The role of host plants in determining the germination of parasitic plants *Cuscuta gronovii* Willd. and *Odontites verna* (Bell.) Dum.

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Abstract

Parasitic plants or their seeds may be able to detect potential hosts nearby using chemosensory mechanisms. Chemodetection could then trigger subsequent germination in the parasite, or direct seedling growth towards the host plant. The objectives of this study were to compare the effects of the seedlings of various host species on the germination percentages of Common Dodder (*Cuscuta gronovii*) and Red Bartsia (*Odontites verna*), and to determine directionality of growth in the seedlings of the parasite. Seeds of *Odontites verna* and host plant Wood Nettle (*Laportea canadensis*) could not be included in the trial due to complications in breaking seed dormancy. The experiment was conducted using 50 seeds of *C. gronovii* with 50 seedlings of five host species in a series of Petri dish trials (in the case of *L. canadensis*, 50 seeds of *C. gronovii* were placed with 0.10g of plant tissue). The host species were Wood Nettle (*Laportea canadensis*), Red Clover (*Trifolium arvense*), Common Plantain (*Plantago major*), Sweetclover (*Melilotus* spp.), Timothy Grass (*Phleum pratense*), and Kentucky Blue-Grass (*Poa pratensis*). The control consisted of 50 parasite seeds being germinated without a host. There were four replicates per treatment. Directional movement was monitored by dividing each Petri dish into four quadrants and noting seedling growth by *C. gronovii* into host seedling quadrants. Analysis using the Kruskal-Wallis statistic failed to reject the null hypothesis ($P = 0.776$). Thus there was no influence of host seedlings on germination in *C. gronovii*. Directional movement towards the host seedlings was observed in all six treatments, with 76% of all parasite seedlings growing towards the host seedlings.

Acknowledgements

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Introduction

Parasitic plants inhabit most ecosystems (Press and Phoenix, 2005). They occur in many plant families, especially Scrophulariaceae, Loranthaceae, Orobanchaceae and Cuscutaceae; thus it is not surprising that there is a certain degree of variation in the mechanisms used for parasitism. Parasitic plants obtain either complete or partial nutrition from a host plant, but usually to the latter's detriment (Baskin and Baskin, 2001). Parasitic plants are classified into two general groups: holoparasites and hemiparasites. Holoparasites usually lack chlorophyll and receive carbon compounds, water and minerals solely from the host plant, whereas hemiparasites are able to survive independently of a host connection (Begon *et al*., 1996). Although able to survive independently, most hemiparasites grow more vigorously when attached to a host (Govier *et al*., 1968). The parasites are mainly generalists in their host selection (Press and Phoenix, 2005). In most cases, parasitism occurs by means of haustorial connections which are responsible for attachment, penetration, and solute transfer from the host organism to the parasite (Stewart and Press, 1990). Haustoria are specialized roots that aid in increasing solute absorption from the host (Göker *et al*., 2007). Haustorial attachment is made with the stem or root of the host, depending on the parasitic species. The parasitic species that are the subjects of my experiment are the holoparasite *Cuscuta gronovii* Willd. ex Roem. & Shult. and the hemiparasite *Odontites verna* (Bell.) Dumort.

Although *Cuscuta gronovii* (Rao and Reddy, 1991; Parker and Riches, 1993) and *Odontites verna* (Govier and Harper, 1964; Parker and Riches, 1993) do not require a host stimulus to germinate, both species do react to chemical stimuli as adults. The reaction of plants to chemical signals is called chemotropism and is common in many parasitic and non-parasitic plant species. Little is known about the detection mechanisms employed by seeds to locate nearby host plants. Host plants may have the potential to trigger the germination of

parasitic seeds via chemotropism and/or direct their growth towards the host plant, ensuring their survival after germination. There would be a survival advantage for populations of any parasitic species to have developed such chemosensory mechanisms in order to maximize surrounding host resources. In the case of *Cuscuta gronovii* this would be especially advantageous because of the relative fragility of seedlings and time frame they need to attach themselves to a host plant. Ability to detect chemical cues by noting presence or absence of host plants would help determine whether or not it is advantageous for the seed to germinate.

