

# Manipulation of light spectral quality disrupts host location and attachment by parasitic plants in the genus *Cuscuta*

Beth I. Johnson<sup>1</sup>, Consuelo M. De Moraes<sup>2</sup> and Mark C. Mescher<sup>2\*</sup>

<sup>1</sup>Biology Department, Juniata College, Huntingdon, PA 16652, USA; and <sup>2</sup>Department of Environmental Systems Science, ETH Zürich, Zürich 8092, Switzerland

## Summary

1. Parasitic plants in the genus *Cuscuta* (dodders) make their living by extracting resources from other plants. While relatively few dodder species are agricultural pests, those that are can be challenging to control, in part due to their intimate physical and physiological association with host plants. Consequently, dodders remain pervasive and economically damaging pests in a variety of crop systems. The development of improved management strategies would be facilitated by greater understanding of the ecological and environmental factors that influence the establishment and perpetuation of dodder infestations.

2. Light cues play an important role in dodder host location and attachment. To better understand the influence of light conditions on parasite ecology, and potential implications for management, we examined how manipulating the ratio of red to far-red wavelengths (R:FR), via both passive filtering of natural sunlight and active spectral manipulation using LEDs, affects host location and host attachment by two dodder species (*C. campestris* on tomato hosts and *C. gronovii* on jewelweed).

3. For both host–parasite combinations, host location and subsequent attachment by dodder parasites was dramatically reduced in high-R:FR environments compared to control conditions (with R:FR characteristic of sunlight) and low-R:FR conditions. Circumnutation by dodder seedlings was also significantly faster under high R:FR.

4. We observed short-term effects of high R:FR on the height and dry mass of tomato host plants (immediately following 7-day exposure), as well as changes in tomato volatile emissions. However, preliminary investigation of long-term effects on host plants suggests that short-term exposure to high R:FR (i.e. during the critical period when dodder seedlings emerge and attach to hosts) has little or no effect on host plant size or fruit yield at the time of harvest.

5. *Synthesis and applications.* Our findings suggest that spectral manipulation during the early stages of crop plant growth (e.g. via light-filtering row covers) may have significant potential to augment existing methods for managing or preventing dodder infestations in agricultural crops. We discuss potential obstacles to the realization of its potential, as well as next steps towards the development and optimization of spectral manipulation methods for use in agroecosystems.

**Key-words:** *Cuscuta*, dodder, light spectral manipulation, light-emitting diodes, non-chemical weed control, parasitic plants, photoselective film, ratio of red to far-red light (R:FR)

## Introduction

Parasitic plants are widespread and ecologically important components of many natural plant communities (Press &

Phoenix 2005; Costea & Stefanović 2009). Furthermore, some parasitic plant species are highly damaging agricultural pests that pose significant threats to regional and global commodity and food production (Cudney, Orloff & Reints 1992; Goldwasser, Lanini & Wrobel 2001; Aly 2007; Parker 2012). Nevertheless, parasitic

\*Correspondence author. E-mail: mescher@usys.ethz.ch

plants currently remain understudied relative to their ecological and economic significance (Pennings & Callaway 2002; Press & Phoenix 2005). In particular, we know relatively little about the ecological and environmental factors that influence the establishment and perpetuation of parasite populations under natural conditions. Improved understanding of these factors thus has significant potential to inform the development of new strategies for managing plant parasites in agricultural (and natural) ecosystems.

For parasites in the genus *Cuscuta* (Convolvulaceae; commonly called dodders), successful parasitism depends critically on efficient host-location mechanisms, as the free-living seedlings of these vine-growing species actively 'forage' for nearby plants (Lyshede 1985; Kelly 1990; Koch, Binder & Sanders 2004) and, being obligate parasites, must rapidly make an attachment to a susceptible host before expending their limited resources (Lanini & Kogan 2005; Costea & Tardif 2006). World-wide, nearly 200 species of *Cuscuta* (Costea 2007 onward) parasitize a broad range of herbaceous and woody host plants. While only 10–15 of these species are considered economically important pests (Parker & Riches 1993; Costea 2007 onward), their broad geographic and host ranges contribute to impacts on a wide range of agricultural crop systems (Dawson *et al.* 1994; Costea & Tardif 2006). For example, dodder infestations have been reported to cause crop losses of 40–75% in alfalfa (Dawson 1989; Cudney, Orloff & Reints 1992; Parker 2012), 50–75% in tomato (Goldwasser, Lanini & Wrobel 2001; Lanini 2004), 85% in niger seed (Mishra *et al.* 2007), 70–90% in carrot (Bewick, Binning & Dana 1988) and 80–100% in cranberry (Sandler 2010).

Several features of dodder ecology complicate management efforts, including their intimate physiological connections with hosts (Dawson *et al.* 1994) and their production of resilient seed banks that are capable of causing new annual infestations for decades (Dawson *et al.* 1994). No currently available control method consistently eradicates dodder infestations; however, several tactics are employed with varying rates of success (and varying costs of application), including hand removal, targeted herbicide application, mowing or burning infested fields, crop rotation with non-host (often monocotyledonous) species, transplanting large greenhouse-grown crop seedlings that can resist attack by dodder seedlings (though not by mature dodder vines), and the use of resistant crop varieties (Cudney, Orloff & Reints 1992; Dawson *et al.* 1994; Goldwasser, Lanini & Wrobel 2001; Lanini & Kogan 2005; Costea & Tardif 2006). Control efforts are generally most effective when two or more of these tactics are deployed in combination (Lanini & Kogan 2005); however, there remains a strong need for effective and less costly control methods (Sandler 2010).

The development of such control strategies would be facilitated by improved understanding of the ecological

and environmental factors that influence the establishment of dodder infestations, including the cues that mediate host location and attachment. Dodder seedlings have previously been shown to exhibit directed growth towards potential hosts in response to light cues (Lane & Kasperbauer 1965; Orr, Haidar & Orr 1996) as well as host plant odours (Runyon, Mescher & De Moraes 2006). With respect to the former, previous work has documented phototropism towards far-red light sources (e.g. Orr, Haidar & Orr 1996), which – under otherwise normal lighting conditions – yields reduced ratios of red to far-red (R:FR) light similar to those created by the differential absorption and reflectance of these wavelengths by plant tissues (Ballaré & Casal 2000). Phototropism towards regions of low R:FR thus likely helps dodder seedlings to grow towards nearby plants (Orr, Haidar & Orr 1996) and perhaps even to discriminate among hosts based on differences in chlorophyll content (Benvenuti *et al.* 2005). Similar light cues have also been implicated in the elicitation of 'shade-avoidance' growth patterns (Ballaré, Scopel & Sánchez 1990) in other (non-parasitic) plant species.

