarify a role for volatiles in seedling choice, however, we found evidence for a dominant role of light cues in the selection of tomato over flax
plants. Dodder seedlings favored tomato-shaped plant models over flax-shaped plant models. The contrasting architectures of these models produced subtle but reliable differences in the ratio of red to far red (R:FR) light wavelengths that were projected on to seedlings—and this difference was also observed between real flax and tomato plants. Thus it appears that flax dodder seedlings respond preferentially to light sources with the strongest reduction in R:FR. R:FR reduction is a function of plant distance and of plant architecture. In a distance choice assay seedlings preferred the nearest model plants. Thus it appears that flax dodder uses R:FR signals to solve the problem of finding the nearest candidate host plant.

INTRODUCTION

Plants forage for resources by responding to environmental cues with growth responses that optimize their access to light, water and nutrients (Hodge, 2009; Sultan, 2010). Parasitic plants, which steal resources directly from other plants (Smith et al., 2013), face the challenge of finding suitable hosts in time and space. Parasites are adept at this task, many of them being capable of selectively infesting preferred hosts among heterogeneous plant communities (Bouwmeester et al., 2007; e.g., Gibson and Watkinson, 1991; Kelly, 1992; Koch et al., 2004). Relatively little is known concerning mechanisms of host location and selection by most parasite species, however, despite the enormous impacts of parasitic weeds on global food production (Aly, 2007; Parker, 2009) and a growing awareness of their importance in natural ecosystems (Fisher et al., 2013; Press and Phoenix, 2005). Much recent work on this topic is focused upon root parasites Striga spp. and Orobanche spp. (Orobanchaceae) which forage for hosts by germinating in response to chemical signals (strigolactones) from nearby host roots (Bouwmeester et al., 2003) (Shen et al., 2006). But parasites vary considerably in their evolutionary and natural histories (Westwood et al., 2010) and other groups are likely to utilize different foraging tactics. For example, stem parasites in the genus Cuscuta (also called “dodder”; Convolvulaceae) do not germinate in response to host cues (Dawson et al., 1994), but are nevertheless faced with the challenge of needing to infest a host plant quickly—within three to five days for some species (Lanini and Kogan, 2005)—or else they die. Recent
studies show that seedlings of *C. campestris* overcome this problem by directing their growth toward nearby host plants (Benvenuti et al., 2005; Runyon et al., 2006). In part this may be explained by a positive growth response of seedlings to light that is reflected or transmitted by chlorophyll-containing leaves (Benvenuti et al., 2005); however experiments by Runyon et al. (2006) demonstrate that seedlings can also grow toward volatile chemical cues from host plants, even in the absence of plant light cues. Remarkably, parasites distinguished volatile cues from good hosts (tomato) and poor hosts (wheat) and grew preferentially toward the odors of the better hosts. When seedlings were presented with individual components of the tomato and wheat volatile blends, they exhibited positive growth responses to two terpenes unique to the tomato blend (β-phellandrene and α-pinene) and to one terpene produced by both tomato and wheat (β-myrcene), but they exhibited a negative growth response to (Z)-3-hexenyl acetate, a compound unique to the wheat blend. These findings suggest that *Cuscuta* parasites are capable of evaluating host plant quality at a distance based upon their volatile cues and of using this information to selectively infest optimal hosts. Moreover they integrate at least two sources of information—light and odor cues—to locate host plants.

These studies pose significant frontiers in our knowledge about the foraging behaviors of parasitic plants. While at least one other *Cuscuta* species responds to far-red light (Orr et al., 1996), it is not known whether other dodder species (yet alone other parasite genera) utilize volatile cues to locate host plants, although all dodder species presumably face strong pressure to locate optimal host plants. For parasites that utilize a limited range of host plant species, a key additional question is whether they recognize and respond preferentially to cues from their preferred hosts. Host recognition based upon visual or olfactory cues is common among herbivorous insects (e.g., De Moraes et al., 2001; Jersáková et al., 2012; Kariyat et al., 2013) but remains largely unexplored in plant parasites, despite our knowledge that plants in general can respond to volatile and light cues from other plants (Baldwin et al., 2006; Sultan, 2010).

Flax dodder (*Cuscuta epilinum*) presents an ideal system for testing these questions because it shows a degree of host specificity that is uncommon among *Cuscuta*
spp. (Pennings and Callaway, 2002), being found nearly exclusively on flax (*Linum usitatissimum*) (Costea and Tardif, 2006; Gaertner, 1950). Reputedly, seedlings of flax dodder do not survive unless they form their first attachments to flax (Parker and Riches, 1993). This led us to hypothesize that flax dodder seedlings would be attracted to the light and/or odor cues of flax plants and furthermore would prefer cues from flax plants over cues from alternative hosts. We tested this idea with a series of no-choice and choice assays that allowed us to observe the relative preferences of flax seedlings for nearby flax and alternative host plants, and then proceeded to examine whether light or volatile cues mediate their preferences. Contrary to our hypothesis, parasites were more attracted to tomato plants (poor hosts) than to flax plants (ideal hosts). These plant species exhibit divergent volatile profiles, but we were not able to detect a role for volatiles in determining parasite preference. Tomato and flax also have contrasting architecture which leads to reproducible differences in light cues they emanate. We recreated these light quality differences using model tomato and flax plants that selectively absorb red light and transmit far-red light (like chlorophyll) and found that *C. epilinum* is more attracted to tomato-shaped models than to the flax-shaped models of equal size. We provide further data showing that seedlings prefer nearby models to more distant models of equal size and shape, which suggests that the seemingly poor choice of tomato over flax may reflect the parasite’s strategy to find the nearest host plant by favoring the incident light with the lowest red to far-red (R:FR) ratio.

Since seedlings do not appear to be specialized for flax-finding, we further assessed the abilities of seedlings to infest a range of host plants not previously paired with this parasite in other studies. Parasites were successful on a wider range of host plants than would be expected based on prior reports.

**RESULTS**

*Flax dodder seedlings orient toward nearby host plants*

To begin investigating the foraging strategies of flax dodder (*C. epilinum*) we first asked whether flax dodder seedlings were capable of directing their growth toward nearby host
plants as was previously reported of *C. campestris* (Benvenuti et al., 2005; Runyon et al., 2006). In a greenhouse no-choice experiment, we studied the directionality of parasite growth relative to nearby flax plants (*L. usitatissimum*), the preferred hosts of flax dodder. Newly-germinated parasite seedlings were planted in the center of circular foraging arenas flanked on one side by a flax plant and oppositely by a plant-free soil-filled pot (to control for the possibility that soil-born cues influence parasite choice). Flax plants were sufficiently far from the seedlings (4.5 cm) as to avoid contact with the seedlings during the experiment. After three days of foraging, 27 of 36 seedlings grew into half of the arena closest to the flax plant (Figure 1), which indicates a non-random growth pattern ($\chi^2 = 9$, df = 1, $P = 0.0027$). This confirmed that flax seedlings respond to non-contact cues produced by flax plants.

**Flax dodder seedlings choose tomato over flax plants**

Having established that flax dodder seedlings grow toward their preferred hosts (flax), we next asked if seedlings could distinguish between inferior host plants and their preferred host plants. In a growth assay similar to the no-choice experiment described above, seedlings were presented with a choice between flax and tomato (*Lycopersicum esculentum*), a poor host species. Flax and tomato plants were paired according to size to minimize the possibility that size differences could bias the assay. Contrary to our expectations, the seedlings grew preferentially toward tomato ($\chi^2 = 5.8182$, n = 44, df = 1, $P = 0.01586$). During this experiment, daytime temperatures in the greenhouse reached a maximum of 48°C, leading us to question whether high temperatures may have altered the outcome of the experiment. But a repeat experiment the following spring when greenhouse temperatures remained cooler (maximum of 36°C) produced the same result: a significant majority of seedlings (35 of 48) grew toward the tomato plants ($\chi^2 = 10.0833$, df = 1, $P = 0.001496$) (Figure 2). Since dodder species may favor larger host plants (Alers-Garcia, 2005), we checked the tomato and flax plants for differences in their total leaf area, but they did not differ (Paired T-test: $T(5) = 0.4088$, $P = 0.6996$).
Flax and tomato plants differ in quantity and quality of volatile chemical emissions

Since volatiles released by host plants are important foraging cues for the related species *C. campestris* (Runyon et al., 2006), we analyzed the odor blends released by tomato and flax plants using GC-MS. These host species differ markedly in their production of volatiles. Tomato volatile chromatograms were dominated by terpenes (β-phellandrene, limonene and 4-carene), whereas flax volatile chromatograms were dominated by green leafy volatiles (GVS’s, especially (Z)-3-hexenyl acetate) and other aliphatic compounds (octanol, nonanol, decanal) (Figure 3). Overall, flax plants emitted more volatiles than tomato plants (T-test: $T_{(8)} = 4.0181$, $P = 0.003851$). These differences were viewed as a potential explanation for seedling preference for tomato over flax—a possibility that we explored in the following experiments.

Progenitor flax plants (*Linum bienne*) and cultivated flax plants (*L. usitatissimum*) differ in volatile cues but are similarly attractive to dodder seedlings

Since volatile factors might contribute to the relatively weak attraction of dodder seedlings to flax hosts, we questioned whether the flax variety employed in our initial experiments might be a poor match for our line of flax dodder (since different flax varieties could vary in their production of visual or olfactory cues). To investigate this possibility we presented the dodder seedlings with a choice between two flax varieties that differ in their volatile profiles. The new flax option was selected from among four lines of *Linum* spp. that differ in their natural histories (two lines of cultivated flax [*L. usitatissimum*] and two lines of pale flax [*L. bienne*], which is the wild ancestral relative of cultivated flax). Headspace volatile collection and analysis by GC-MS revealed that cultivated and progenitor flax do differ in their volatile production (Figure 4). *Linum bienne* 1 (LB1) was selected for use in a subsequent dodder foraging assay since it released four times less (Z)-3-hexenyl acetate (a compound known to repel *C. campestris* seedlings (Runyon et al., 2006)) than our original flax cultivar (LU1) (T-test: $T_{(4)} = 3.3737$, $P = 0.02795$). LB1 also emits less (E)-β-ocimene than LU1 (T-test: $T_{(4)} = 7.4344$, $P = 0.001748$) and tends to be more ramified and shorter than the other varieties tested.
Despite the volatile differences between Lb1 and Lu1, flax dodder seedlings did not distinguish between them in a greenhouse choice assay ($\chi^2 = 1.4848$, $n = 33$, df = 1, $P = 0.223$) (Figure 5). Lu1 plants were weakly favored by the seedlings, but it should be noted they were also larger than Lb1 plants despite being planted a week later to offset differences in germination time (Figure 5).