Cuscuta gronovii (commonly known as dodder) is a member of the family Cuscutaceae and is distributed across North America, being the most prevalent native species of dodder in Canada (Costea and Tardif, 2006) (Figure 1). *Cuscuta gronovii* occurs in a variety of habitats including moist fields, riparian areas and disturbed communities (Connecticut Botanical Society, 2005; Costea and Tardif, 2006). The plant consists of slender, twining, yellow-green stems with clusters of small white flowers that appear from July to October (Plate 1). The seeds of *C. gronovii* are light brown in color with a slight depression in the centre. On average there are three seeds per capsule (but they can contain between one and four) and are on average 1.65 mm in diameter. Most seeds in the genus *Cuscuta* have a demonstrated physical dormancy where scarification increases germination rates (Gaertner, 1950). *Cuscuta gronovii,* however, forms non-dormant seeds, although scarification does increase germination rates (Baskin and Baskin, 2001). *Cuscuta gronovii* is a well-known agricultural weed that parasitizes many crops, including; cranberries, alfalfa, sunflowers and chrysanthemums (Kim *et al*., 2004; Lanini and Kogan, 2005; Costea and Tardif, 2006). It is considered a holoparasite, even though it possesses chlorophyll which is critical to the early seedling stage (Parker and Riches, 1993). In order to survive, *Cuscuta* seedlings must become attached to a

host within about twelve days after germination (Parker and Riches, 1993). Seedlings attach to hosts by coiling around the stems in a counter-clockwise rotation. Attachment to the host occurs in a number of phases; a prehaustorium first appears within the parasite stem near the vascular tissue, these eventually develop into outgrowths that adhere to the host stem (Christensen *et al*., 2003) (Plate 2). Once the haustorium has a reached a certain depth within the host stem, hyphal projections extend into the tissues and enter the xylem (Dawson *et al*., 1994) (Plate 3, pg. 5). Once the parasite has adhered to the host, its root decays and the plant becomes a complete epiphyte (Parker and Riches, 1993).

Studies done by Gaertner (1950), Kelly (1990) and De Moraes *et al*. (2006) have shown that seedlings of some species of *Cuscuta* can direct their growth towards specific chemicals emitted by host plants. Chemical preference has also been established in *Cuscuta pentagona*, directing its growth preferentially towards commercial species of tomato plants (De Moraes *et al*. in Pennisi, 2006). This chemical gradient is responsible for allowing *Cuscuta* to 'sniff out' its host and attach itself to the plant.

The hemiparasite *Odontites verna* (commonly known as red bartsia) can be found in all provinces across Canada, and is present in Manitoba near Gimli and in other small, isolated pockets throughout the province (Figure 2). *Odontites verna* is a small herb in the snapdragon family: Scrophulariaceae. It invades hay fields, pastures and roadsides (Manitoba Agriculture, Food and Rural Initiatives, 2006), with a preference for wet ground adjacent to waterways (Parker and Riches, 1993). The plant is short in stature (15-25 cm in height), purple-green in color, with lanceolate leaves that are up to 5 cm in length (Plate 4). The flowers are small, pink, with the upper petals fused to form a hood, emerging from July into the fall. The seeds of *O. verna* are white and irregularly-shaped with vertical striations. The seed capsules contain numerous seeds, about 1400 per plant (Manitoba Agriculture, Food

and Rural Initiatives, 2006), and are 1.5 mm in length. Seeds of *Odontites verna* have a physiological dormancy and require a period of 28 days at temperatures between -5º C to 5ºC to germinate (Chancellor 1973). The hemiparasite attaches to the root of its host by means of haustoria (Plate 5). The haustoria develop laterally on the terminal end of the fine roots (Govier *et al*., 1968). Multiple haustoria can be found in areas where the root of *O. verna* is in close contact with the host root (Govier *et al*., 1968), indicating a possible chemical gradient to initiate haustorial projections. As a hemiparasite, the species does not require attachment to a host in order to survive (Parker and Riches, 1993; Govier *et al*., 1968; Atsatt and Strong, 1970).

The purpose of this experiment was to determine whether or not the presence of potential hosts can change germination rates. Determining whether the rate and proportion of parasite seed germination are influenced by nearby host plants would provide further insight into the effects of chemotropism in both species.

The objectives of this study were to:

- Determine the germination requirements of parasites *Cuscuta gronovii* and *Odontites verna*, and hosts *Laportea canadensis*, *Plantago major, Melilotus* spp., *Trifolium arvense, Phleum pratense* and *Poa pratensis*
- Compare the effects of the presence of seedlings of various host species on the germination percentages of *Cuscuta gronovii* and *Odontites verna*
- Determine whether there is a directionality of seedling growth in *Cuscuta gronovii* and *Odontites verna* towards potential host species