In addition to the phototropic responses discussed above, exposure to far-red light (700–800 nm) in the absence of other wavelengths has been shown to mediate twining of dodder vines around host stems (Lane & Kasperbauer 1965) and the production of haustoria (the organs that penetrate host plant tissues) (Furuhashi, Kanno & Morita 1995; Tada, Sugai & Furuhashi 1996; Furuhashi *et al.* 1997), while exposure to red light (620–700 nm) in the absence of other wavelengths inhibits twining (Lane & Kasperbauer 1965) and haustoria production (Furuhashi, Kanno & Morita 1995; Furuhashi *et al.* 1997). However, simultaneous exposure to red and far-red light can have variable effects depending on the specific treatment. For example, Haidar, Orr & Westra (1997) discovered that *brief exposure* to red light followed by a flash of far-red *inhibits* twining and prehaustoria formation, while *prolonged exposure* to red light followed by far-red *promotes* twining and prehaustoria. Dodder growth responses can also be influenced by other factors including phytohormone levels (Haidar, Orr & Westra 1997, 1998), exposure to other wavelengths of light (e.g. blue light, Furuhashi, Kanno & Morita 1995; Haidar, Orr & Westra 1998) and tactile cues (Tada, Sugai & Furuhashi 1996).

The current study builds on previous findings by exploring the effects of altered light quality on rates of successful parasitism (location of and attachment to host plants) for two parasite–host combinations, *C. campestris* on tomato (*Solanum lycopersicum*) and *C. gronovii* on jewelweed (*Impatiens capensis*), under semi-natural conditions. In particular, we aimed to determine whether parasite establishment can be effectively disrupted by altering ratios of R:FR light. This question has applied relevance, as it is possible to envision the implementation of such manipulations in agricultural settings (e.g. via light-filtering row covers), particularly given the relatively

short temporal window during which dodder seedlings must emerge and attach to susceptible host seedlings (Dawson *et al.* 1994).

To explore the potential of this approach, we employed two different methods of light spectral manipulation: (i) passive filtration of ambient sunlight and (ii) active manipulation via LED light sources. Using each of these manipulation methods, we tested rates of successful host location (i.e. directed growth towards host plants) by dodder seedlings and (separately) rates of successful attachment once the parasite encountered a host under three different lighting conditions, including (i) control conditions approximating the spectral characteristics of natural sunlight; (ii) low R:FR (relative to sunlight), reflecting spectral conditions similar to those encountered beneath the shade of plants (which have previously been suggested to serve as a positive phototropic cue for dodder seedlings); and (iii) high R:FR, representing spectral conditions unlikely to occur in natural settings and whose effects on dodder performance have not previously been explored. We also examined the effects of spectral manipulation on parasite circumnutation patterns, the short- and long-term growth of tomato host plants and host plant biochemistry (including phytohormone levels and volatile emissions).

## Materials and methods

### STUDY ORGANISMS

We employed two dodder species and appropriate hosts: *Cuscuta campestris* with tomato (*Solanaceae: Solanum lycopersicum* var. Halley 3155) and *Cuscuta gronovii* with jewelweed (*Balsaminaceae: Impatiens capensis*). *Cuscuta campestris* seeds were collected in 2010 by W.T. Lanini from infested tomato (Yolo County, CA, USA); *C. gronovii* seeds were collected by J.D. Smith in 2010 from infested jewelweed (Centre County, PA, USA). Dodder seeds were refrigerated until use, and germination was induced via scarification with concentrated sulphuric acid (1 h). Tomato plants were grown from seeds in an insect-free growth chamber at 23 °C under a 16-h photoperiod (425  $\mu\text{mol m}^{-2} \text{s}^{-1}$  provided by 215-W cool white fluorescent tubes; see Fig. S1 in Supporting Information). Naturally occurring jewelweed seedlings were collected in April 2013 (University

Park, PA, USA), transplanted to greenhouse trays with 5 g of Osmocote® fertilizer (NPK: 14-14-14; The Scotts Company, Marysville, OH, USA) and grown outdoors until used for experiments.

### PASSIVE LIGHT MANIPULATION

We used various photosensitive films to create three experimental light treatments: (i) a low-R:FR treatment employed Roscolux #4330 Calcolor 30 Cyan polyethylene terephthalate film (Rosco Laboratories, Stamford, CT, USA); (ii) a high-R:FR treatment employed Solatrol polyethylene film (BPI Visqueen Horticultural Products, Ayrshire, Scotland); and (iii) a control treatment, with R:FR similar to sunlight but reduced light transmission approximating the other treatments (Table 1), employed a non-selective film, E-Colour+ #130 Clear (Rosco Laboratories) together with Agribon+ AG-19 row cover shade cloth (Johnny's Selected Seeds, Waterville, ME, USA). A PS-300 spectroradiometer (Apogee Instruments, Logan, UT, USA) was used to document the photosynthetic photon flux density of each R:FR treatment and of unfiltered sunlight (Fig. S2).

All experiments were conducted outdoors in opaque boxes (55.9 cm L  $\times$  30.5 cm W  $\times$  10.1 cm H<sub>1</sub>  $\times$  20.3 cm H<sub>2</sub>) made from white corrugated plastic, which enclosed greenhouse trays containing dodder and host seedlings. The tops of the boxes were covered with one of the R:FR treatment films. A DC-powered fan (80 mm  $\times$  80 mm  $\times$  25 mm; Sunon Inc., Brea, CA, USA) inside each box ran from 06:00–22:00 daily to exhaust excessive heat.

### Foraging assay

To determine whether the ratio of R:FR influences the ability of *C. campestris* seedlings to locate tomato hosts, we measured the direction of dodder seedling growth relative to the position of nearby tomato seedlings within a circular foraging arena. Seven days after planting, tomato seedlings with expanded cotyledons were transplanted to greenhouse trays and moved to an insect-free greenhouse for 2–4 days before being transported outside for experiments. On the first day of the experiment, newly germinated *C. campestris* seedlings (2–3 cm long) were planted 4.5 cm from the stem of tomato hosts ( $n = 84$  high R:FR, 83 low R:FR, 79 control). Plants were watered daily. After 7 days, we defined a circular perimeter around the base of each parasite seedling and recorded the half (and quadrant) of the resulting 'foraging arena' in which the seedling resided with respect to the location of the

**Table 1.** The ratio of red (650–670 nm) to far-red (710–740 nm) wavelengths (R:FR), photosynthetic active radiation (summation of radiation between 400 and 700 nm, PAR), percentage transmission and average daily temperature for the photosensitive film and ELVIS treatments