*Dodder seedlings fail to locate flax plants with obstructed light cues*

Since seedlings did not distinguish between flax varieties that differ in their volatile profiles, we next tested whether the parasites would show any measurable response to volatiles from flax plants. To do this, we modified the original no-choice experiment by enclosing each flax plant in a transparent plastic tube that blocked the transmission of light cues with opaque baffles but permitted the diffusion of flax volatiles through an opening towards the foraging arena (assay concept adapted from (Runyon et al., 2006)) (Figure 6.A). Control tubes were placed opposite the ones over flax plants. The seedlings grew toward control and flax tubes with nearly equal numbers ($\chi^2 = 0.1818$, $n = 22$, df = 1, $P = 0.6698$) (Figure 6.B), indicating that volatiles from the flax plants were neither attractive nor repellent to dodder seedlings.

*Dodder seedlings locate flax plants with obstructed volatile cues*

Since parasite response to flax volatiles did not match their directed response to intact flax pants, we hypothesized that light cues might serve as the dominant foraging stimuli for flax dodder seedlings. To test this, we modified the above no choice experiment by enclosing flax seedlings in transparent plastic chimneys (tubes) that permitted the transmission of light cues but greatly reduced the incidence of volatile cues by channeling volatiles up 27cm above the foraging arena surface. Empty chimneys were placed opposite the flax tubes to control for their effects on light; and as a positive control, no chimneys were used in half of the replicates. Seedlings in both groups (treatment and positive control) grew toward the flax plants, although the statistical
significance of their choice at this replication level was marginal (see analysis in Figure AD). Notably, the two groups responded to the flax plants with an equal efficiency irrespective of the presence of the chimneys (Figure 7) ($\chi^2 = 0$, n=21, df = 1, $P = 1$), indicating there was not a significant effect of directing volatile cues away from the plane of foraging.

_Dodder seedlings fail to make a clear choice between tomato and flax plants with obstructed volatile cues_

Since seedlings easily located flax plants inside of the volatile-blocking clear chimneys, we tested whether seedling choice between tomato and flax plants would be affected by volatile-blocking chimneys. As a positive control, chimneys were omitted in half of the replicates. Contrary to our prior experiments in which seedlings preferred tomato to flax, the seedlings in this experiment did not make a significant choice in either the chimney group ($\chi^2 = 0.36$, n=25, df = 1, $P = 0.5485$), nor positive control group ($\chi^2 = 1.96$, n=25, df = 1, $P = 0.1615$), and nor was the choice significant for the pooled data ($\chi^2 = 0.32$, n=50, df = 1, $P = 0.5716$). Overall more plants grew toward tomato than flax (Figure 8), which was consistent with our earlier findings. There was no statistical difference between the chimney and control group responses (Bernard’s exact test: $P=0.2047$), but the third day the trend toward tomato appeared stronger in the positive control (no chimney) group (Figure 8).

_Light cues differ between tomato and flax plants_

Being unable to establish a clear role for volatiles in mediating dodder preference for tomato, we began to explore the alternative hypothesis that light cues from host plants might underlie dodder’s preference for tomato over flax. As mentioned, the related parasite _C. campestris_ is attracted to light transmitted through green leaves, which enriched in far-red wavelengths relative to red wavelengths due to chlorophyll activity.
(Benvenuti et al., 2005). Thus it is conceivable that differences in the R:FR light cues from potential hosts could allow dodder seedlings to distinguish among them.

To test for R:FR differences in tomato and flax, we measured light transmission and reflection from leaf pieces as well as from intact plants using a spectroradiometer (StellarNet PS-100, by Apogee). Light cues from intact plants (which are composed of a combination of reflected and transmitted sunlight) were measured by placing the sensor’s fiber optic cable in the position of the dodder seedlings in our foraging assay: 8.5 cm away from the base of the host plant, angled 15° above level. The tip of the fiber optic cable was capped with a cosine-corrected diffuser that gathered light from a 180° field of view. Spectral curves from both plant species were similar overall, having higher transmission of green and far-red light, which is characteristic of green leaves (Figure 9).

To quantify the relative effects of each plant on the R:FR ratio, we averaged the percent transmission of far red (710-740 nm) and red light (650-680) and used these values to calculate an index of R:FR ratio change:

$$\delta_{R:FR} = \frac{\%R}{\%FR}$$

Separate δR:FR values were calculated for each replicate to allow for statistical comparisons. Tomato plants effected a stronger reduction of R:FR than flax plants (T-test: $T_{(18)} = 5.0805, P = 7.803e-05$) although the difference was numerically small (δR:FR tomato: 0.949 ± 0.016 versus δR:FR flax: 0.971 ± 0.005) (Figure 9).

To test whether these differences might be attributable to leaf properties (as opposed to plant architecture or other factors) we measured the transmission of light through leaves from each plant, and we also measured the reflection of light from leaf disks (disks were used to control for differences in leaf shape and size). δR:FR values from tomato and flax plants were not different with respect to transmission (Welch T-test: $T_{(10.73)} = 0.6379, P = 0.5369$) or reflection (T-test: $T_{(18)} = 1.7321, P = 0.1004$) (Figure 10). This indicated that differences in overall δR:FR can not be explained by these inherent leaf properties. We next turned to plant architecture—which is markedly divergent between tomato and flax—as a potential explanation for the observed δR:FR differences between these species.
**Dodder seedlings prefer tomato-shaped plant models to flax-shaped models**

Since it would be difficult to separate the effects of plant architecture on R:FR from other qualities that could vary between individual plants (e.g., leaf angle, total surface area, chlorophyll content), we constructed simplified model plants out of a plastic film (CC30 Cyan, Lux #4330 by Rosco) that mimics the effect of chlorophyll on R:FR by selectively absorbing red and transmitting far-red light. Each plant was modeled with the same total surface area (6 inches$^2$), but they differed in architecture: flax plants were represented by a planar linear form six inches tall by one inch wide; tomato plants were represented by a planar T-shaped form: three inches tall and four inches wide at the top (Figure 11). We measured the effect of each model on $\delta$R:FR at a distance of 8.5 cm and found subtle but significant differences (T-test: $T_{(14)} = 7.152, P = 4.919e-06$) similar to those created by real tomato and flax plants (Figure 11).

This confirmed that plant architecture is a key variable in determining a plant’s overall R:FR light cue, and this relationship likely contributes to the differences we observed between tomato and flax light cues. It also raises the question of whether flax dodder seedlings can distinguish the subtle R:FR differences that arise from plant architecture.

**Dodder seedlings distinguish between equal-sized model plants that differ in their architecture**

To determine whether host plant architecture contributes to dodder choice, we presented flax dodder seedlings with a choice between model plants with divergent architecture (see description in prior section) that represented simplified forms of flax and tomato plants. Seedlings grew preferentially toward plant models with the tomato-type architecture ($\chi^2 = 4.2353, n=36, df = 1, P = 0.03959$) (Figure 12).
Dodder seedlings use R:FR cues to orient toward the nearest model

The seedlings’ preference for the model shape that produced the lowest R:FR signal might reveal a general pattern for seedlings to orient toward incidental light with the lowest R:FR ratio. This strategy could be beneficial if lower R:FR signals are characteristic of closer host plants, since seedlings have a very limited foraging range (Benvenuti et al., 2005; Lanini and Kogan, 2005). Indeed, we found that R:FR reduction by tomato and flax wanes rapidly as their distance to the sensor increases (Figure 13) (Mixed Model Repeated Measures ANOVA: species \( F_{(1,8)} = 12.26, P = 0.0081 \); distance \( F_{(3,24)} = 77.29, P < 0.0001 \); species*distance \( F_{(3,24)} = 7.35, P < 0.0012 \)). The same was true of the model plants across multiple distances (Figure 13) (Mixed Model Repeated Measures ANOVA: architecture \( F_{(1,14)} = 9.3, P = 0.0085 \); distance \( F_{(2,28)} = 720.9, P < 0.0001 \), architecture*distance \( F_{(2,28)} = 5.8, P = 0.0078 \)).

To see if dodder seedlings can detect the nearest plant based upon light cues (in the absence of plant volatile cues), we conducted an additional choice test between near (6.5 cm) and far (10.5 cm) model plants of the same architecture. \( \delta \)R:FR values are measurably different between these two distances (Figure 13). Irrespective of architecture, seedlings showed a strong preference for the nearest plant model (Figure 14) (Chi Square Goodness of Fit: \( \chi^2 = 7.0488, n = , df = 1, P = 0.007932 \)).

Flax dodder seedlings are capable of infesting a wider range of host plants than previously reported

Since seedlings respond to R:FR signals that are not specific to their preferred hosts (flax), we wondered whether they are capable of utilizing species other than flax as their primary host plants. In a greenhouse experiment we found that C. epilinum seedlings could grow on more than half of the plant species that we presented to it (i.e., 10 of 16 species; see Table 1). No growth was observed on Poaceous hosts, which is consistent with reports that grasses are poor hosts for Cuscuta spp. (Dawson et al., 1994), nor on cotton or soybean (Table 1). Some hosts that supported initial growth of the parasites proved to be poor long-term hosts, either because they themselves died (e.g., onion, flax),
or because they acquired resistance that staunched parasite growth (e.g., tomato, radish, pumpkin). Other species proved to be moderate to good hosts throughout the lifecycle of the parasite, including mustard, safflower, fava bean, sweet clover, and alfalfa. Few flowers and fruits were produced by parasites growing on flax in this experiment because these hosts succumbed quickly to the parasites. It is noteworthy that flax plants that were allowed to mature one additional week before being infested were not killed by their parasites and supported vigorous parasites that outperformed all other parasite-host combinations reported in Table 1 (flowers and fruits totaled 963 but are not directly comparable to other values due to differences in transplanting dates and total (fewer) hosts, as these plants were grown for seed production).