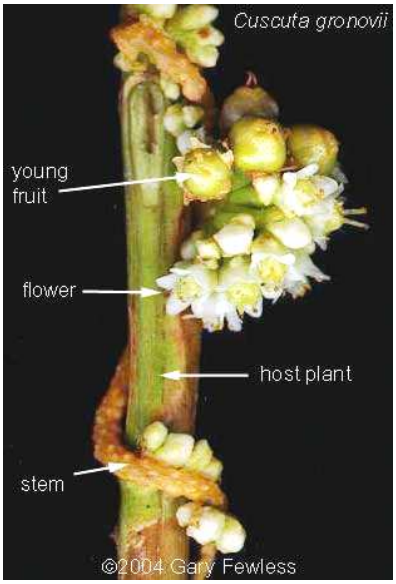

Plate 1: Photograph illustrating attachment of *Cuscuta gronovii* to host plant with description of plant parts
(Source: University of Wisconsin-Green Bay, 2002-2006)

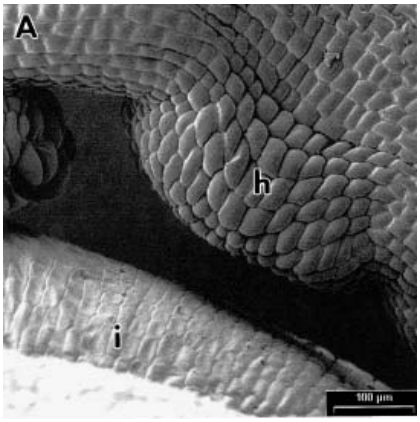

Plate 2: Budding growth of haustoria (h) from stem of *Cuscuta pentagona* L. (A) towards host stem (i)
(Adapted from Vaughn, 2002)

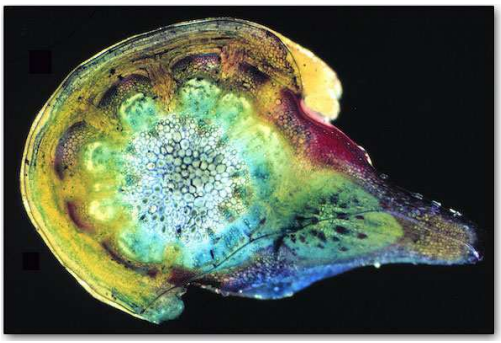

Plate 3: Cross-section of *Cuscuta salina* haustoria penetrating the tissue of *Chenopodium* sp.
(Source: http://www.flickr.com/)

Plate 4: *Odontites verna*
(Source: Donegal Wildlife Project, 2005)

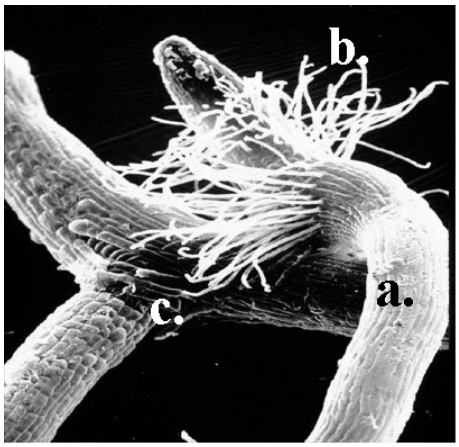

Plate 5: Scanning electron microscope image of *Agalinis purpurea* (Family Scrophulariaceae) root (a.) with haustoria (b.) attached to host root (c.)
(Source: SIUC College of Science, Parasitic Plant Connection, 2001)