Source	Treatment	Ratio of R:FR	PAR	Transmission (%)	Avg. daily temperature (°C)
Sunlight	(Reference)	0.691	1934.9	100	–
Photosensitive film	Control	0.659	1377.4	71.2	21.5
	Low	0.391	1280.8	66.2	21.8
	High	1.697	1484.4	76.7	21.7
	Control	0.690	156.2	–	–
ELVIS	Low	0.395	155.6	–	–
	High	1.703	155.9	–	–



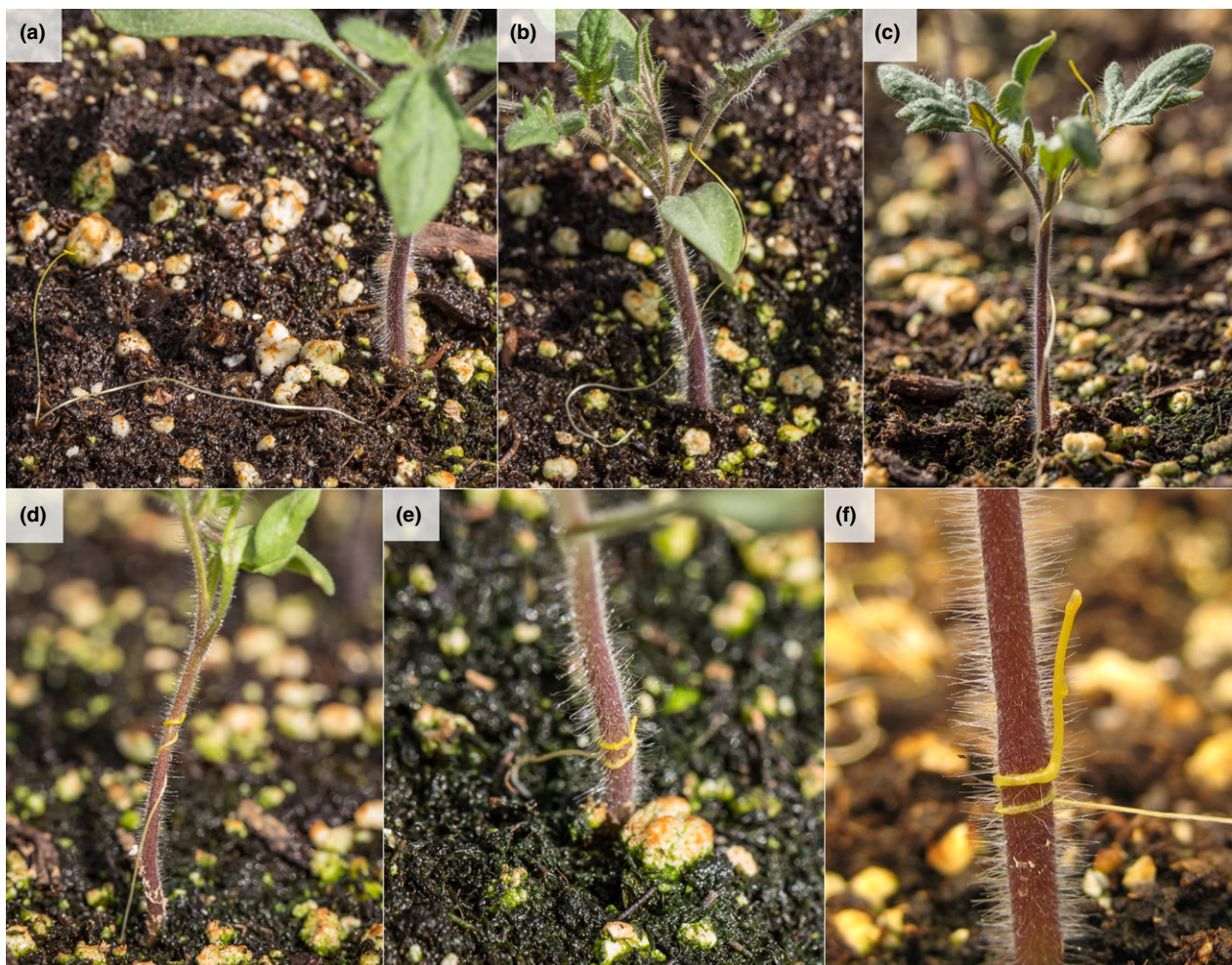
tomato host (modified from the methods of Runyon, Mescher & De Moraes 2006). Data were analysed by chi-square in Minitab [version 14.1, Minitab Inc., State College, PA, USA].

### Parasitism assay

To determine whether R:FR influences the ability of *Cuscuta* seedlings to successfully attach to hosts, we measured rates of parasitism over time for *C. campestris* on tomato hosts and *C. gronovii* on jewelweed. These assays employed the same treatments described above, but with newly germinated *Cuscuta* seedlings planted 1 cm from the stem of their respective host plants to ensure contact with the host (*C. campestris*:  $n = 180$  for all treatments; *C. gronovii*:  $n = 22$  high R:FR, 21 low R:FR, 22 control). After 7 days (*C. campestris*) or 10 days (*C. gronovii*), the progression of dodder parasitism was categorized as *No Host Contact*, *Unsuccessful Attachment* (seedlings that contacted, twined or coiled around the host, but without evidence of haustoria formation) or *Successful Attachment* (seedlings that formed prehaustoria or mature haustoria) (Fig. 1). Data were analysed using binary logistic regression when test assumptions were met.

### Effects of altered R:FR on plant growth and biochemistry

To assess how exposure to altered R:FR affects short-term plant growth, *C. campestris* seedlings and tomato hosts were grown (separately) for 7 days beneath each of the three light treatments. At the conclusion of the experiment, we measured parasite seedling length ( $n = 30$  high R:FR, 30 low R:FR, 29 control). For tomato hosts, we measured the number of expanded leaves, height and dry mass ( $n = 30$  for all treatments), as well as constitutive levels of phytohormones (total jasmonic acid, JA; *cis*- and *trans*-isomers of JA; and total salicylic acid, SA;  $n = 11$  for all treatments) (see Appendix S1 for detailed methods).