**DISCUSSION**

Considerable research attention has been directed toward the foraging mechanisms of root parasites (Yoneyama et al., 2013), but aerial parasites have received far less attention. Yet discoveries in the last decade revealed that some aerial parasites (*C. campestris*) can respond to both light and odor cues to locate nearby host plants and/or discriminate between plants that vary in species or condition (Benvenuti et al., 2005; Runyon et al., 2006). We investigated whether a related parasite that is far more host-specific than *C. campestris*—*C. epilinum* (flax dodder) (Costea and Tardif, 2006)—might use similar strategies to selectively infest its preferred host, flax.

*Background: The association of flax dodder with flax is ancient*

Flax (*L. usitatissimum*) was cultivated in south-west Asia 9,500 or more years ago, and archeological finds indicate that non-domesticated flax was harvested by humans even earlier (Zohary, 2012). Multiple lines of evidence suggest that the wild ancestor of cultivated flax is pale flax (*L. bienne* Mill. syn *L. angustifolium* Huds.) (Allaby et al., 2005), which is interfertile with cultivated flax but has smaller seeds and shorter, more
ramified branches than cultivated varieties due to human selection of the later for oil and fiber production.

Flax cultivation over time has also evidently selected for flax-like traits in ‘linilocus’ weed varieties that associate primarily with flax (Hjelmqvist, 1950). For example, *Camelina sativa var linicola* flowers in synchrony with flax and has winnowing and seed properties that favor its propagation along with flax in human agriculture (Barret, 1983; Hjelmqvist, 1950). Flax dodder (*C. epilinum*) is another linilocus weed that may have evolved traits adaptive to infesting flax fields under the selection of human agricultural practices. *C. epilinum* seeds are distinct from other *Cuscuta* spp. in lacking dormancy mechanisms that would otherwise prevent it from germinating when sewn with flax seed (other *Cuscuta* spp. exhibit physical and/or physiological dormancy that must be overcome by scarification and/or cold stratification before seeds germinate (Gaertner, 1956; 1950; Jayasuriya et al., 2008)). This enables flax dodder seedlings to attack the flax plants while they are young and vulnerable (Hegi, 1927). Another potentially adaptive trait unique to *C. epilinum* is the production of fused double seeds that approach the size of flax seeds and are therefore more difficult to separate from flax seeds than single dodder seeds (Barret, 1983; Hegi, 1927; Hjelmqvist, 1950). While it is difficult to know conclusively whether these attributes were shaped by selection through agriculture, archeological evidence would suggest that dodder—presumably *C. epilinum* or its progenitor—co-occurred with flax in Europe during the bronze age and on through more recent eras (Hegi, 1927; Hellwig, 1997; Latalowa, 1998; Pals and Van Dierendonck, 1988)—which may allow three or more millennia for evolutionary adaptation of *C. epilinum* as a flax pest. Moreover, the fact that flax dodder grows nearly exclusively upon flax plants (Hegi, 1927; Parker and Riches, 1993)—and does so with great vigor—suggests that flax dodder is indeed well adapted to utilize this resource. Thus is conceivable that that *C. epilinum* may also possess adaptations that enable it to forage selectively for flax plants.
Flax dodder seedlings grow toward nearby host plants

Multiple instances of selective host acquisition by *Cuscuta* spp. (e.g., *C. europea*, *C. salina*, *C. campestris*, *C. gronovii* (Kelly, 1992; Koch et al., 2004; Pennings and Callaway, 1996; Schoolmaster, 2005)) would suggest that dodgers actively forage for their hosts, and a number of foraging mechanisms have been proposed. Foraging might involve post-contact responses to host surface chemistry (Kelly, 1992) or to the success of initial haustoria at withdrawing nutrients (Koch et al., 2004); alternatively, foraging may involve pre-contact host location and evaluation. This appears to be the case for seedlings of *C. campestris*, which grow toward light and volatile cues from nearby host plants (or cuttings) (Benvenuti et al., 2005; Runyon et al., 2006) and may even discriminate between the volatiles of poor and preferred hosts (Runyon et al., 2006). Pre-contact host location and evaluation may be key to survival in parasites throughout the genus *Cuscuta* given that seedlings of all *Cuscuta* spp. face pressure to locate and infest amenable hosts before exhausting their seed resources (Dawson et al., 1994). In this study we found that seedlings of *C. epilinum*, like those of *C. campestris*, are capable of using non-contact cues to grow toward nearby host plants—flax and tomato in this case (Figures 1 and 2). *C. epilinum* and *C. campestris* represent distinct subgenera within the genus *Cuscuta* (Cuscuta and Grammica, respectively (McNeal et al., 2007)), and thus our findings support the idea that pre-contact host location may be a normative foraging behavior among *Cuscuta* spp.

Flax dodder seedlings do not forage selectively for flax plants despite volatile differences between flax and tomato

Although flax dodder seedlings grew toward flax plants in no choice experiments, when presented with a choice between flax and tomato (an inferior host plant), seedlings grew preferentially toward the tomato (Figure 2). This result was unexpected in light of flax dodder’s historical association with flax (discussed above) and the putative dependence of flax dodder seedlings on reaching a flax host (Parker and Riches, 1993). Initially we thought that volatiles cues from tomato and flax might pose a possible explanation for
this preference. The tomato volatile profile contains terpenes that are attractive to *C. campestris* seedlings, whereas the flax volatile profile is dominated by a compound that is repellant to *C. campestris* seedlings: (Z)-3-hexenyl acetate. (Runyon et al., 2006) (Figure 3). (Z)-3-hexenyl acetate is commonly released by wheat and other monocot crops that are unusable host plants for all *Cuscuta* spp. (Runyon et al., 2006; Tamiru et al., 2011; Tooker and De Moraes, 2007), and by plants that sustain herbivore damage (De Moraes et al., 2001; Mescher et al., 2006; Rose et al., 1996; Tooker and De Moraes, 2007; e.g., Turlings et al., 1990). Thus the wider ecological context for the major component of flax volatiles could pose risks to seedlings that would orient toward them. In an evolutionary view these risks might balance or overwhelm the benefits of responding exclusively to flax volatiles.

Despite these potential risks and unlike *C. campestris*, flax dodder seedlings do not appear to avoid (Z)-3-hexenyl acetate. This was evident in the seedlings’ inability to choose between Lu1, our original flax variety, and LB1, an ancestral flax line that releases fourfold less (Z)-3-hexenyl acetate than Lu1 plants (Figures 4 & 5).

*Dodder seedlings respond to host light cues, which are a function of host plant architecture and distance*

This raised the key question of whether flax dodgers respond at all to volatile cues when they forage for hosts—and if not, whether responses to light cues can account for their ability to locate nearby hosts. We found no evidence that obscuring volatile cues from flax plants reduces their attractiveness to dodder seedlings, nor that seedlings can respond to flax odors when flax light cues are obscured (Figures 6 and 7). Also there was no clear effect of blocking tomato and flax odor cues simultaneously on seedling’s ability to choose between these plants (Figure 8), although the data suggest that a weak effect of the transparent volatile tubes may have occurred by redirecting volatiles and/or constraining plant architecture. We can not rule out the possibility that volatiles play a small but undetected role in attracting flax dodger seedlings, but it appears that other host cues such as light reflection are more central to parasite foraging responses.
The importance of light-based cues was confirmed in our experiments with plant models that mimic the spectral properties of leaves by absorbing red and reflecting far-red light (Figure 10). A phototropic response to low R:FR light by *C. campestris* and *C. planifora* has been reported previously (Benvenuti et al., 2005; Orr et al., 1996), thus it is likely that *C. epilinum*’s attraction to the plant models is also due to the low R:FR signal from the model plants. This is further supported by the observation that in choice tests with real plants as well as with plant models, seedlings consistently preferred targets with the lowest R:FR ratios (i.e., tomato plants and tomato-type models).

Notably, we observed that tomato plants have stronger effects on $\delta R:FR$ than flax. Since tomato and flax leaves have similar reflectance and transmission properties (Figure 10) this effect difference is likely due to plant architecture. We confirmed that $\delta R:FR$ varies as a function of architecture by recording light signals from model tomato and flax plants (see diagram in Figures 12A). Although the models of each type had equal surface area, the tomato-type models effected a lower $\delta R:FR$ (Figure 11). This results from the inverse relationship between distance and light intensity because the flax surface points are, on average, farther from the sensor than are the tomato surface points, resulting in a less intense light signal. The overall effect can also be explained from the perspective of the sensor: the tomato model occupies more of the sensors ‘field of view’ than does the flax model.

This relationship between architecture and $\delta R:FR$ presented an intriguing explanation for the observed parasite preference for tomato over flax (Figure 2). In a choice test between model tomato and flax plants of equal size, dodder seedlings preferred the tomato type architecture (Figure 14). This confirmed that host shape and associated light cues play a significant role in guiding dodder seedlings.

Although this architectural effect reduced the ability of seedlings to make the “correct” choice in our foraging assays, in nature a similar effect may benefit dodders by limiting the attractiveness of tall, slender grasses (which are non-hosts) relative to herbaceous plants (which are generally more susceptible hosts (Dawson et al., 1994)). Tropic responses to low R:FR light may also help seedlings to select healthier host plants:
*C. campestris* seedlings prefer light transmitted by dark green leaves to light transmitted by light green leaves (Benvenuti et al., 2005).