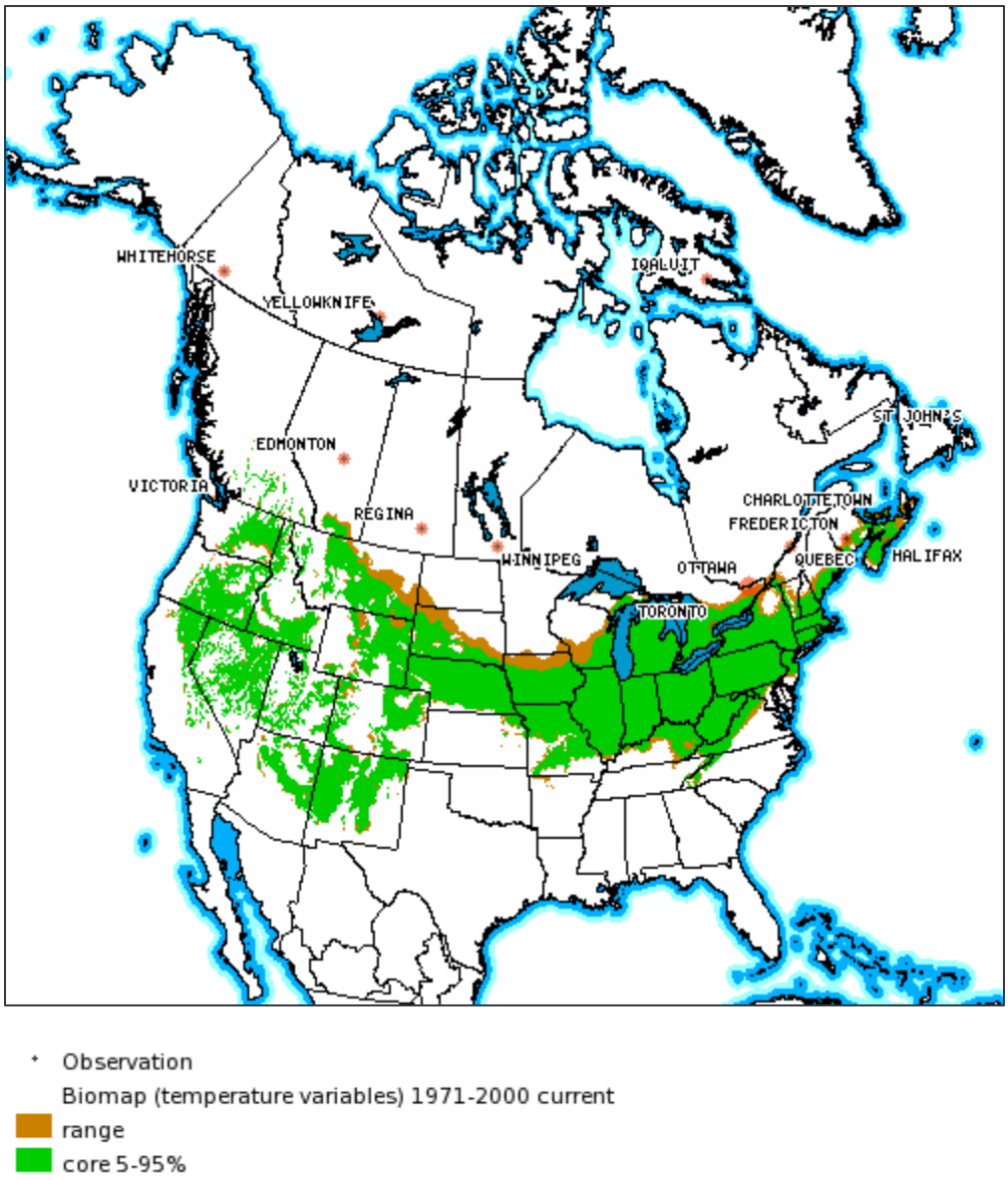

Figure 1: Distribution of *Cuscuta gronovii* in North America
(Natural Resources Canada, 2006)

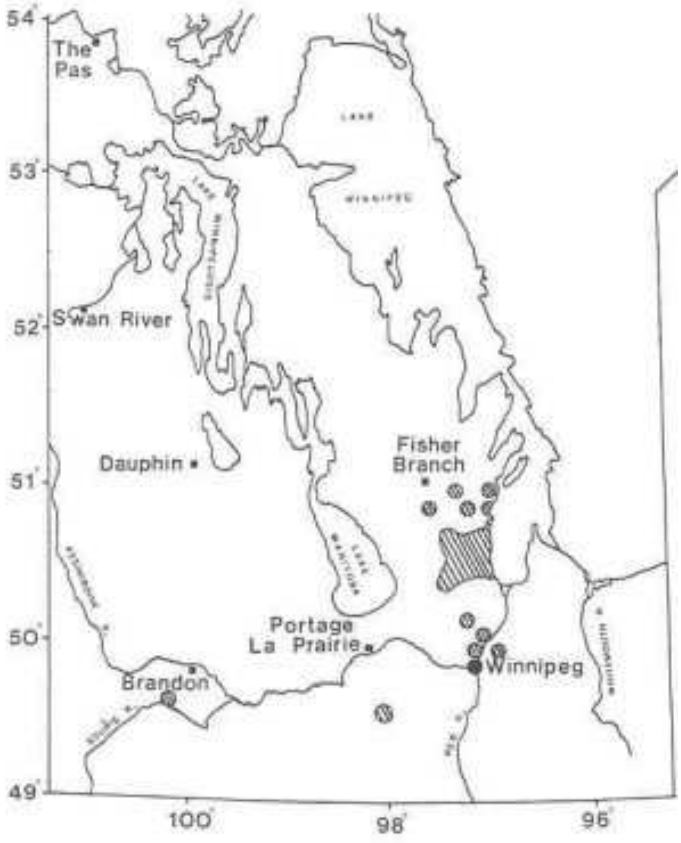

Figure 2: Distribution of *Odontites verna* in Manitoba
(Manitoba Agriculture, Food and Rural Initiatives, 2006)