**Fig. 1.** Primary dodder parasitism progression categories: (a) *No Host Contact*; (b–d) *Unsuccessful Attachment*; (e, f) *Successful Attachment*. Description of each stage of parasitism: (a) *No Host Contact* – the parasite does not touch any part of the host (including trichomes); (b) *Contact* – the parasite contacts the host but does not entwine more than 360°; (c) *Loose Twine* – the parasite entwines more than 360° but not in a tight coiling fashion; (d) *Close Coil* – the parasite entwines more than 360° as a tight coil (usually to the stem or petiole), but no haustorial swellings are present; (e) *Prehaustoria* – the parasite coils tightly and small haustorial swellings are observed at the interface between the parasite and host tissue; (f) *Haustoria* – haustorial swellings are prominent (the parasite has formed connections to host xylem and phloem) and a new parasitic shoot possessing an apical budlike scale emerges from the site of attachment. Photograph credits: Nick Sloff (a–e), Beth Johnson (f).

As a preliminary attempt to determine whether (relatively) brief exposure to altered R:FR affects the longer-term growth of and fruit yield of tomato plants, we grew tomato seedlings in the treatment boxes for 7 days and then transplanted them to a common garden where they were exposed to natural sunlight until they were harvested 54 days later (we characterize this attempt as preliminary because we were limited to three tomatoes for each treatment). At the conclusion of the experiment, we measured total above-ground dry mass, fruit yield, fruit fresh and dry mass, as well as numbers of open and senesced flowers and unopened flower buds. Short- and long-term effects of R:FR on plant growth were analysed using either the general linear model (GLM) with treatment as a fixed factor and Tukey's test as a post hoc analysis or nonparametric analyses (Kruskal–Wallis, Mann–Whitney *U*-test) when GLM assumptions were not met.

To examine R:FR effects on the constitutive production of tomato host volatiles, we collected volatiles from potted tomatoes (each pot contained four 18-day old plants) exposed to one of the R:FR treatments ( $n = 12$  pots for each treatment). R:FR was manipulated by affixing photosensitive film to the outside of glass collection domes to prevent film-related odours from contaminating plant samples. Collections occurred in three separate 48-h replicates (four pots per treatment per replicate) conducted in a greenhouse in ambient sunlight (University Park, PA, USA; July 2013, approximately a 16-h photoperiod) and employed a push–pull collection system (Analytical Research Systems, Gainesville, FL, USA) (methods in Appendix S1). Data were analysed using a GLM with replicate as a random blocking factor.

#### ACTIVE LIGHT MANIPULATION

To actively manipulate light spectral quality and intensity, we developed a custom LED light array called ELVIS (Effulgent LED Variable Intensity Spectrum), comprising emitters with different spectra (UV, green, red, deep red, far-red, infrared, neutral white and warm white) (LED Engin Inc., San Jose, CA, USA) driven with a constant current controller (TheLEDart.com, Brooklyn, NY, USA) (Fig. S3). The intensity of each channel was regulated using an open-source microcontroller (Arduino, Ivrea, Italy) and pulse-width-modulated breakout board (ADA Fruit, New York City, NY, USA). The R:FR of each ELVIS treatment was similar to the R:FR of the photosensitive films, while light intensity (the amount of photosynthetically active radiation) was similar across all ELVIS R:FR treatments (Table 1 and Fig. S4).

#### Parasitism assay

We conducted a parasitism assay similar to that described above for passive light manipulation, except that plants were instead

exposed to the high, low and control R:FR treatments generated by ELVIS (16-h photoperiod;  $n = 15$  for each treatment). At the conclusion of the experiment, parasitism progression was categorized as above (Fig. 1) and data were analysed as in the previous parasitism assay.

#### Circumnutation assay

To assess how R:FR affects the foraging behaviour of *C. campestris* seedlings, newly germinated seedlings (2–3 cm long) were planted in general-purpose potting soil and subjected to high, low and control R:FR treatments generated by ELVIS in the absence of host plants (24-h photoperiod;  $n = 8$  for each treatment; Fig. S4). Light, temperature and relative humidity were monitored using a HOBO<sup>®</sup> model U12-012 data logger (Onset Computer Corporation, Bourne, MA, USA). Seedlings were photographed from above at 4 min intervals with a Nikon D200 camera (Nikon Corporation, Tokyo, Japan). We calculated the time required by each seedling to complete each 360° rotation (using the photograph time stamps), and then determined the average circumnutation period for individual seedlings over the duration of normal circumnutation movements until seedlings fell and made contact with the soil. Data were analysed with a GLM (R:FR treatment was a fixed factor and temperature was a covariate in the model) and with Tukey's test as a post hoc analysis.

## Results

#### PASSIVE LIGHT MANIPULATION

##### Foraging assay

A significant proportion of *C. campestris* seedlings grew towards the target tomato hosts under the control R:FR treatment (arena half:  $\chi^2 = 30.39$ ,  $P < 0.000$ ; arena quadrant:  $\chi^2 = 84.19$ ,  $P < 0.000$ ), and similar results were observed for the low-R:FR treatment (arena half:  $\chi^2 = 18.33$ ,  $P < 0.000$ ; arena quadrant:  $\chi^2 = 49.10$ ,  $P < 0.000$ ) (Table 2). However, seedlings subjected to the high-R:FR treatment exhibited no clear evidence of directed growth towards tomato hosts (arena half:  $\chi^2 = 0.762$ ,  $P = 0.383$ ; arena quadrant:  $\chi^2 = 3.333$ ,  $P = 0.343$ ) (Table 2).

##### Parasitism assay

*Cuscuta campestris*: Almost all seedlings subjected to the control (98.9%) and low-R:FR (94.5%) treatments had reached the *Successful Attachment* category after 7 days,

**Table 2.** The number of *C. campestris* seedlings occupying the focal half and quadrant of the foraging arena after 7 days of foraging beneath the photosensitive film treatments

Photosensitive Film R:FR Treatment	Seedlings choosing arena half				Seedlings choosing arena quadrant					
	Toward Tomato	Away	$\chi^2$	$P$	Toward tomato	Left	Right	Away	$\chi^2$	$P$
Control	64	15	30.39	0.000	55	7	10	7	84.19	0.000
Low	61	22	18.33	0.000	48	10	16	9	49.10	0.000
High	38	46	0.76	0.383	19	28	17	20	3.33	0.343



while few seedlings subjected to the high-R:FR treatment (15.0%) had done so (Table 3). Indeed, 25.0% of seedlings exposed to high R:FR were not in contact with the host and 60.0% remained in the *Unsuccessful Attachment* category (Table 3) – in preliminary assays (not presented here), we observed that seedlings remaining in this category at day 7 almost never go on to successfully attach. Using binary logistic regression to compare the proportion of *C. campestris* seedlings at the *Haustoria* stage (in the *Successful Attachment* category) vs. all other stages of parasitism revealed no statistical difference between the control and low-R:FR treatments ( $Z = -1.03$ ,  $P = 0.305$ ), while there was a clear difference between the control and high-R:FR treatments ( $Z = -9.86$ ,  $P < 0.000$ ).

