The Plant Vigor Hypothesis applies to a holoparasitic plant on a drought-stressed host

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The Plant Vigor Hypothesis applies to a holoparasitic plant on a drought-stressed host

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Abstract

Parasitic plants extract resources from host vascular tissues but their responses to environmental fluctuation experienced by the host are poorly studied. Three frequently-cited hypotheses for effects of environmental stress on plant resistance to herbivores predict decreased, increased, or fluctuation in herbivore performance in response to drought stress. We tested which hypothesis best accounts for how drought stress applied to a perennial herb affects growth of the holoparasite, *Cuscuta gronovii* (common dodder), an obligate shoot parasite. *Verbesina alternifolia* (wingstem) supporting single, young *C. gronovii* were exposed to continuous, pulsed, or no water stress for 32 d and then dry mass of each parasite was determined. Consistent with the Plant Vigor Hypothesis, *C. gronovii* grew significantly better on well-watered hosts. Continuous and pulsed drought stress of the host resulted in similar growth reduction relative to no drought stress. In addition to reducing absolute growth of the holoparasite, continuous and pulsed drought stress reduced the growth of the holoparasite relative to host growth. Although functionally similar to insect phloem feeders, growth of holoparasites such as *C. gronovii* is constrained by source-sink relations. Our results suggest drought stress experienced by a host weakens source strength and reduces uptake by the holoparasite acting as a sink.

Keywords: *Cuscuta gronovii*, drought, host resistance, phloem feeders, *Verbesina alternifolia*, parasitic plant
Introduction

Approximately 1% of flowering plant species in 18 families are considered to be parasitic (Nickrent 2002; Westwood et al. 2010). This designation is based on formation of a haustorium, an organ bridging host and parasite phloem or xylem (Kujit 1969). Through these structures parasites extract a variety of resources from their hosts, including photosynthates, solutes, and metabolites (Watling and Press 2001; Smith et al. 2013). Some parasitic plants, termed holoparasites, are functionally non-photosynthetic. Because these species are fully dependent on hosts for resources transported in the host’s xylem and phloem streams, they may be subject to fluctuations in resource availability following water stress experienced by the host.

Parasitic plants are important constituents of many terrestrial ecosystems and economically important pests (Parker 2012). Nonetheless, the ecology of parasitic plant-host responses to drought is poorly known. By contrast, drought-mediated interactions between herbivores and host plants have been examined extensively (Huberty and Denno 2004, Joern and Mole 2005, Mody et al. 2009, Gutbrodt et al. 2011). Similarities in feeding mode of holoparasitic plants and herbivorous insects (Pennings and Callaway 2002; Pennings and Simpson 2008) and hormone signaling in parasitized hosts (Runyon et al. 2010) suggest that hypotheses for effects of plant drought stress on herbivore performance may predict responses of holoparasitic plants.

Two long-standing hypotheses postulated to explain patterns of herbivore performance predict opposite effects of environmental stress experienced by the host plant. The Plant Stress Hypothesis assumes that stress increases availability of nutrients
or decreases secondary metabolites, resulting in greater herbivore preference for or
performance on stressed plants (White 1969). The **Plant Vigor Hypothesis** predicts
greater herbivore abundance on rapidly-growing, vigorous plants or plant modules for a
variety of reasons, including greater concentrations or accessibility of quality nutrients
(Price 1991). A third hypothesis, the **Pulsed-stress Hypothesis**, predicts the best
performance by phloem, mesophyll, and xylem feeders (sap feeders) on plants
experiencing intermittent drought stress because drought induces increases in plant
nitrogen compounds that become available to sap feeders when turgor is regained
(Huberty and Denno 2004).

Here we test the three hypotheses (above) for effects of host plant stress on
holoparasite-host interactions. Because their haustoria bridge the parasite and host
vascular tissues, holoparasites may be postulated to feed in a manner similar to phloem
and xylem feeding herbivores (Marquardt and Pennings 2010; Pennings and Callaway
2002; Pennings and Simpson 2008). We relate holoparasites to phloem-feeding
herbivores because feeding guild is one of the best predictors of insect response to plant
stress (Huberty and Denno 2004; Koricheva et al. 1998) and holoparasites acquire
resources primarily from host phloem (Jeschke et al. 1994).

We conducted a greenhouse study of the effects of drought stress of the perennial
plant *Verbesina alternifolia* (L.) Britton ex Kearney (wingstem) on resistance and
tolerance to parasitism by the holoparasite *Cuscuta gronovii* Willd. Ex Schultes (common
dodder). The impact of drought stress on host response to parasitism (tolerance) has been
published elsewhere (Evans and Borowicz 2013). Here we focus on effects on
holoparasite growth as a measure of quantitative resistance to determine which of the
three hypotheses best predicts behavior of this holoparasite-host system.