Methods

Seed Collection

Seeds were collected in mid-September, 2006, with the exception of a few host seeds which had been donated by the local seed harvesting company, PickSeed. The seeds of the parasite *Cuscuta gronovii* were collected in Kildonan Park, Winnipeg and those of *Odontites verna* were collected adjacent to Hwy 9, 12 km south of Gimli, Manitoba. The host seeds were collected from various locations: *Plantago major* (common plantain) and *Melilotus* spp. (sweetclover) were both collected in a field in St. Francis Xavier; *Trifolium arvense* (red clover), *Phleum pratense* (Timothy grass), and *Poa pratensis* (Kentucky bluegrass) were donated by PickSeed; seeds and plant tissue of *Laportea canadensis* (wood nettle) were collected from plants being parasitized by dodder in Kildonan Park, Winnipeg.

Germination Pre-Trial

A germination pre-trial test was set up to determine germination requirements of seeds of host and parasite species. One Petri dish (9cm diameter) was used for each species following the procedures of Anderson (1968) and Staniforth (pers. comm.). Each Petri dish contained two filter papers, 50 seeds, and sufficient distilled water to keep the seeds moist. The germination chamber used was model CMP4030 (Controlled Environments Ltd., 2001, Winnipeg, Manitoba). The chamber was set to simulate local natural spring growing conditions: 14 hours of light at 25°C and 10 hours of dark at 15°C. These Petri dishes were examined and the number of germinated seedlings was recorded on alternate days for two weeks. The results of the pre-trial tests confirmed that the germination of most species would take place in the standard germination chamber under 'spring' conditions. However,

the seeds of *Odontites verna* and *Laportea canadensis* had to be placed in cold storage in order to break their dormancy.

Chancellor (1973) has shown that 28 days at temperatures between -5º C to 5º C are needed to break seed dormancy in *O. verna*. There was no information that could be obtained to determine the length of cold storage required to break the seed dormancy in *L. canadensis*, thus all seeds were stratified for 28 days. Upon removal from cold storage, a repetition of the pre-trial was conducted to test germination.

During the second pre-trial germination for seeds of *O. verna* and *L. canadensis*, it was found that the germination rate was 10% for *O. verna* and 1% for *L. canadensis* (n = 100). Due to the low germination rates from the pre-trial, the seeds were placed back into cold storage for another 30 days to further attempt dormancy breakage. The seeds were again tested for germination rates upon removal from cold storage. The third pre-trial of both species yielded no germination for *O. verna* seeds and only 7% germination of *L. canadensis* seeds. To induce higher germination rates, a fourth pre-trial was set up for both species involving the use of 10^{-3} M concentration of gibberellic acid (GA_3) (Dr. Eva Pip, pers. comm.). Gibberellic acid has been used in various studies to induce germination (Chauhan *et al.*, 2006; Stevens *et al.*, 2006). This treatment yielded minor improvements in germination for *O. verna* (4%), but a decrease in germination of *L. canadensis* (2%). These germination rates were not sufficient for the two species to be used in the study. The study was continued using *Cuscuta gronovii* as the only parasite.

Germination Trial of Cuscuta gronovii

Host-parasite studies conducted by Gaertner (1950) have shown that *C. gronovii* will naturally (or artificially) become attached to any of the host species proposed in this study with the exception of *Phleum pratense* and *Plantago major*.

Host seeds were placed in the germination chamber set to the same conditions as used in the pretrial: 14 hours of light at 25°C and 10 hours of dark at 15°C. Once the host seeds germinated, 50 seedlings of each species were placed in Petri dishes containing two pieces of filter paper and 50 *C. gronovii* seeds. A treatment containing 0.10 g of *Laportea canadensis* plant material with 50 seeds of *C. gronovii* was also included.