*Cuscuta gronovii*: All seedlings in the control and low-R:FR treatments had reached the *Successful Attachment* category after 10 days, compared to 27.2% of seedlings in the high-R:FR treatment (Table 3). The majority of seedlings in the high-R:FR treatment (68.2%) were not in contact with their hosts (Table 3). (These data were not analysed using binary logistic regression as this test requires a larger sample size than was available from our data set.) The initial parasitism success by *C. gronovii* seedlings beneath the control and low-R:FR treatments resulted in extensive infestations by day 25 compared to the relatively mild infestation observed for the high-R:FR treatment (Fig. 2).

#### Effects of altered R:FR on plant growth and biochemistry

Exposure to the R:FR treatments did not affect parasite (*C. campestris*) seedling length (GLM:  $F = 1.23$ ,  $P = 0.296$ ; Fig. S5a). For tomato hosts, we observed no short-term effects on the number of fully expanded leaves (Kruskal–Wallis:  $H = 3.80$ , d.f. = 3,  $P = 0.284$ ; Fig. S5b) or on phytohormones (total JA GLM:  $F = 0.88$ ,  $P = 0.427$ ; *cis*-JA GLM:  $F = 0.96$ ,  $P = 0.394$ ; *trans*-JA GLM:  $F = 1.74$ ,  $P =$

0.195; SA GLM:  $F = 0.38$ ,  $P = 0.685$ ; Fig. S6). However, R:FR treatment did have short-term effects on host plant height (Fig. S5c), with tomatoes exposed to low R:FR being tallest and those exposed to high R:FR shortest (Mann–Whitney:  $U_{\text{control vs low}} = 776.0$ ,  $\text{median}_{\text{control}} = 5$ ,  $\text{median}_{\text{low}} = 5$ ,  $P < 0.018$ ;  $U_{\text{low vs high}} = 1245.0$ ,  $\text{median}_{\text{low}} = 5$ ,  $\text{median}_{\text{high}} = 4$ ,  $P < 0.000$ ;  $U_{\text{control vs high}} = 1160.0$ ,  $\text{median}_{\text{control}} = 5$ ,  $\text{median}_{\text{high}} = 4$ ,  $P < 0.000$ ). Plants exposed to control R:FR also had greater dry mass than plants in either the low- or high-R:FR treatments (GLM:  $F = 11.69$ ,  $P < 0.000$ ) (Fig. S5d).

In longer-term trials, tomato plants initially exposed to high R:FR for 7 days and then to natural sunlight had significantly more unopened buds at harvest (54 days later) compared to control plants or those initially exposed to low-R:FR conditions (GLM:  $F = 32.3$ ,  $P < 0.001$ ; Fig. S7a). However, none of the other traits



**Fig. 2.** *Cuscuta gronovii* parasitizing jewelweed hosts after exposure to high R:FR (left tray), low R:FR (centre) and control R:FR (right tray). Plants grew beneath the photosensitive film treatments for the first 10 days and then grew in full sunlight for an additional 15 days. Note the striking differences in parasite infestation and host plant size among the R:FR treatments. Photograph credit: Beth Johnson.

**Table 3.** Parasitism progression of *Cuscuta* seedlings after exposure to the photosensitive film and ELVIS treatments

		Parasitism progression (% in each category)						
		No host contact	Unsuccessful attachment			Successful attachment		
Source	R:FR treatment	No contact	Contact	Loose twine	Close coil	Prehaustoria	Haustoria	<i>n</i>
<i>C. campestris</i> on tomato hosts								
Photoselective film	Control	0.6	0	0.6	0	27.2	71.7	180
	Low	4.4	0.6	0	0.6	27.8	66.7	180
	High	25.0	16.7	35.0	8.3	11.1	3.9	180
<i>C. gronovii</i> on jewelweed hosts								
Photoselective Film	Control	0	0	0	0	0	100	22
	Low	0	0	0	0	0	100	21
	High	68.2	4.6	0	0	13.6	13.6	22
<i>C. campestris</i> on tomato hosts								
ELVIS	Control	26.7	0	6.7	0	20.0	46.7	15
	Low	20	0	0	0	53.3	26.7	15
	High	20	13.3	66.7	0	0	0	15

that we measured (including fruit yield and weight) differed as a result of exposure to the R:FR treatments (open flowers GLM:  $F = 0.05$ ,  $P = 0.949$ ; senesced flowers GLM:  $F = 0.48$ ,  $P = 0.641$ ; fruit yield GLM:  $F = 0.07$ ,  $P = 0.930$ ; fruit fresh mass GLM:  $F = 0.25$ ,  $P = 0.786$ ; fruit dry mass Kruskal–Wallis:  $H = 0.37$ , d.f. = 2,  $P = 0.832$ ; total above-ground dry mass GLM:  $F = 0.02$ ,  $P = 0.982$ ; Fig. S7b–g).

We observed a marginal treatment effect on the total volatile emissions by tomato, with a tendency for lower emissions under high R:FR compared to low R:FR (GLM:  $F = 2.50$ ,  $P = 0.097$ ; Tukey's test: control vs. low:  $P = 0.392$ ; control vs. high:  $P = 0.646$ ; low vs. high:  $P = 0.082$ ; Fig. S8a). Three individual compounds, (+)-4-carene (GLM:  $F = 3.43$ ,  $P < 0.044$ ),  $\beta$ -phellandrene (GLM:  $F = 3.48$ ,  $P < 0.043$ ) and  $\beta$ -caryophyllene (GLM:  $F = 9.54$ ,  $P < 0.001$ ), also showed significant differences in production across treatments, with each exhibiting lower emissions under high R:FR compared to low R:FR (Fig. S8b). Compounds present but not affected by R:FR included  $\alpha$ -pinene,  $p$ -cymene,  $\beta$ -myrcene, ( $E$ )- $\beta$ -ocimene, ( $Z$ )- $\beta$ -ocimene,  $\alpha$ -terpinolene, nonanal, octanoic acid, pentadecane, 2,4-bis(1,1-dimethylethyl)-phenol and 16 unidentified compounds (data not shown).

#### ACTIVE LIGHT MANIPULATION

##### Parasitism assay

After 7 days, the majority of *C. campestris* seedlings subjected to the control and low-R:FR ELVIS treatments had reached the *Successful Attachment* category (66.7% and 80.0%, respectively), while none of the seedlings subjected to the high-R:FR treatment had done so (Table 3). The majority of seedlings beneath the high-R:FR treatment (80.0%) remained in the *Unsuccessful Attachment* category (Table 3). (These data were not analysed using binary logistic regression as this test requires a larger sample size than was available from our data set.)