Through transport of the small seeds, *Cuscuta* spp. have spread to many parts of the
world and are economically important weeds for many crops, including cranberry,
clovers, alfalfa, and soybean (Parker 2012). *Cuscuta gronovii* is the most common and
abundant species of *Cuscuta* in North America (Sandler 2001). Stems of this annual
twine around a host and connect to its vascular tissue via searching hyphae that
differentiate into either xylem or phloem elements (Birschwilks et al. 2006). Both
*Verbesina alternifolia* (Asteraceae) and *C. gronovii* (Cuscutaceae) grow in thickets,
woods, and bottomlands throughout the Midwestern and Eastern United States (Gleason
and Cronquist 1991) in areas subject to prolonged or repeated episodes of drought.

**Materials and methods**

Methods were provided previously (Evans and Borowicz 2013) and details pertinent
to performance of *C. gronovii* are summarized here. *Cuscuta gronovii* and *Verbesina
alternifolia* are both native plants of central Illinois and were found to be in a host-
parasite relationship naturally. Seeds of both species collected in Lexington, McLean
County, Illinois, USA in October 2010 were cold stratified for 278 d (*V. alternifolia*) or
188 d (*C. gronovii*) in plastic bags with damp Perlite™ in a 3°C refrigerator prior to
germination. On day 0 (22 d after cold stratification ended) *V. alternifolia* seedlings were
transplanted individually into 1.7 L pots containing 1 : 1 MetroMix™ 510 : Perlite™ on
greenhouse benches with 14 h of supplemental light. On days 11 and 22 each *V.
Alternifolia was fertilized with 1 g (12-4-8 N-P-K) of a slow-release granular fertilizer. No pests were observed during the course of the experiment.

C. gronovii seeds were placed on top of a mixture of soil, sand, and Perlite\textsuperscript{TM} to germinate. On day 12, C. gronovii seedlings (3-6 cm long) were placed singly in small, 1 dram glass shell vials filled with water and haphazardly assigned to 90 V. alternifolia. Vials were placed within 2 cm of the main stem of the host. The seedling was considered to be attached when it completed one tight $360^\circ$ loop around the host stem and this date was recorded. Bamboo skewer sticks were placed in each pot. Velcro\textsuperscript{TM} strips were used if necessary to secure aggressively growing parasites to the bamboo stick in order to prevent spread to other pots. Therefore, the parasite could only attach to the assigned host. C. gronovii made multiple attachments on the host; however the number of attachments was not recorded. Because V. alternifolia seeds were collected from the field we anticipated some variation in growth rates due to genotypic differences and conditions experienced by the mother. On day 13 we recorded the length of the longest leaf on each host as a measure of pre-treatment host size.

Thirty V. alternifolia were randomly assigned to each of three treatments (control, persistently drought stressed, or pulsed drought stressed). On day 25, each pot was watered to excess with 500 ml of water and weighed 30 minutes later to determine initial saturation mass (ISM), a benchmark for all treatments. Control plants were maintained at >85\% of ISM. Continuously stressed treatment pots were watered to 45\% of ISM after the pot was < 40\% of ISM. Pulse-stressed pots received no water until any of 10 sentinel pots declined to 35\% ISM, then watered to 100\% ISM. Pulse-stressed pots were brought
to ISM twice and the experiment was terminated on d 57 when these pots needed a third pulse of water. Pulsed and continuous drought treatments received similar amounts of water over the course of the experiment. *Cuscuta gronovii* were removed from host shoots and dried at 60°C to constant weight. Shoots and roots of *V. alternifolia* were also harvested, dried, and weighed.

Eleven *C. gronovii* failed to attach to the host at least 4 d before drought stress began and were excluded prior to analysis, ensuring that all parasites were well-established at the onset of drought stress. Twenty-six controls, 28 pulse-stressed, and 25 continuously-stressed pots were included in the analyses (*n*=79). Data were transformed to meet the assumptions of normality and homoscedasticity. All statistical analyses were performed using SAS 9.2 © 2008.

Dry mass of *C. gronovii* was analyzed by analysis of covariance with length of the longest host leaf as covariate to determine the effects of host water stress on resistance to parasitism. To determine if the parasite was limited by the amount of the host plant available, parasite mass g⁻¹ of total host mass (shoot and root mass combined) was analyzed with ANCOVA using duration of attachment to the host as the covariate. When shade treatment was significant, differences among means were analyzed using orthogonal comparisons (Sokal and Rohlf 2012).

**Results**

Over 45% of the *C. gronovii* attached within one day and 90% within three days of placement. Drought stress applied to hosts significantly affected the dry mass of *C. gronovii* (*F*<sub>2,75</sub> = 61.73, *P*<0.0001). Mean mass of *C. gronovii* on well-watered hosts was
>2x that of those on drought-stressed hosts ($F_{1,75} = 123.28$, $P < 0.0001$; Fig. 1A).

However, holoparasite growth did not differ between pulsed- and continuously-stressed hosts ($F_{1,75} = 0.68$, $P = 0.4116$). *Cuscuta gronovii* mass increased significantly with the length of the longest host leaf prior to the start of treatments ($F_{1,75} = 4.06$, $P = 0.0474$).