There were four replicates for each treatment:

Treatment 1: 50 seeds of *C. gronovii* alone (Control)
Treatment 2: 50 seeds of *C. gronovii* + 50 seedlings of *Melilotus* spp.
Treatment 3: 50 seeds of *C. gronovii* + 50 seedlings of *P. major*
Treatment 4: 50 seeds of *C. gronovii* + 50 seedlings of *P. pratense*
Treatment 5: 50 seeds of *C. gronovii* + 50 seedlings of *P. pratensis*
Treatment 6: 50 seeds of *C. gronovii* + 50 seedlings of *T. arvense*
Treatment 7: 50 seeds of *C. gronovii* + 0.10g of *L. canadensis* plant tissue

H = Host

P = Parasite

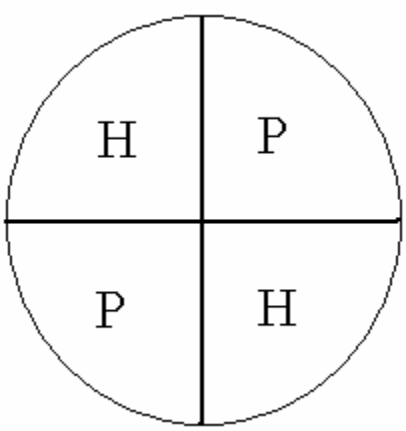

Figure 3: Arrangement of parasite seeds and host seedlings in each Petri dish

Each Petri dish was sectioned into four quadrants, each containing 25 seeds of either the host or the parasite, adjacent to each other (Figure 3). The seeds were arranged in this fashion in order to allow for maximum dispersion of host seed exudates to come in contact with the parasite seeds.

The data collected consisted of daily and cumulative germination scores for seeds of the parasite, as well as the directional growth of the parasite seedlings into or away from the host quadrants.

Statistical Analysis

The data collected for the germination of *C. gronovii* were analyzed using the non-parametric Kruskal-Wallis test. The Kruskal-Wallis non-parametric analysis of variance test was used to compare mean germination values for *C. gronovii* sown in the presence of host seedlings. The null hypothesis was there is no influence of host seedlings on germination in *C. gronovii*. This analysis was done using SPSS version 12.0.

Results

Germination Pre-trial

Seeds of all host and parasite species germinated under natural spring conditions (14 hours of light at 25°C and 10 hours of dark at 15°C), with the exception of those of *Laportea canadensis* and *Odontites verna*. Two trials of 30 days in cold storage (5ºC) did not result in significant dormancy breakage of seeds of either species (Table 1). Treatment with the growth hormone gibberellic acid (10^{3}M) failed to increase germination rates to a sufficient level for these two species to be used in the study.

Table 1: Germination pre-trial of host and parasite seeds (n = 50)

	No Cold Storage	**30 Dayes of Cold Storage**	**60 Days of Cold Storage**	**Gibberellic Acid (103M)**
Laportea canadensis	0%	1%	7%	2%
Odontites verna	0%	10%	0%	4%
Cuscuta gronovii	6%			
Melilotus spp.	92%			
Plantago major	78%			
Phleum pratense	98%			
Poa pratensis	40%			
Trifolium arvense	96%			

Germination Trial for Seeds of C. gronovii in the Vicinity of Germinated Seeds of Various Host Species

The germination trial of dodder seeds with seedlings of various host species yielded similar but very low germination rates for all host treatments. The control had the highest mean percent germination (4.5%). The lowest germination for seeds of *C. gronovii* was in the treatment using *T. arvense* as a host (mean percent germination of 2%). The greatest difference was found between the control and the *Trifolium arvense* treatment (Figure 3, Table 2).

The Kruskal-Wallis test failed to reject the null hypothesis that there would be no influence of host seedlings on germination of seeds of *C. gronovii* ($P = 0.776$) (Appendix III).