##### Circumnutation assay

R:FR treatment significantly influenced the period of circumnutation of *C. campestris* seedlings (GLM<sub>Treatment</sub>:  $F = 7.09$ ,  $P < 0.005$ ; Fig. S9). The average circumnutation period was shortest in high-R:FR environments (92.1 min  $\pm$  2.9) and significantly longer in control (101.3 min  $\pm$  1.8) and low-R:FR environments (104.6 min  $\pm$  3.0). Ambient temperature, a covariate in the model, did not contribute to this result (GLM<sub>Temperature</sub>:  $F = 2.42$ ,  $P = 0.136$ ).

#### Discussion

To the best of our knowledge, this study is the first to explore the potential applied use of light spectral manipulation techniques as a pest management strategy

to disrupt host acquisition by parasitic plants. Our results demonstrate that manipulation of light spectral quality can significantly impact both the ability of parasitic dodder seedlings to locate nearby hosts and their ability to make successful attachments following host contact. In particular, experimental treatments that produced R:FR ratios significantly higher than those observed in sunlight – representing light conditions that are not found in nature and whose effects on parasite performance have not previously been documented – resulted in dramatic reductions in host location (Table 2) and attachment success (Table 3). Indeed, the results of our foraging assay were consistent with completely random growth by parasite seedlings under high-R:FR conditions, as only 23% of seedlings occupied the quadrant of the foraging arena nearest the host in high-R:FR treatments (compared to a 25% expectation from random chance and the 70% of seedlings occupying this quadrant under control light conditions). Moreover, even when seedlings were placed directly adjacent to hosts, relatively few (ranging from 0 to 27% across several assays) were able to successfully attach. Furthermore, our limited investigation of impacts on host plants suggests that spectral manipulation during the period of maximal susceptibility to dodder infestation might have relatively minor impacts on longer-term plant growth and yield (Fig. S7).

Despite the strong effects of R:FR on parasite host location and attachment, we observed no effect of our treatments on the growth (i.e. length) of parasite seedlings. Circumnutation by parasite seedlings was significantly faster in high-R:FR conditions compared to the other treatments (Fig. S9), but it is unclear whether and how this effect relates to the observed reduction in parasite foraging success. We also observed significant short-term effects of our R:FR treatments on host plants, as tomato plants exposed to high R:FR for 7 days and harvested immediately were significantly shorter and had reduced dry mass compared to controls, although they did not exhibit differences in the number of expanded leaves or constitutive levels of phytohormones (Figs S5 and S6). In a separate assay, we found that tomatoes exposed to high R:FR released significantly fewer quantities of the plant volatiles (+)-4-carene,  $\beta$ -phellandrene and  $\beta$ -caryophyllene compared to those in low-R:FR conditions (Fig. S8). Interestingly, dodder has previously been reported to exhibit directed growth towards  $\beta$ -phellandrene (Runyon, Mescher & De Moraes 2006). Thus, it is conceivable that the effects of high R:FR on volatile emissions may attenuate olfactory cues that play a role in host location by the parasite (although the primary impact of high R:FR on parasite foraging success is most likely caused by the disruption of light cues). In longer-term trials, in which tomatoes were exposed to high R:FR for the first 7 days and harvested 54 days later, most measured traits did not differ among treatments, including the number of open and senesced flowers, fruit yield, fruit fresh and dry mass, and total above-ground dry mass (Fig. S7).

The only long-term impact of brief exposure to altered R:FR that we observed was an increase in the number of unopened buds at the time of harvest (Fig. S7). As discussed above, our data on long-term effects on host plants are based on very small sample sizes (of a single host species) and must be considered preliminary; however, our overall results are certainly encouraging with respect to the prospect that spectral manipulation during early stages of crop plant growth might provide a viable method of preventing dodder infestations in agricultural crops.

The current study directly addressed two dodder species that are significant agricultural pests: *C. gronovii*, a major pest of cranberry and blueberry in the United States, and *C. campestris*, an important world-wide pest that attacks numerous crop species as well as ornamental plants (Dawson *et al.* 1994; Costea & Tardif 2006). Furthermore, the similar effects of spectral manipulation observed for the interactions of these species with two different host plants (jewelweed and tomato, respectively) suggest that high R:FR might be expected to similarly disrupt interactions of these dodder species with other hosts, as well as host acquisition by other economically important dodder species. However, we note that both species examined here are in the *Cuscuta* subgenus *Grammica*, and thus, further investigation will be required to confirm that spectral manipulation has similar effects on host acquisition by agriculturally important parasites in the subgenera *Cuscuta* (e.g. *C. epithymum*, *C. europaea* and *C. planiflora*) and *Monogyna* (e.g. *C. japonica* and *C. reflexa*) (Parker & Riches 1993; Lanini & Kogan 2005).

Spectral manipulation techniques have previously been employed effectively for the control of other classes of pests and pathogens in greenhouses (Raviv & Antignus 2004; Nguyen *et al.* 2009) and to control plant growth without the use of growth regulators (Decoteau *et al.* 1988; Rajapakse *et al.* 1999; Paul & Moore 2006; Lykas, Kittas & Katsoulas 2008). Large-scale manipulation of spectral quality under field conditions to control dodder infestations will no doubt pose significant technical challenges. However, it is certainly possible to envision the integration of passive manipulation techniques (entailing the use of photoselective materials to filter sunlight) into existing agricultural technologies, including for example row covers, high tunnels, soil sterilization materials and plastic mulches ('plasticulture'). In addition, dedicated-use light-filtering canopies could potentially be employed for the treatment of localized infestations. Deployment of active manipulation methods in field settings is currently less practical than passive manipulation; however, the rapid development of new lighting technologies such as LEDs and lasers may make such methods more feasible in the future.

Assuming that effective methods for the manipulation of light spectral quality can be developed, they have the potential to mitigate some of the limitations of existing dodder control methods. For example, management strategies based on spectral manipulation could provide a non-

invasive, chemical-free alternative to herbicide use that is ideal for organic production. Moreover, photoselective films could potentially be reused in consecutive growing seasons (reducing costs and waste) and can be made from recyclable materials. And emerging lighting technologies such as high-power LEDs that might eventually be employed in active manipulation are extremely energy-efficient compared to other horticulture lighting options (e.g. high-pressure sodium and metal halide lights). Indeed, it is conceivable that the deployment of spectral manipulation techniques – alone or in combination with other management tactics – could prove relatively inexpensive compared to the considerable costs associated with currently available methods for dealing with established dodder infestations (e.g. hand removal or the use of genetically engineered resistant crop varieties and corresponding selective herbicides) or the costs of reduced crop quality and yield associated with the failure to control such infestations.