In addition to finding hosts of suitable shape or status, a critical challenge for obligate parasites is to locate candidate hosts that are within the range of their limited growth abilities. For small-seeded dodders like *C. campestris*, this range measures only five centimeters or less (Benvenuti et al., 2005; Lanini and Kogan, 2005) (more distant hosts may be reached but are seldom infested, presumably because extended growth exhausts seed resources) (Benvenuti et al., 2005). In light of this challenge it is significant that δR:FR varies as a function of distance (Price and Wilcut, 2007; H. Smith et al., 1990). Smith (1990) showed that distance to a leaf canopy affects not only the R:FR ratio but also the equilibrium of interchanging R- and FR-absorbing phytochrome conformations (also known as Pr and Pfr, which exist in dynamic equilibrium in plants), thus indicating that plants have a physiological mechanism to perceive the proximity of neighboring plants. Whereas autotrophic plants use these assessments to trigger elongation and shade avoidance (Schmitt and Wulff, 1993), dodders apparently use this information to orient toward potential host plants (Orr et al., 1996). It was not previously known, however, whether dodder parasites use R:FR cues to assess the relative distances of candidate hosts. In our choice assay between model plants of two distances the seedlings grew preferentially toward the closest models (Figure 14). This finding presents strong evidence that parasites utilize light cues to select the nearest host plant.

In light of these results it is interesting to consider the potential contribution of seedling growth movements to finding host plants. Prior to contacting their hosts, dodder seedlings exhibit a rotational growth pattern called circumnutation that is thought to contribute to foraging efficiency (Mescher et al., 2009). The base of a circumnutating parasite remains anchored in the ground while the apical tip travels a circular path of approximately three centimeters in diameter (Johnson, 2013). This movement could facilitate evaluation of host distance in the following way: circumnutation causes the distance between parasites and their potential host plants to oscillate. Distance oscillation causes δR:FR from hosts to also oscillate. δR:FR oscillation from near hosts will have a greater amplitude than δR:FR oscillation from more distant hosts because δR:FR is more
affected by distance at close ranges than at far ranges (see the trendlines in Figure 13 and in H. Smith et al., 1990). The amplitude of $\delta$R:FR oscillation is thus a function of distance and theoretically could be exploited by seedlings to gauge distance to host plants. Thus it might be possible for dodder seedlings to perceive host proximity by virtue of their rotational growth movements. Albeit more sophisticated than simply growing toward the lowest R:FR light source, this speculative pathway for information transduction could enable seedlings to choose optimally even when the nearest hosts do not reduce R:FR as much as more distant leaf canopies (such as from trees), and thus would present an advantage in complex light environments.

Host range of C. epilinum is broader than previously thought

*C. epilinum* is widely considered one of the most host-specialized species in the genus *Cuscuta* (Parker and Riches, 1993). Although there are reports of if it infesting plants other than flax (Baráth and Csiky, 2012; Gaertner, 1950; Hegi, 1927) (Table 3), Parker and Riches (1993) inferred that flax dodder seedlings need to attach to a preferred host (i.e., flax) before they can utilize less suitable hosts. To encompass this distinction they introduced the terms ‘primary host’—which are susceptible to seedling parasites—and ‘secondary hosts’—which can resist seedlings but not mature parasite vines. Our host range study indicates that the list of primary host species for flax dodder is much broader than expected. Seedlings grew on 10 of 16 host species representing seven different plant families (Table 1). Furthermore, they flowered and set seed on seven of these plant species, although overall success of the parasites varied widely on these plants. Some of the host species that initially permitted parasite growth acquired resistance as they matured. For example, tomato hosts halted parasite growth after only a few weeks; eventually the parasites dried up and died before they could flower. It appears that host susceptibility is a dynamic trait that that varies in response to age and/or prior exposure to parasites. As such, assigning host species to traditional categories of susceptibility (i.e., primary, secondary and non-host) may frequently lead to oversimplification. Even flax, the most compatible host species, illustrates this point because it is difficult for dodder seedlings to become established on fully grown flax plants (Hegi, 1927), whereas the
young flax plants in our experiment succumbed so quickly that the parasites subsequently starved (resulting in minimal flower production). It may be that our decision to employ young host plants (14 days post planting) resulted in a greater number of compatible parasite-host combinations than would have been observed if we used older hosts. This could explain why we observed parasites growing quite successfully on *Medicago sativa* which was previously reported resistant (Table 3).

These data indicate that it may be possible for flax dodder seedlings to utilize a broad range of host species as long as they germinate nearby in time and space. It would serve them well to function as generalists at this nascent stage considering their limited foraging range. Some authors have suggested that limited motility may explain why parasitic plants as a whole tend to utilize broad host ranges (Pennings and Callaway, 2002)—and seedlings are the least mobile of all dodder life stages. Seedlings that infest relatively poor hosts nevertheless gain time and resources to reach more distant host plants. Thus in general it may be advantageous for parasites in the seedling stage to prioritize proximity over other host factors when selecting their first host. To this end R:FR light cues may be more useful to seedlings than volatile cues because light cues are more reliably affected by distance than volatile cues which can travel relatively unchanged in air currents.

Optimal foraging strategies may change as parasites develop from resource-poor seedlings to mature vines since the later are capable of crossing larger distances to reach optimal host plants. Thus we might predict that mature vines should be more responsive than seedlings to host-specific cues that indicate host quality, such as volatile profiles (Runyon et al., 2006) or surface chemistry (Kelly, 1992). At least two studies have noted non-random host use by mature dodder vines (Kelly, 1992; Koch et al., 2004). It would be interesting to see if these vines use different foraging strategies than their seedlings.

**CONCLUSIONS**

Our findings indicate that although flax dodder grows particularly well on flax, seedlings of this parasite do not orient exclusively toward flax in exclusion of other host plants, nor
are they exclusively dependent upon flax hosts in this early stage of life. Rather, seedlings are attracted to objects or plants that project low R:FR light—a non-species-specific indicator of green plants—and appear to select those with the lowest R:FR signature. In nature this strategy may increase the seedlings’ odds of finding nearby, suitable hosts. It is possible that flax dodder becomes more selective in later life stages—the widely reported association with flax hosts supports this idea—but more work is needed to explore this possibility.

**METHODS**

*Seeds*

*C. epilinum* seeds were provided by the Old Botanical Garden of Göttengen University (Germany). *Linum usitatissimum* (‘Rehab 94’) seeds were kindly donated by the Albert Lea Seed House (Albert Lea, MN). Other *Linum* spp. seeds were obtained through the National Plant Germplasm System (NPGS).

<table>
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<th>Species</th>
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<th>Source</th>
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<tr>
<td>LU3</td>
<td>L. usitatissimum</td>
<td>PI 523051</td>
</tr>
<tr>
<td>LB1</td>
<td>L. bienne</td>
<td>PI 253971</td>
</tr>
<tr>
<td>LB2</td>
<td>L. bienne</td>
<td>PI 231886</td>
</tr>
</tbody>
</table>

Tomato (*Solanum esculentum*) ‘Halley 3155’ seeds were purchased from Orsetti Seeds (Hollister, CA).

Other seeds required for the host specificity project (listed in Table 1) were already in cold storage in the De Moraes and Mescher lab.
Plant growing conditions

*C. epilinum* seeds for all experiments were germinated on moist filter paper disks inside covered 9 cm petri dishes at a density of approximately 50 seeds per dish. Petri dishes were sealed with Parafilm to retain moisture and kept in a reach-in growth chamber for 5 to 6 days, or long enough for seedlings to reach a length of 2-3cm. The chamber was set to a 16-hr photoperiod with light/dark temperatures of 25/23°C.

Host plants for foraging assays and volatile collections were grown in an insect-free walk-in growth chamber set to 16-hour photoperiod with 23º/21ºC light/dark temperatures and 50% relative humidity. All host plants were grown in plastic green pots with general purpose potting soil (100g dry weight) (Pro-Mix BX with mycorrhizae, Premier Horticulture Inc.) mixed with SCOTTS 14-14-14 Osmocote (3g) and SCOTTS Micromax Micronutrients (0.9g) (Griffin Greenhouse Supplies, Morgantown, PA). Plants for the *Linum* spp. volatile collections were sown directly into their final pot and thinned to a density of four per pot, whereas all other plants were sown into trays of potting soil and transplanted individually as seedlings. Pot volumes were 200ml for the host range study and 600ml for all other experiments.

Seedling foraging assays (choice and no choice)

Choice tests to detect dodder seedling preferences were conducted in a Pennsylvania State University greenhouse (coordinates 40.802895,-77.861883) during spring and summer months. Greenhouse temperatures were imperfectly regulated by air conditioning units set to 21ºC, and typically they ranged from 20-38ºC; however, during the initial choice test between tomato and flax temperatures reached high as 48ºC. Supplemental growth lights were turned off during experiments so that light cues from target plants would be natural (i.e., not altered by the distinct spectral qualities of greenhouse lights) and to avoid the possibility of bias resulting from phototropic responses to growth lights (Benvenuti et al., 2005; Orr et al., 1996).
Foraging arenas consisted of a filter paper disk 9 cm in diameter supported by a cardboard disk of equal size that rested upon the edges of the two pots containing target plants or controls. Boundary lines for quadrants and halves were traced upon the filter paper disk with pencil to facilitate nonbiased categorization of seedling choice. Seedlings were planted in a soil-filled fluorocarbon Teflon straw (# ASTM-D-3296-03 by Zeus, Raritan, NJ) 10 cm tall by 0.6 cm wide that fit through a small hole in the center of the arena and rested in a vial of water. Soil in the straw tip was watered by a wick that reached from the soil column down to the water. In the fully assembled arena, the radicle (base) of the seedling was on level with the filter paper disk, and target plants were 8.5 cm to either side. Teflon straws were autoclaved and replenished with soil between experiments.