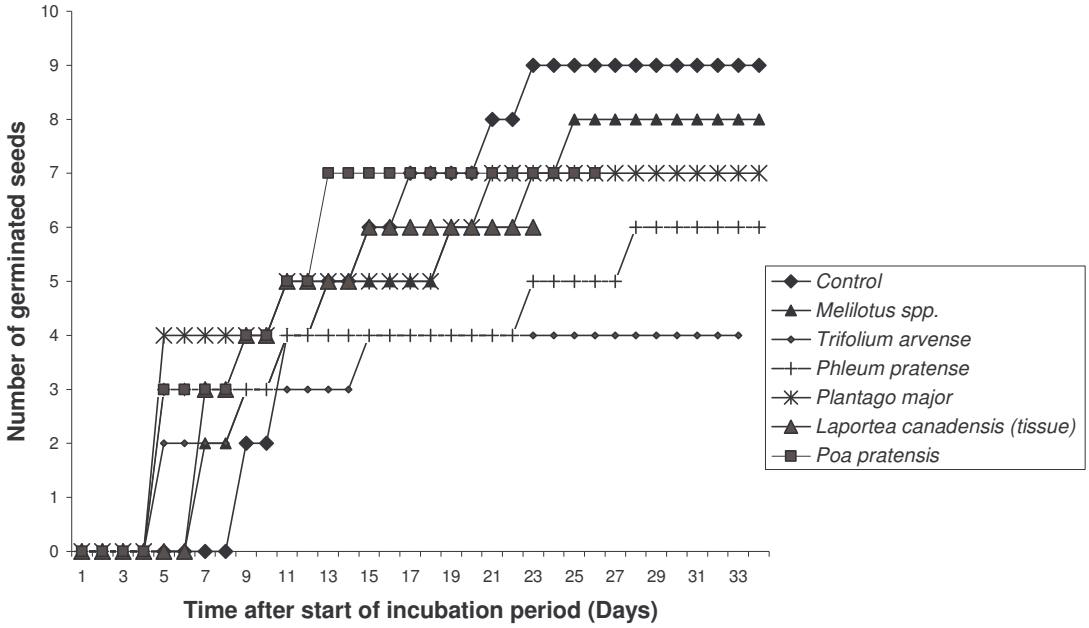

Figure 4: Cumulative germination for *Cuscuta gronovii* in the presence of seedlings of host species for a period of 34 days (n = 200)

Table 2: Mean germination of *Cuscuta gronovii* in the presence of host seedlings (n = 4 replicates of 50 seeds each)

Control	**Mean Seed Germination**	**Mean Percent Germination**	**Standard Deviation**
Cuscuta gronovii	2.25	4.5%	± 1.7

Host Seedlings	**Mean Seed Germination**	**Mean Percent Germination**	**Standard Deviation**
Melilotus spp.	2.00	4.0%	± 1.2
Plantago major	1.75	3.5%	± 1.0
Poa pratensis	1.75	3.5%	± 1.0
Phleum pratense	1.50	3.0%	± 1.5
Laportea canadensis (plant tissue)	1.50	3.0%	± 0.6
Trifolium arvense	1.00	2.0%	0.0

Directional growth of seedlings of C. gronovii in all treatments

Directional growth in the stems of dodder seedlings was observed in each treatment. The highest proportion of seedlings that grew into the host quadrants within the Petri dishes was recorded for *Phleum pratense* and *Trifolium arvense* treatments (Table 3). In total, 76% of all germinated seedlings of *C. gronovii* grew towards host species and became entwined around the host plants (Plates 7 & 8).

Table 3: Number of seedlings of *C. gronovii* that grew into host seedling quadrants within the Petri dishes (n = 200)

	Number of seeds germinated (total)	**Seedlings that moved into host quadrants**	**Seedlings that did not move into host quadrants**
Phleum pratense	6	6	0
Trifolium arvense	4	4	0
Melilotus spp.	8	7	1
Plantago major	7	5	2
Laportea canadensis (tissue)	6	4	2
Poa pratensis	7	3	4

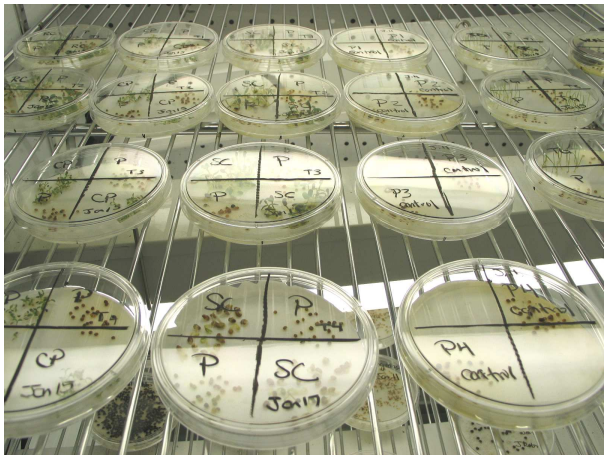

Plate 6: Germination trials for *C. gronovii*

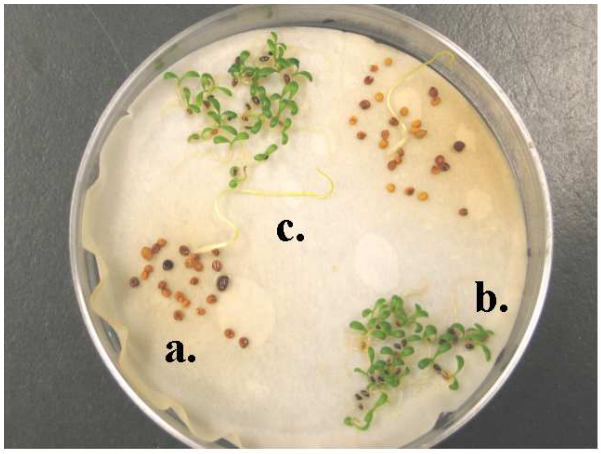

Plate 7: Treatment showing the seed arrangement for *Cuscuta gronovii* (a.), seedlings of *Plantago major* (b.), and seedlings of *C. gronovii* (c.)