Analysis of parasite mass g$^{-1}$ of host indicated that parasite growth was not strictly linked to the amount of host available. Water treatment significantly affected the parasite:host ratio ($F_{2,75} = 3.9$, $P = 0.01$) and *C. gronovii* on well-watered hosts produced more than 25% more dry mass per gram of dry host mass compared to growth on drought-stressed hosts ($F_{1,75} = 7.78$, $P = 0.0067$). Growth of *C. gronovii* per gram of host did not differ between continuously-stressed and pulse-stressed hosts ($F_{1,75} = 0.04$, $P = 0.8458$; Fig. 1B). Although most *C. gronovii* attached to hosts within 3 days, the duration of attachment ranged from 37 days to 44 days and longer duration of attachment resulted in greater final mass ($F_{1,75} = 6.98$, $P = 0.0100$).

**Discussion**

Well-watered *V. alternifolia* grew most rapidly (Evans and Borowicz 2013) and supported the largest *C. gronovii*, consistent with the Plant Vigor Hypothesis. Interim measurements were not taken and it is possible that pulse-stressed hosts supported more rapid *C. gronovii* growth during intervals of relief from drought. Nonetheless, holoparasite growth over the course of three bouts of intermittent drought stress was similar to growth on continuously-stressed plants, refuting the Pulse-stress Hypothesis.
Often portrayed as mutually exclusive, the Plant Vigor and the Plant Stress hypotheses may each usefully describe patterns of herbivore performance if type of tissue consumed is considered (White 2009). Increased performance of phytophages accessing nutrients translocated from mature tissue (“senescence feeders”) is likely to be predicted by the Plant Stress Hypothesis and increased performance of phytophages obtaining nutrients translocated into new growth (“flush feeders”) is likely to be predicted by the Plant Vigor Hypothesis (White 2009). Unlike individual insects, one \textit{Cuscuta} plant maintains multiple haustoria of varying ages on one or more hosts. Due to prolonged attachment and extensive growth, \textit{Cuscuta} and other holoparasites may feed simultaneously as flush-feeders from recent attachments and early in the season when hosts are growing, and as senescence-feeders from older haustoria and later in the season as hosts reallocate metabolites. This hypothesis would be readily tested with \textit{Cuscuta} and suggests avenues for future research. Regardless, in the present experiment, \textit{C. gronovii} clearly performed better on more vigorous, unstressed hosts.

Although holoparasites and aphids feed in analogous ways from the same plant part, the analogy may break down for mechanism of resource acquisition. Using fine stylets, aphids pierce phloem and feed passively due to high turgor pressure within the sieve elements (Guerrieri and Digilio 2008). Transfer of proteins (Haupt et al. 2001), fluorescent dyes (Birschwilks et al. 2006), viruses (Hosford, 1967), and mRNA (Roney et al. 2007) from host to other species of \textit{Cuscuta} strongly suggests symplasmic continuity. Both \textit{C. gronovii} and aphids may have direct contact with host cell contents; however movement to \textit{Cuscuta} occurs because the holoparasite creates a very efficient nutrient sink (Wolswinkel 1984; Vaughn 2006; Smith et al. 2013). Unlike aphids, which are
much smaller than their hosts, *C. gronovii* mass can exceed that of its host (Fig. 1b). By creating a strong sink, the holoparasite deprives host roots of carbon, leading to increased host photosynthesis and flow of resources to the holoparasite (Jeschke et al. 1994; Jeschke and Hilpert 1997). Drought-stressed plants close stomata to reduce transpiration and photosynthesis declines (Xu et al. 2010). This reduces uploading and translocation of resources in the phloem stream, decreasing access by the holoparasite. Resistance to parasitism, as measured by holoparasite growth, is consequently greater when hosts are drought stressed.

Many climate change models predict increased variability in precipitation and regional droughts (Dore 2005; IPCC 2007). While the impact of drought on plant-herbivore systems has received considerable attention (e.g., Mody et al. 2009; Johnson et al. 2011; Grinnan et al. 2013), less is known about drought effects on parasitic plants as consumers. Similar to insects, holoparasites can be potent pests (Dawson et al. 1994; Sandler 2001; Parker 2010) and have also been considered as agents of biocontrol (Shen et al., 2005; Yu et al. 2009, 2011). Studies that examine the impact of the abiotic environment on holoparasite-host interactions provide context for better understanding the role of holoparasites in natural communities and also as pests and biocontrol agents. Our study indicates that whether continuous or episodic, drought will reduce performance of holoparasites on stricken hosts and the impact is greater than would be accounted for through reduced host growth.
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Data accessibility

Data will be deposited at ISU ReD: Research and eData at http://ir.library.illinoisstate.edu
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Fig. 1. Least squares mean ($\pm$ s.e.) of C. gronovii (a) dry mass, and (b) dry mass g$^{-1}$ dry host mass when the host V. alternifolia was well watered, pulse-stressed, or continuously drought stressed. *** = $P < 0.0001$. ** = $P < 0.01$. 
C. gronovii mass (g)

control pulsed continuous

C. gronovii:host mass ratio

drought-stress treatment

b

ns

ns

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