Host plants grown in growth chambers were transferred to the greenhouse one or more days before each experiment to allow a period of adjustment. Replicates were distributed evenly across four greenhouse tables (6 ft wide by 8 ft long) such that all seedlings were 60 cm or farther from the next nearest replicate. The absolute orientation of each replicate was systematically varied (NE, WN, SW, SE) to control for possible biases from environmental stimuli (e.g., reflectance from tree canopies outside of the greenhouse). Plant ages and experiment dates are shown in Table 2. Dodder seedlings two to three cm long were planted in the foraging arenas and allowed to forage until all seedlings fell and settled in a region of the disk (2 or 3 days depending upon greenhouse temperatures). Choices were categorized two ways—by quadrant and by half—according to the location of the tip of the seedling when viewed from above.

Experiments four and five (Table 2) required accessories to obscure olfactory and visual cues respectively. Transparent tubes were constructed by rolling 8.5x11 inch acetate sheets (3M transparency film #PP2500) along their long axis to produce tubes that were 11 inches tall by eight inches in circumference. For the reduced volatile assay, tubes were placed over flax plants and planted about one centimeter into the soil to minimize volatile leakage under the bottom edge. A small one centimeter hole was punched into the base of the tube and the tops were left open to facilitate convectional transfer of volatiles up and out of the tubes. For the olfactory cue assay, an oval-shaped 8x4 cm hole
was cut in each tube to allow volatile dispersal toward the seedlings. An opaque baffle was inserted along one side of the tube to block light cues from reaching the parasite. The baffle was constructed of a strip of black craft foam (by Creatology, distributed through Michaels, USA) that was notched along its sides so that volatiles could easily diffuse around it. The tops of tubes were covered with petri dishes to promote volatile diffusion toward the seedlings.

**Volatile collections**

For the characterization of plant volatiles, host plants were enclosed in 2.5-L glass domes with Teflon bases that excluded the soil and roots. Domes were steadily flushed with clean, filtered air at a rate of 1.5 liters per minute (LPM) through a port in the top of the dome. Volatiles were trapped by pulling air out of ports near the base of the domes through filters containing a non-polar, general adsorbant (Hayesep Q, 35mg/filter, Supelco, Bellefont, PA, USA) at a rate of 1.0 LPM—slow enough to maintain a slight positive pressure within the dome that prevented the influx of unfiltered air. Plants were placed in domes overnight to allow equilibration prior to the start of collections. Sampling was automated by push-pull systems (Analytical Research Systems, Gainsville, FL, USA) that sampled over the course of two days; data from the second day is reported in this paper to ensure that plants had sufficient adjustment time.

Flax cultivar volatiles were collected in a growth chamber (16/8 hour light/dark cycle, 23/21°C temperature cycle, 50% humidity, high pressure sodium and metal halide lamps) in two 24 hour periods from three replicates per species. Collections were started 21 days after sewing the seeds. To minimize within-species variation and maximize the amount of volatiles trapped, we collected from four plants per pot. Afterward we oven-dried and weighed above ground plant tissues to normalize volatile quantities by weight.

Tomato and flax volatiles were collected in conjunction with foraging experiment 3 (Table 2)—in the same location and with the same cohort of host plants—to synchronize plant condition and environmental parameters between experiments. Volatiles were sampled for 11 hrs by day and seven hours by night from six individual tomato and flax
replicates. Afterward, the size of each plant was estimated by quantifying total leaf area (rather than measuring dry mass, since tomato and flax may differ in their distribution of mass between supporting and photosynthetic tissues). Excised leaves were taped to white paper and scanned as .pdf files at a resolution of 600 dpi. Files were then converted to .jpg files (using Preview, version 5.03) and leaf areas were measured using the free software ImageJ (version 1.45).

Gas chromatography and volatile analysis

Filters containing volatiles were eluted with 150 μL of dichloromethane. To each sample we added 5 μL of an internal standard solution (n-octane [40 ng/L] and nonyl-acetate [80 ng/L] in dichloromethane). Sample components were separated with Agilent model 6890 gas chromatographs (GC) coupled to a quadrupole mass spectrometer (MS) for identification and quantification. MS was used for quantification rather than FID to enable a semiquantitative separation of β-phellandrene and limone, which coelute on HP-1 columns. Each compound was quantified relative to the internal standard by dividing the area of the major ion by the area of the largest nonyl acetate ion and multiplying by a factor of 400 (using a method developed in MassHunter [2014] software by Agilent). Response values were also corrected for numbers of collection hours, differences in push/pull rates, and overall plant size (leaf area or dry weight).

Host range study

Host plants germinated in a walk-in growth chamber (described above) were transplanted after two weeks and relocated to an insect-free glasshouse where temperature was maintained at a constant 24°C with 50% relative humidity. Supplemental grow lights were turned off to prevent spectral disruption of parasite attachment; thus photo-period was determined by natural day length from April 18 through July 2 (at coordinates 40.820475,-77.858606). One flax dodder seedling was placed at the base of each host plant. Seedling progress was recorded after seven, 14, and 21 days. Data from the few
replicates in which seedlings perished due to disease or mishandling were discarded. Host plants were combined to one tray per species so that successful parasite seedlings could access additional plants. At 13 weeks, during the flowering stage of the parasites, the glasshouse abruptly overheated and ended the experiment (no plants survived). Flowers and fruits were counted to measure overall parasite success on each plant species.

**Statistical Analyses**

Seedling foraging assays were analyzed by 2-way Chi Square Goodness of Fit tests to establish whether observed choice distributions (among halves or quadrants adjacent to the targets and controls) were significantly different from the a random (50:50) distribution (as in Runyon et al., 2006). Quadrant data was analyzed by 2-way Chi Square rather than 4-way Chi Square (as in Runyon et al., 2006) because there were no control pots (or tubes) adjacent to the side quadrants, making the null hypothesis of equal distribution between quadrants only valid between the quadrants adjacent to target hosts and/or controls. For low samples sizes with predicted values less 10, a binomial exact test was used in place of the Chi Square test. Contingency tables were tested with Bernard’s Exact test.

We used the statistical software R (3.0) to run all statistical analyses.
Figure 1. Flax dodder seedlings orient toward nearby flax plants. [A] Photograph of no-choice assay showing a dodder seedling growing towards the nearby flax plant. [B] Summary of data showing final locations of parasite tips after three days of foraging. [C] Statistical results tables showing Chi Square Goodness of Fit tests.
Figure 2. Flax dodder seedlings prefer tomato to flax. [A] Photograph of choice test after setup. Leaf area between flax and tomato plants after three days did not differ (Paired T-test: $T_{(5)} = 0.4088$, $P = 0.6996$). [B] Summary of data showing final locations of parasite tips after three days of foraging. [C] Statistical results tables showing Chi Square Goodness of Fit tests.
Figure 3. Tomato and flax plant produce different volatile blends. [A] Volatile components identified in flax and tomato chromatograms from daytime headspace collections. Response values represent mean intensities (from five replicates) of major ion peaks normalized relative to leaf area and to a 400 ng internal standard (nonyl acetate). [B] Total volatile emissions from each plant species. Asterisks indicate significant difference (T-test: $T_{(8)} = 4.0181, P = 0.003851$).
Figure 4. Flax (*Linum* spp.) volatile emissions vary between and within species. [A] Major volatile blend components. [B] Minor volatile blend components. Volatiles were collected for 24 hours by headspace volatile analysis and analyzed by GC-MS. Each species is represented by 12 individuals divided evenly among three replicates. Data represent intensities of the major m/z ion from each compound normalized relative to an internal standard and plant size (dry weight).
A. 

![Figure 5. Flax dodder seedlings did not choose between cultivated flax (Lu1) and wild flax (LB1). [A] Summary of data showing final locations of parasite tips after three days of foraging. [B] Statistical results tables showing Chi Square Goodness of Fit tests or binomial exact tests (for predictions less than 10). [C] The host types differed in size (Paired t-test: $T_{35} = -8.1084$, $P = 1.507e-09$), which could account for the non-significant preference for Lu1 hosts.](image-url)
**Figure 6. Flax dodder seedlings do not orient toward flax volatiles.** [A] Top-down schematic of foraging assay (showing tubes and baffles that block light cues but permit passage of volatile cues) with data summaries. [B] Statistical results tables showing Chi Square Goodness of Fit tests or binomial exact tests (for predictions less than 10).
Figure 7. Dodder seedling foraging is not affected by removal of flax volatile cues. [A] Schematic of no-choice experiment with volatile-removing chimneys in half of the replicates. Data are summarized by half and quadrant. [B] Statistical results tables showing Chi Square Goodness of Fit tests or binomial exact tests (for predictions less than 10). Seedling orientation toward flax is marginally non-significant at this replication level (but similar to trend in Figure 1). [C] Results from Bernard’s exact test of independence for two-way contingency table. Treatment groups are not different, indicating that volatile removal does not hinder parasite foraging for flax plants.
Figure 8. Effect of volatile cue reduction on dodder choice. [A] Schematic of choice experiment with volatile-removing chimneys over plants in half of the replicates. Data are summarized by half and quadrant. [B] Statistical results tables showing Chi Square Goodness of Fit tests or binomial exact tests (for predictions less than 10). Seedling orientation toward flax was not significant in this experiment but tomato appear to be favored as in data shown in Figure 1. [C] Bernard’s exact tests of independence for 2x2 contingency tables showing that volatile reduction treatment did not significantly alter dodder seedling distributions.
Figure 9. Light cues differ between flax and tomato plants. Tomato and flax plants alter the intensity and quality of light incident to dodder seedlings (or spectroradiometer, in this case). Measurements were taken outdoors on a sunny afternoon at a distance of 8.5 cm from plants to approximate the vantage point of dodder seedlings in choice tests. [A] Full spectrum chart showing average of 10 plants per species. [B] Graph of differential change index between red light (R: 650-680 nm) and far-red light (FR: 710-740 nm) defined as \( \delta \text{R:FR} = \frac{\% \text{R}}{\% \text{FR}} \). Tomato plants have a stronger effect on R:FR than flax plants (T-test: \( T_{(18)} = 5.0805, P = 7.803\times10^{-5} \)).
Figure 10. Tomato and flax leaves alter R:FR ratios in reflected and transmitted sunlight. [A] Reflection δR:FR of leaf disks relative to a white reflective standard. [B] Transmission δR:FR of flax and tomato leaves relative to sky light. Treatment effects are not significant.
Figure 11. Model plants resembling flax and tomato architecture produce different light cues. Models (depicted in Figure 12) mimic the effect of chlorophyll on R:FR by selectively absorbing red light and transmitting far-red light. [A] Full spectrum chart showing average of readings from 8 model plants of each type at a distance of 8.5 cm from the spectroradiometer sensor. Sensor was positioned at ground level to simulate the dodder seedling perspective. [B] Graph of differential change index between red light (R: 650-680 nm) and far-red light (FR: 710-740 nm) defined as $\delta R:FR = (%R) / (%FR)$. Tomato models have a stronger effect on the R:FR ratio than flax models (T-test: $T_{(18)} = 5.0805, P = 7.803e-05$).
Figure 12. Dodder seedlings distinguish between equal-sized model plants that differ in their architecture. [A] Schematic of model plants constructed with equal areas of photoselective plastic film (CC30 Cyan, Lux #4330 by Rosco) (for spectral properties see Figure 11). [B] Summary of dodder seedling growth data when presented a choice between both model types at a distance of 8.5 cm. [C] Chi Square Goodness of Fit test results.
Figure 13. Distance affects magnitude of δR:FR from real and model plants. [A] δR:FR from flax and tomato plants measured at four distances from the spectroradiometer sensor. [B] δR:FR from model flax and tomato plants at distances corresponding to those employed in dodder seedling choice tests with model plants. Plots represent means ± s.e. from 5 replicates of each species (plot A) or 8 replicates of each model (plot B). Measurements were taken outdoors on a sunny afternoon with a spectroradiometer in a level position directed toward the plants.