Despite these potential advantages, there are also a number of limitations and challenges regarding the application of spectral manipulation techniques for dodder control in agriculture. First, while the current results suggest that spectral manipulation might effectively control initial host acquisition by dodder seedlings, it seems likely that such techniques will prove less valuable in preventing the spread of mature dodder vines from one host to another – or, on perennial hosts, the annual regrowth of parasites from haustorial tissues. Thus, spectral manipulation is likely to be most effectively employed as a strategy for preventing the initiation of dodder infestations or the perpetuation of existing infestations across growing seasons in annual crops. A second concern is that the timing and duration of the critical period during which most parasite seedlings germinate and seek hosts – which is highly temperature-dependent (Allred & Tingey 1964) – may prove difficult to predict for a given region. These occurrences pose potential challenges for efforts to limit the duration of spectral manipulation (to minimize effects on host plants). However, dodder seedlings, in contrast to mature vines, must typically attach to relatively early developmental stages of host plants (Koch, Binder & Sanders 2004; Meulebrouck *et al.* 2009). Thus, for most crop species, the stage at which plants become invulnerable to parasitism by dodder seedlings might be used to define the maximal period over which spectral manipulation would be required, which could be reduced further to the extent that peak periods of dodder germination can be predicted or tracked. But, while dodder seeds typically germinate early in the growing season, a low rate of parasite seedling emergence is likely to continue beyond the period of spectral manipulation, potentially leading to significant infestations if these late-emerging parasites make their first attachments to susceptible weed species – or to young axillary shoots of crop plants – and having become established via these routes subsequently spread to mature crop plants. Moreover, while our results suggest that manipulating light spectral quality can greatly reduce



rates of successful host acquisition by dodder, and further optimization of the techniques employed might yield even more effective control, it is unlikely that light spectral manipulation will prove 100% effective in preventing parasite establishment. Thus, it seems likely that spectral manipulation techniques might be best employed in combination with other control methods, such as subsequent monitoring and physical control of incipient infestations, in the context of an integrated management approach (Sandler 2010).

These challenges notwithstanding, our results suggest light spectral manipulation is an intriguing approach that has the potential to eventually provide a useful tool for the prevention and management of dodder infestations in agriculture. Exploring this potential will require further work to devise and test methods of spectral manipulation that can be deployed at scales relevant for use in agricultural fields (as discussed above) and the evaluation of feasibility of incorporating such methods into the management of particular crop systems. Field studies and economic analyses can then be employed to assess the efficacy and cost-effectiveness of spectral manipulation techniques and assess their current and future potential to complement existing management approaches. These applied efforts will be facilitated by continued research exploring the mechanisms of host acquisition by plant parasites. Finally, we note that the critical dependence of host acquisition by dodder seedlings on appropriate lighting environments has practical implications beyond agriculture, including for the rearing of dodder from seed for research purposes or conservation efforts. The latter point is particularly valuable given the important, if often overlooked, ecological roles that dodder species play in many natural systems (Pennings & Callaway 2002; Press & Phoenix 2005; Costea & Stefanović 2009).

## Acknowledgements

We thank Tom Lanini and Jason Smith for supplying *Cuscuta* seeds; Shawn Johnson for designing the ELVIS array; Dennis Decoteau for providing the spectroradiometer; Ariella Haber for statistical assistance; Heike Betz, Max Michkofsky, Jason Smith and Erica Smyers for technical assistance; and several anonymous reviewers for helpful comments on the manuscript. Funding was provided by the David and Lucile Packard Foundation and the National Science Foundation (CAREER Grant No. 0643966 awarded to C.M.D.M., Graduate Research Fellowship awarded to B.I.J.).

## Data accessibility

All data supporting this study, including data from parasite foraging and attachment assays, host plant performance assays, host plant chemical analyses and information regarding light spectral characteristics, are available from Dryad Digital Repository doi: 10.5061/dryad.1d2c6 (Johnson, De Moraes & Mescher 2016).

## References

Allred, K.R. & Tingey, D.C. (1964) Germination and spring emergence of dodder as influenced by temperature. *Weeds*, **12**, 45–48.