Statistics:

**Plot A**, Mixed Model Repeated Measures ANOVA: species $F_{(1,8)} = 12.26, P = 0.0081$; distance $F_{(3,24)} = 77.29, P < 0.0001$; species×distance $F_{(3,24)} = 7.35, P < 0.0012$.

**Plot B**, Mixed Model Repeated Measures ANOVA: architecture $F_{(1,14)} = 9.3, P = 0.0085$; distance $F_{(2,28)} = 720.9, P < 0.0001$, architecture×distance $F_{(2,28)} = 5.8, P = 0.0078$.

Significant differences between distance categories (Tukey HSD $P < 0.05$) averaged across plant types are indicated with lowercase letters.
Figure 14. Dodder seedlings are most attracted to the nearest plant model. [A] Data summaries from seedling choice between near (6.5 cm) and far (10.5 cm) plant models, quantified by half and quadrant. [B] Statistical results table for data split by plant type (Chi Square Goodness of Fit and Binomial Exact Tests). [C] Statistical results table for pooled data.
Table 1. *Cuscuta epilinum* establishment and reproduction on flax and various non-preferred host plants. Host plants were infested 14 days after planting by placing one newly germinated parasite seedling at the base of each host.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species (variety)</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Foraging</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sustained contact with host</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Attaching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Attempted to formhaustoria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Growing strong** / weak*** / failed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reproduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total parasite flowers and/or</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>fruits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALLIACEAE†</strong></td>
<td><em>Allium cepa</em>† (Borretana)</td>
<td>7 of 12</td>
<td>7 of 12</td>
<td>5 / 0 / 2</td>
<td>0 (hosts died)</td>
</tr>
<tr>
<td><strong>BRASSICACEAE</strong></td>
<td><strong>Raphanus sativus†</strong> (Cherry Belle)</td>
<td>9 of 13</td>
<td>8 of 13</td>
<td>7 / 0 / 1</td>
<td>0 (poor growth)</td>
</tr>
<tr>
<td></td>
<td><em>Brassica juncea</em>† (Florida Broadleaf)</td>
<td>12 of 13</td>
<td>9 of 13</td>
<td>8 / 0 / 1</td>
<td>292</td>
</tr>
<tr>
<td><strong>COMPOSITAE†</strong></td>
<td><em>Carthamus tinctorius</em>† (Orange Grenade)</td>
<td>8 of 13</td>
<td>6 of 13</td>
<td>4 / 2 / 0</td>
<td>124</td>
</tr>
<tr>
<td><strong>CUCURBITACEAE†</strong></td>
<td><em>Cucurbita pepo</em>† (Connecticut Field)</td>
<td>3 of 11</td>
<td>3 of 11</td>
<td>1 / 1 / 1</td>
<td>3 (poor growth)</td>
</tr>
<tr>
<td></td>
<td><em>Cucurbita pepo</em>† (Dixie)</td>
<td>4 of 10</td>
<td>5 of 10</td>
<td>0 / 0 / 5</td>
<td>0 (parasites died)</td>
</tr>
<tr>
<td></td>
<td><em>Vicia fava</em>† (Banner)</td>
<td>12 of 12</td>
<td>11 of 12</td>
<td>9 / 0 / 2</td>
<td>223</td>
</tr>
<tr>
<td><strong>FABACEAE</strong></td>
<td><em>Melilotus officinalis</em> (sweet clover)</td>
<td>13 of 13</td>
<td>7 of 13</td>
<td>1 / 3 / 4</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td><em>Medicago sativa</em> (Charger)</td>
<td>13 of 13</td>
<td>7 of 13</td>
<td>6 / 0 / 1</td>
<td>603</td>
</tr>
<tr>
<td></td>
<td><em>Glycine max</em> (Williams 82)</td>
<td>9 of 13</td>
<td>8 of 13</td>
<td>0 / 0 / 13</td>
<td>0 (parasites died)</td>
</tr>
<tr>
<td><strong>MALVACEAE†</strong></td>
<td><em>Gossypium hirsutum</em> (unlisted)</td>
<td>8 of 12</td>
<td>7 of 12</td>
<td>0 / 1 / 6</td>
<td>0 (parasites died)</td>
</tr>
<tr>
<td><strong>LINACEAE</strong></td>
<td><em>Linum usitatissimum</em> (Rehab 94)</td>
<td>9 of 15</td>
<td>9 of 15</td>
<td>9 / 0 / 0</td>
<td>6 (hosts died)</td>
</tr>
<tr>
<td><strong>POACEAE</strong></td>
<td><em>Triticum aestivum</em> (McNeal)</td>
<td>4 of 13</td>
<td>4 of 13</td>
<td>0 / 0 / 4</td>
<td>0 (parasites died)</td>
</tr>
<tr>
<td></td>
<td><em>Zea mays</em> (non-Bt field corn)</td>
<td>9 of 12</td>
<td>8 of 12</td>
<td>0 / 2 / 6</td>
<td>0 (parasites died)</td>
</tr>
<tr>
<td><strong>SOLANACEAE</strong></td>
<td><em>Solanum lycopersicum</em> (Halley 3155)</td>
<td>11 of 14</td>
<td>8 of 14</td>
<td>7 / 0 / 1</td>
<td>0 (parasites died)</td>
</tr>
</tbody>
</table>

*strong: new parasite shoots are longer than 2.5 cm in combined length
**weak: new parasite shoots are less than 1 cm in combined length
***failed: no parasite growth beyond the point of attachment
† Indicates a species or taxon not previously tested for compatibility with *C. epilinum*; earlier records are summarized in Table 3.
<table>
<thead>
<tr>
<th>Date</th>
<th>Assay</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2011</td>
<td>21-day <strong>flax</strong> (no choice)</td>
<td>preferred flax</td>
</tr>
<tr>
<td>July 2011</td>
<td>20-day <strong>flax</strong> vs. 17-day <strong>tomato</strong></td>
<td>preferred tomato</td>
</tr>
<tr>
<td>June 2012</td>
<td>21-day <strong>flax</strong> vs. 18-day <strong>tomato</strong></td>
<td>preferred tomato</td>
</tr>
<tr>
<td>Sept. 2012</td>
<td>22-day <strong>flax light cues</strong> (reduced volatiles) (no choice)</td>
<td>preferred flax light cues (no effect of reducing volatiles)</td>
</tr>
<tr>
<td>May 2013</td>
<td>21-day <strong>flax olfactory cues</strong> (blocked light cues) (no choice)</td>
<td>no preference for flax volatiles</td>
</tr>
<tr>
<td>May 2013</td>
<td>19-day <strong>flax</strong> vs. 26-day <em>L. bienne</em></td>
<td>no preference</td>
</tr>
<tr>
<td>April 2014</td>
<td>17-day <strong>flax</strong> vs. <strong>tomato</strong> (blocked volatile cues)</td>
<td>no preference (no effect of reducing volatiles)</td>
</tr>
<tr>
<td>May 2014</td>
<td><strong>flax model</strong> vs. <strong>tomato model</strong></td>
<td>preferred tomato model</td>
</tr>
<tr>
<td>May 2014</td>
<td>near <strong>model</strong> vs. far <strong>model</strong></td>
<td>preferred near model</td>
</tr>
</tbody>
</table>
### Table 3. *Cuscuta epilinum* hosts and non-host species reported in previous studies.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Host Status</th>
<th>Test Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALSAMINACEAE</td>
<td><em>Impetiens sultanii</em> Hook</td>
<td>+</td>
<td>1</td>
<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td>BRASSICACEAE</td>
<td><em>Camelina sativa</em></td>
<td>+?</td>
<td>4</td>
<td>(Hegi, 1927)</td>
</tr>
<tr>
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<td>-</td>
<td>1</td>
<td>(Gaertner, 1950)</td>
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<tr>
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<td><em>Lathyrus aphaca</em></td>
<td>±</td>
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<td>(Hegi, 1927)</td>
</tr>
<tr>
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<td><em>Medicago sativa</em></td>
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<td>1</td>
<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td>FABACEAE</td>
<td><em>Trifolium hybridum</em> L</td>
<td>-</td>
<td>1</td>
<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td></td>
<td><em>Trifolium pratense</em> L</td>
<td>±</td>
<td>3,2</td>
<td>(Hegi, 1927; Nobbe and Simon, 1904)</td>
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<tr>
<td></td>
<td><em>Vicia hirsute</em></td>
<td>±</td>
<td>1</td>
<td>(Hegi, 1927)</td>
</tr>
<tr>
<td>LINACEAE</td>
<td><em>Linum usitatissimum</em> L</td>
<td>+</td>
<td>1</td>
<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td></td>
<td><em>Avena sativa</em> L</td>
<td>-</td>
<td>1</td>
<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td>POACEAE</td>
<td><em>Hordeum vulgare</em> L</td>
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<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td></td>
<td><em>Lolium temulentum</em></td>
<td>+?</td>
<td>4</td>
<td>(Hegi, 1927)</td>
</tr>
<tr>
<td>POLYGONACEAE</td>
<td><em>Fagopyrum esculentum</em></td>
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<td>(Gaertner, 1950)</td>
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<td>SOLANACEAE</td>
<td><em>Nicotiana tabacum</em></td>
<td>-</td>
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<td>(Gaertner, 1950)</td>
</tr>
<tr>
<td>URTICACEAE</td>
<td><em>Cannabis sativa</em></td>
<td>+?</td>
<td>4</td>
<td>(Hegi, 1927)</td>
</tr>
<tr>
<td></td>
<td><em>Humulus lupulus</em> L.</td>
<td>+?</td>
<td>4</td>
<td>(Hegi, 1927)</td>
</tr>
<tr>
<td></td>
<td><em>Urtica sp.</em></td>
<td>+?</td>
<td>4</td>
<td>(Hegi, 1927)</td>
</tr>
</tbody>
</table>

**Key to host status:** + susceptible host; - non-susceptible host; ± poor host permitting temporary parasite growth; +? potentially susceptible host (see method 4 notes)

**Methods:**
1 – Gaertner (1950) infested hosts with dodder vines or vine cuttings
2 – Hegi (1927) did not specify an infestation method
3 – Nobbe and Simon (1904) infested hosts with dodder seeds and seedlings
4 – Hegi’s (1927) ‘infrequent host’ records are presumably based on field observations. The inclusion of a grass species in this list raises questions about whether these plants are truly susceptible hosts, since dodder vines can twine around grasses and other non-host plants as they spread (Dawson et al., 1994).
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ABSTRACT

Parasitic plants in agricultural settings have devastating effects on crop yields and are notoriously difficult to control. New control strategies are needed for parasitic weeds around the world, including for dodder species (Convolvulaceae: Cuscuta spp.) that are noxious pests in North America. Insights into the ecology of these parasites suggest that biocontrol and volatile-based foraging disruption are two strategies that could be developed for improved management. Our research highlights significant challenges to these approaches that should be considered in their development: biocontrol may be beset by the transfer of secondary metabolites from parasites into host plants; and volatile-based parasite diversion will need to overcome the strong appeal of host plant light cues. Alternatively, light-based manipulations may be key to disrupting parasite host-finding and attachment. We present some concepts for implementing this idea.
INTRODUCTION

An estimated one percent of all angiosperms are parasitic on other plants (Heide-Jørgensen, 2008). Only a handful of these species pose serious threats to human food production, most being members of *Striga, Orobanche, Phelipanche, Cuscuta* (Heide-Jørgensen, 2008; Parker and Riches, 1993). Yet these weeds inflict billions of dollars of losses upon farmers around the globe each year (Aly, 2007; Parker, 2009). Managing parasitic weeds is notoriously difficult, due in part to the challenge of selectively eradicating pests that are physically connected and biologically related to crops we intend to protect. Conventional chemical and mechanical control measures are frequently insufficient to avoid economic harm due to these pests, and novel control tactics are needed (Aly, 2007).

Prospects for new control methods are improving with recent insights into the chemical ecology of parasitic plants. The discovery that parasitic plants uptake mRNA’s from their hosts (David-Schwartz et al., 2008; Roney et al., 2007), for example, presents a range of opportunities to disrupt the translation of vital parasite RNAs through silencing RNAs from transgenic crops (Runo et al., 2011; Yoder et al., 2009). Early attempts at this produced mixed but encouraging results that parasites might be controlled in this manner (Alakonya et al., 2012; Aly et al., 2009; Bandaranayake and Yoder, 2013; de Framond et al., 2007; Tomilov et al., 2008). Another strategic albeit older insight—that *Striga* and *Orobanche* parasites germinate in response to short-range chemical cues (strigolactones) from host plant roots (Yoneyama et al., 2013)—has likewise stimulated a surge of research to exploit this process via artificial stimulants that induce “suicidal” seed germination (Logan and Stewart, 1991; Wigchert and Zwanenburg, 1999) and via trap crops or intercrops that release germination stimulants but resist parasite infestation (e.g. cowpea, *Desmodium* spp.) (Bouwmeester et al., 2003; Khan et al., 2010).

Whereas the bulk of this research is focused on management of broomrape and witchweed root parasites (Orobanchaceae), less research has explored novel ecological strategies to thwart dodder aerial parasites (Convolvulaceae). In part this reflects the smaller economic impact of these parasites relative to *Striga* and *Orobanche* spp. on a global scale; nevertheless in the United States dodder is the most pernicious parasitic
weed (Dawson et al., 1994). Dodder infests major economic crops including tomato, potato, alfalfa, citrus, clover, cranberry, sugarbeet and onion, and can impose yield losses of 50-100% in these crops (Lanini and Kogan, 2005). Infestations pose not only acute but also chronic management problems since dodder seeds remain viable for twenty or more years in the soil (Lanini and Kogan, 2005). The ideal management solution is to consistently pant only resistant species (e.g., corn or wheat) where dodder seed banks exist, but this is frequently impractical (e.g., in cranberry bogs or perennial alfalfa fields) (Costea and Tardif, 2006). In susceptible crops, infestations of dodder can be moderated by a combination of herbicidal sprays (if selective herbicides are available), tilling, mowing, hand-picking (on small-scale operations), and, in drastic scenarios, burning the crop (Lanini and Kogan, 2005)—but these methods are costly and only partially effective. There is considerable room for improvement via ecologically-based control strategies.

Two such strategies that have been proposed are biocontrol of dodder (Sandler, 2010; Tóth et al., 2008) and disruption of dodder seedling foraging (Runyon et al., 2010). Research presented in this dissertation holds implications for developing each of these methods that are discussed in the following sections.

**PROSPECTS FOR BIOLOGICAL CONTROL OF DODDER**

Selective control of dodder via insects or pathogens that specialize on these parasites is an attractive option in light of the difficulty of controlling weeds that are intimately associated with valuable host crops. One of the major selection criteria for an ideal biocontrol organism is that it must be capable of overcoming the target plant’s defenses, which typically include both physical and chemical forms of resistance. Plants ward off attack with constitutively present defenses as well as with more aggressive defenses that can be induced by chemical signatures of attack by insects or disease (Dou and Zhou, 2012; Howe and Jander, 2008; Walling, 2000). While the intensity of such defenses can vary widely due to a range of biotic and abiotic factors (Bezemer and van Dam, 2005; De Vos et al., 2006; Falk et al., 2007), in general they pose effective barriers.
to non-specialist herbivores and pathogens. Hence biocontrol programs frequently rely upon specialists organisms that can tolerate or avoid the defenses of their preferred host plants (Macho and Zipfel, 2014; Walling, 2008).

But any organism deployed for the control of plants that are parasitic faces an unusual challenge: the metabolite constituents of parasitic plants are unpredictable. Most parasitic plant species have access to diverse plant chemistries because they infest a wide range of host species (Parker and Riches, 1993). And many studies have shown that parasitic plants obtain not only primary metabolites from their host plants, but also secondary metabolites that mediate allelopathic interactions such as deterring herbivores or inhibiting pathogens (Smith et al., 2013; Stermitz, 1998). Thus, whereas autotrophic plants are limited to defending themselves with the compounds in their metabolic repertoire, parasitic plants are seemingly not: parasites may also defend themselves with their host’s metabolites.

The idea of host plant secondary metabolites bolstering parasite defenses is not new (Atsatt, 1977), but tests of this hypothesis are few (Adler et al., 2001; Stermitz et al., 1989). Adler (2001) found that quinazolidine alkaloids originating in *Lupinus albus* transfer into the root parasite *Castilleja indivisa*, which gains protection against caterpillars. Research presented in this dissertation (Chapters 2 & 3) represents another test of this hypothesis. We found that glucosinolates transfer from Brassicaceous hosts into dodder and thereby alter the palatability of dodder for aphids. This presents new support for the hypothesis that host-derived secondary metabolites can enhance parasite defense against insects. A similar benefit to plant defense against pathogens is conceivable but has not been explicitly tested.

The implication of this work for biocontrol programs is that success may vary as a function of the parasite’s host species. It may be necessary to identify different control organisms for different parasite-host pairs.

In the case of dodder, specialist weevils in a subgroup of the genus *Smicronyx* have been identified as candidates for biocontrol because they are specific to *Cuscuta* spp. and have a direct impact upon seed production (Mahmood et al., 1982; Tóth et al., 2008)—
which is a major priority for biocontrol of parasitic plants (Sauerborn et al., 2007). They feed selectively on dodder and are capable of reducing seed production since the larvae of some species feed within developing dodder fruits (Anderson, 1962; Tóth et al., 2008). Despite numerous references to Smicronyx spp. as potential biocontrol organisms, this possibility has not been successfully attempted (Parker, 1991), and it is possible that host species effects limit the potential usefulness of Smicronyx spp. in agricultural systems.