- Aly, R. (2007) Conventional and biotechnological approaches for control of parasitic weeds. *In Vitro Cellular and Developmental Biology – Plant*, **43**, 304–317.
- Ballaré, C.L. & Casal, J.J. (2000) Light signals perceived by crop and weed plants. *Field Crops Research*, **67**, 149–160.
- Ballaré, C.L., Scopel, A.L. & Sánchez, R.A. (1990) Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. *Science*, **247**, 329–332.
- Benvenuti, S., Dinelli, G., Bonetti, A. & Catizone, P. (2005) Germination ecology, emergence and host detection in *Cuscuta campestris*. *Weed Research*, **45**, 270–278.
- Bewick, T.A., Binning, L.K. & Dana, M.N. (1988) Postattachment control of swamp dodder (*Cuscuta gronovii*) in cranberry (*Vaccinium macrocarpon*) and carrot (*Daucus carota*). *Weed Technology*, **2**, 166–169.
- Costea, M. (2007 onward) Digital Atlas of *Cuscuta* (Convolvulaceae). Wilfrid Laurier University Herbarium, Ontario, Canada, [http://www.wlu.ca/page.php?grp\\_id=2147&p=8968](http://www.wlu.ca/page.php?grp_id=2147&p=8968). (Accessed 2 January 2015).
- Costea, M. & Stefanović, S. (2009) *Cuscuta jepsonii* (Convolvulaceae): an invasive weed or an extinct endemic? *American Journal of Botany*, **96**, 1744–1750.
- Costea, M. & Tardif, F.J. (2006) The biology of Canadian weeds. 133. *Cuscuta campestris* Yuncker, *C. gronovii* Willd. ex Schult., *C. umbrosa* Beyr. ex Hook., *C. epithymum* (L.) L. and *C. epilinum* Weihe. *Canadian Journal of Plant Science*, **86**, 293–316.
- Cudney, D.W., Orloff, S.B. & Reints, J.S. (1992) An integrated weed management procedure for the control of dodder (*Cuscuta indecora*) in alfalfa (*Medicago sativa*). *Weed Technology*, **6**, 603–606.
- Dawson, J.H. (1989) Dodder (*Cuscuta* spp.) control in established alfalfa (*Medicago sativa*) with glyphosate and SC-0224. *Weed Technology*, **3**, 552–559.
- Dawson, J.H., Musselman, L.J., Wolswinkel, P. & Dörr, I. (1994) Biology and control of *Cuscuta*. *Review of Weed Science*, **6**, 265–317.
- Decoteau, D.R., Kasperbauer, M.J., Daniels, D.D. & Hunt, P.G. (1988) Plastic mulch color effects on reflected light and tomato plant growth. *Scientia Horticulturae*, **34**, 169–175.
- Furuhashi, K., Kanno, M. & Morita, T. (1995) Photocontrol of parasitism in a parasitic flowering plant, *Cuscuta japonica* Choisy, cultured in vitro. *Plant and Cell Physiology*, **36**, 533–536.
- Furuhashi, K., Tada, Y., Okamoto, K., Sugai, M., Kubota, M. & Watanabe, M. (1997) Phytochrome participation in induction of haustoria in *Cuscuta japonica*, a holoparasitic flowering plant. *Plant and Cell Physiology*, **38**, 935–940.
- Goldwasser, Y., Lanini, W.T. & Wrobel, R.L. (2001) Tolerance of tomato varieties to lespedeza dodder. *Weed Science*, **49**, 520–523.
- Haidar, M.A., Orr, G.L. & Westra, P. (1997) Effects of light and mechanical stimulation on coiling and prehaustoria formation in *Cuscuta* spp. *Weed Research*, **37**, 219–228.
- Haidar, M.A., Orr, G.L. & Westra, P. (1998) The response of dodder (*Cuscuta* spp.) seedlings to phytohormones under various light regimes. *Annals of Applied Biology*, **132**, 331–338.
- Johnson, B.I., De Moraes, C.M. & Mescher, M.C. (2016) Data from: Manipulation of light spectral quality disrupts host location and attachment by parasitic plants in the genus *Cuscuta*. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.1d2c6>.
- Kelly, C.K. (1990) Plant foraging: a marginal value model and coiling response in *Cuscuta subinclusa*. *Ecology*, **71**, 1916–1925.
- Koch, A.M., Binder, C. & Sanders, I.R. (2004) Does the generalist parasitic plant *Cuscuta campestris* selectively forage in heterogeneous plant communities? *New Phytologist*, **162**, 147–155.
- Lane, H.C. & Kasperbauer, M.J. (1965) Photomorphogenic responses of dodder seedlings. *Plant Physiology*, **40**, 109–116.
- Lanini, W.T. (2004) Economical methods for controlling dodder in tomatoes. *Proceedings of the California Weed Science Society*, **56**, 57–59.
- Lanini, W.T. & Kogan, M. (2005) Biology and management of *Cuscuta* in crops. *Ciencia e Investigación Agraria*, **32**, 165–179.
- Lykas, C., Kittas, C. & Katsoulas, N. (2008) *Gardenia jasminoides* height control using a photosensitive polyethylene film. *HortScience*, **43**, 2027–2033.
- Lyshede, O.B. (1985) Morphological and anatomical features of *Cuscuta pedicellata* and *Cuscuta campestris*. *Nordic Journal of Botany*, **5**, 65–77.
- Meulebrouck, K., Verheyen, K., Brys, R. & Hermy, M. (2009) Limited by the host: host age hampers establishment of holoparasite *Cuscuta epithymum*. *Acta Oecologica*, **35**, 533–540.
- Mishra, J.S., Moorthy, B.T.S., Bhan, M. & Yaduraju, N.T. (2007) Relative tolerance of rainy season crops to field dodder (*Cuscuta campestris*)

- and its management in niger (*Guizotia abyssinica*). *Crop Protection*, **26**, 625–629.
- Nguyen, T.H.N., Borgemeister, C., Max, J. & Poehling, H.-M. (2009) Manipulation of ultraviolet light affects immigration behavior of *Ceratothripoides claratris* (Thysanoptera: Thripidae). *Journal of Economic Entomology*, **102**, 1559–1566.
- Orr, G.L., Haidar, M.A. & Orr, D.A. (1996) Smallseed dodder (*Cuscuta planiflora*) phototropism toward far-red when in white light. *Weed Science*, **44**, 233–240.
- Parker, C. (2012) Parasitic weeds: a world challenge. *Weed Science*, **60**, 269–276.
- Parker, C. & Riches, C.C. (1993) *Parasitic Weeds of the World: Biology and Control*. Oxford University Press, United Kingdom.
- Paul, N.D. & Moore, J.P. (2006) Manipulation of light spectrum for crop growth regulation. *Acta Horticulturae*, **711**, 357–362.
- Pennings, S.C. & Callaway, R.M. (2002) Parasitic plants: parallels and contrasts with herbivores. *Oecologia*, **131**, 479–489.
- Press, M.C. & Phoenix, G.K. (2005) Impacts of parasitic plants on natural communities. *New Phytologist*, **166**, 737–751.
- Rajapakse, N.C., Young, R.E., McMahon, M.J. & Oi, R. (1999) Plant height control by photosensitive filters: current status and future prospects. *Horttechnology*, **9**, 618–624.
- Raviv, M. & Antignus, Y. (2004) UV radiation effects on pathogens and insect pests of greenhouse-grown crops. *Photochemistry and Photobiology*, **79**, 219–226.
- Runyon, J.B., Mescher, M.C. & De Moraes, C.M. (2006) Volatile chemical cues guide host location and host selection by parasitic plants. *Science*, **313**, 1964–1967.
- Sandler, H.A. (2010) Managing *Cuscuta gronovii* (swamp dodder) in cranberry requires an integrated approach. *Sustainability*, **2**, 660–683.
- Tada, Y., Sugai, M. & Furuhashi, K. (1996) Haustoria of *Cuscuta japonica*, a holoparasitic flowering plant, are induced by the cooperative effects of far-red light and tactile stimuli. *Plant and Cell Physiology*, **37**, 1049–1053.

Received 5 August 2015; accepted 12 February 2016  
Handling Editor: Jennifer Finn

## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Fig. S1.** Light conditions for germinating tomato seeds.

**Fig. S2.** Light conditions of the photosensitive film treatments.

**Fig. S3.** Schematic of the ELVIS system.

**Fig. S4.** Light conditions of the ELVIS system.

**Fig. S5.** Effect of R:FR treatment on short-term plant growth.

**Fig. S6.** Effect of R:FR treatment on host plant phytohormone production.

**Fig. S7.** Effect of R:FR treatment on long-term plant growth.

**Fig. S8.** Effect of R:FR treatment on the constitutive release of tomato host volatiles.

**Fig. S9.** Effect of R:FR treatment on dodder seedling circumnutation.

**Appendix S1.** Detailed methodology of phytohormone extraction and volatile collection.