As a very preliminary test of this hypothesis, we confined field-collected dodder weevils to a novel dodder-host species pair. Adult and larval Smicronyx spp. were collected from flower clusters of Cuscuta gronovii along a stream in Huntington County, PA (40.636258, -77.843862). Parasites grew on jewelweed (Impatiens capensis) and other stream-side vegetation. The weevils species were identified by systematist Robert Anderson (Canadian Museum of Nature) as S. posticus Dietz, S. tychoides LeConte, and S. congestus Casey. (Note: weevils identified as S. tychoides also bear resemblance to the description of S. sculpticullis in Anderson (1962) and therefore identification is pending verification with reference specimens.) We caged a few adult weevils of each type to intact flowers of Cuscuta campestris grown on tomato (Lycopersicum esculentum var. Halley 3155) in a greenhouse. Although this host-host-parasite combination was not present at the collection site, the weevils nevertheless inflicted heavy damage on the flowers by feeding on the peduncles and ovules. Buds with damaged peduncles were frequently aborted. Seeds that were damaged by feeding through the ovule were also aborted. In a few instances the weevils produced offspring in the flower clusters. This was verified by the presence of larvae and seed-feeding in some of the flowers. More testing is needed to determine if these weevil species can complete their lifecycle on the tomato-C. campestris pair, as well as on other parasite-crop pairs. We had only limited success rearing field-collected larvae out to adulthood as many of the larvae succumbed either to desiccation or to fungi. If the rearing conditions were optimized, it should be possible to determine the importance of host species for Smicronyx success.

It is worth noting that secondary metabolite transfer represents only one of multiple ways that host plant identity could alter interactions between dodder and insect biocontrol agents. Host plants may also modulate the floral volatiles of their parasites (Troncoso et
al., 2010), the phenology of parasite flowering (Dawson et al., 1994), and the other significant nutritional factors such as total nitrogen content (Marvier, 1998; Rowntree et al., 2014)—all which could impact the ability of biocontrol organisms to flourish on dodder.

Currently the only registered biocontrol organism for dodder is the pathogen *Alternaria destruens* (Smolder®, by Sylvan BioProducts). One study shows that it can enhance control of dodder in citrus crops when combined with glyphosate (Cook et al., 2009), but it has otherwise performed unreliably in tests with cranberry crops (Sandler, 2010). Whether host-specific metabolites contribute to the variability in the efficacy of *A. destruens* is a matter for further research.

**PROSPECTS FOR DISRUPTION OF DODDER FORAGING**

One of the most remarkable discoveries concerning the ecology of dodder is the finding that seedlings of *C. campestris*—the most important weedy dodder (Dawson et al., 1994)—can respond to plant odors with directed growth responses (Runyon et al., 2006), including positive responses toward sources of select tomato volatiles (β-phellandrene, α-pinene and β-myrcene) and away from sources of (Z)-3-hexenyl acetate, a volatile characteristic of wheat and other non-host grassy plants.

This raises the question of whether it is possible to disrupt parasite foraging by selecting or engineering crop cultivars with repellent odor blends (Runyon et al., 2010). Conceivably these could be used in conjunction with a highly attractive trap crop to create a “push-pull” system for protecting crop plants—a strategy that has been successful against insect pests in some systems (Khan et al., 2010). It should be possible to select or engineer crop varieties with volatile profiles favorable for this purpose: there is good evidence that plant volatile phenotypes reflect heritable genetic traits (Degen et al., 2004; Kariyat et al., 2012), and furthermore there is considerable research attention given to manipulating crop volatile production to favor ecological and/or industrial purposes (i.e., attraction of beneficial pollinators and predators, production of perfume components) (Dudareva and Negre, 2005; Lange and Ahkami, 2012).
While prospects for manipulating crop volatile are encouraging, another key question is whether volatile-based diversion would be are sufficiently influential to overcome dodder’s attraction to host plant light cues. As noted in Chapter 4, light cues from leaves are attractive to foraging *C. campestris* seedlings due to a chlorophyll-mediated reduction in the red:far red (R:FR) light ratio (Benvenuti et al., 2005). The integration of these two types of information (volatiles and light cues) by dodder seedlings has not been studied, and relative importance of these two types of cues are unknown.

We conducted foraging assays with *C. campestris* seedlings that shed some light on this problem. First we conducted a no-choice experiment with *C. campestris* placed adjacent to one of two lines of tomatoes (*Solanum lycopersicum*, variety “Castlemart”) that differ in their volatile production: wildtype tomatoes, which release normal levels of terpenes (that are attractive to *C. campestris* seedlings), and mutant odorless-2 tomatoes, which do not release detectable levels of these compounds (Kang et al., 2010). Dodder seedlings grew toward each of these targets with equal efficiency (17 of 20 seedlings grew to the correct half in each group). Thus the volatile cues from the wildtype tomato did not increase the attractiveness of these plants in our assay. We hypothesized that volatile cues could nevertheless enable seedlings to choose between two plants with similar light cues. But in a choice test between wildtype and odorless-2 tomatoes, the seedlings did not make a significant choice: 10 grew toward the wildtype plants and 7 grew toward the odorless-2 plants (Exact Binomial Test: $P = 0.6291$). If the observed trend to favor wildtype plants is not random, a (logistically unfeasible) sample size of 136 replicates would be required to obtain significant results at the $\alpha = 0.05$ confidence level. Even then, this difference could be attributed to subtle differences in light cues from the two tomato lines, since the mutant plants have divergent leaf shape and trichome morphology (Kang et al., 2010). These results suggest that volatile cues play a relatively minor role as compared to light cues in attracting *C. campestris* seedlings.

This is likely to be true in the case of flax dodder (*Cuscuta epilinum*), as well. We found no evidence that volatile cues contribute to the attractiveness of flax plants for *C. epilinum* seedlings, nor did blocking volatile cues from host plants result in significant
differences in the seedlings choice between tomato and flax (Chapter 4). In fact, the seedlings’ preference for tomato, a poor host plant, could be reproduced in a choice test between model tomato and flax plants that mimic plant light cues (low R:FR) without releasing any plant volatiles. Considering that plants with a low, branching architecture (like tomato) produce a stronger R:FR signal at the ground level (where seedlings are foraging) than similar sized plants with a tall, linear architecture (like flax), a parsimonious explanation for these data is that seedlings are positively phototropic toward light with the lowest R:FR signature. This could be adaptive because nearby plants produce a stronger reduction in R:FR than far-off plants of equal size (Chapter 4) and it is critical for dodder seedlings to locate nearby hosts (Benvenuti et al., 2005). Thus dodders may ultimately prioritize light over volatile signals due to strong selection pressure to locate the nearest host plant.

The preponderance of light cues among other host cues in attracting dodder seedlings suggests that volatile-based crop protection strategies would have little likelihood of making a marked difference on parasite infestation rates. We have not tested the relative strength of repulsion via (Z)-3-hexenyl acetate versus light-mediate attraction, and this could still produce information useful for dodder control. But in Runyon’s study (2006) seedlings grew toward wheat plants—in spite of their production of (Z)-3-hexenyl acetate—when other host choices were not present. When interpreted in light of the above studies, this may also indicate that light cues from nearby plants (even wheat) are more influential than odor cues from the same, thus limiting the utility of volatile-based management schemes.

On the other hand, the attraction of dodder seedlings to light signals that are common to all photosynthetic plants could be exploited to divert parasite seedling attack from crop plants to trap crops. Since seedlings of *C. epilinum* and *C. campestris* grow toward the lowest R:FR ratio, and R:FR reduction appears stronger at shorter distances (Chapter 4, Benvenuti et al., 2005), a high-density trap crop may efficiently reduce parasite attachment to crop plants. In practice, the trap crop would need be absolutely resistant to dodders, since some poor host species (such as tomato) may permit parasites to grow temporarily and access more suitable but distant host plants. Most members of
the Poaceae are completely resistant to dodder and would make suitable candidates for this purpose (Dawson et al., 1994), although their potential to function as weeds themselves would need to be evaluated for each cropping system. One alternative that could avoid this complication would be to employ artificial trap plants as decoys, or perhaps even a continuous fence of low R:FR transmitting plastic, to draw seedlings away from crop plants. Our foraging experiments in Chapter 4 demonstrate that seedlings can be strongly attracted to artificial plants with low R:FR profiles.

Another approach would be to disrupt the reception or transmission of light cues that mediate foraging and attachment. It is likely that dodder’s perception of R:FR ratios occurs via phytochrome B (Furuhashi et al., 1997); if phytochrome B reception or signal cascades could be inhibited in dodder, this would likely inhibit host recognition and/or the formation of haustoria—but we are not aware of chemical means to accomplish this, and it would be difficult to effectively implement such a control strategy because seedlings emerge throughout the growing season (Dawson et al., 1994; Sandler, 2010). Rather than disrupting light perception by seedlings, it may be more feasible to alter light cue transmission. We have noted that seedlings in artificial light conditions fail to attach to host plants when light is provided exclusively by low-far-red sources such as florescent bulbs, high pressure sodium bulbs and metal halide bulbs, whereas seedlings illuminated by incandescent bulbs, a high FR source, attach efficiently. Sunlight also contains ample FR light to facilitate dodder attachment, but selective removal of FR wavelengths (e.g., with photoselective plastic) can completely disrupt foraging and attachment (Johnson, 2013). This suggests that manipulation of ambient light is another promising tool to prevent dodder infestations.

CONCLUSIONS

Two proposed ecological control concepts for dodder face considerable challenges for implementation. Biocontrol programs will need to account for the diversity of secondary metabolites that parasites obtain from their host plants that could influence parasite-antagonist interactions. Further research is needed to determine if host identity poses a
substantial problem to specialist *Smicronyx* weevils for dodder control in agricultural settings. We have also identified a significant hurdle for volatile-based control concepts in the preponderance of light signals among other host plant cues for seedling foraging. Yet light-mediated host choice presents new opportunities for controlling dodders. Further field studies are merited to determine whether parasite foraging can be efficiently disrupted with photosynthetic trap crops, decoy plants and/or environmental light manipulations.
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