Plate 8: A seedling of *Cuscuta gronovii* (a.) twining around host seedlings of *Plantago major*

Discussion

Germination Pre-trial

Results of the germination pre-trial revealed the conditions necessary for the germination of most host and parasite species, with the exception of *Odontites verna* and *Laportea canadensis*. Relatives of *O. verna* are known to possess a physiological dormancy in which they require exposure to cool temperatures before germination occurs (Martin, 1946). As such, the lack of germination by *O. verna* during the first pre-trial was not surprising. However, the lack of germination after cold storage in the second and third pre-trials was unexpected. There are several possible reasons why the seeds of *L. canadensis* did not germinate. In addition, a lack of information regarding the germination requirements in this species contributed to difficulties experienced. A possible reason why seeds of *Odontites verna* and *Laportea canadensis* did not germinate is that the seed collection may have included immature seeds. Immature seeds are unlikely to germinate because the embryo inside may be under-developed or the endosperm of the seed may not contain sufficient reserves to support the seedling (Koning, 1994). Another factor that may have influenced the germination of *L. canadensis* may have been that the seeds were collected from plants already parasitized by *Cuscuta gronovii.* Several authors have observed that the overall performance of parasitized host plants can be negatively affected by the presence of the parasite (Press *et al.*, 1999; Koskela *et al.*, 2001; Press and Phoenix, 2005; Shen *et al.*, 2006). The viability and reproductive fitness of the host plant is often compromised (Press *et al.*, 1999; Shen *et al.*, 2006). A study conducted by Koskela *et al.* (2001) compared parasitized and non-parasitized populations of *Urtica dioica* (stinging nettle) to determine if there was a reduction of overall plant performance between the two. The study found that parasitized populations of *U. dioica* produced fewer flowers and also had an overall reduction in biomass. It may be

possible that the seeds produced by the host plant were non-viable as a result of resource consumption by the parasite. This would reduce the nutrients available to the host and compromise proper seed formation.

Germination of Cuscuta gronovii Amongst Seedlings of Host Species

Germination rates were similar for all treatments. The greatest number of seedlings germinated in the control treatment and the lowest number germinated for *Trifolium arvense*. The Kruskal-Wallis analysis of variance failed to reject the null hypothesis that there is no influence of host seedlings on the germination rates of *Cuscuta gronovii* (Appendix III).

The results failed to support the presence of a chemosensory mechanism in *C. gronovii* germinating in the presence of seedlings of host species. The thickness and impermeability of the seed coat in seeds of *C. gronovii* may inhibit the detection of host plants. Most species in the family Cuscutaceae contain seed coats consisting of four layers; the external epidermis, hypodermis, palisade and parenchyma cells. Over time, the palisade layers of cells in *Cuscuta* become thick-walled and mature into macrosclereids (Hutchison and Ashton, 1979). Variations in the number of layers of palisade cells accounts for variations in the physical dormancy of species in this family (Baskin and Baskin, 2001). Studies on dormancy in *Cuscuta gronovii* by Gaertner (1950) show that the permeability of mature, non-dormant seeds decreases over time as they dry and their palisade cells become macrosclereids. This may suggest that the seeds collected in this experiment may have 'dried out', thus reducing their permeability to water and solutes, and in turn showed no responses to chemicals released by the seedlings of potential host plants.

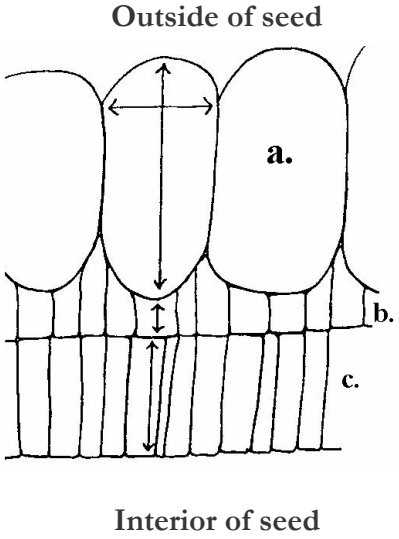

Figure 5: Diagram showing epidermal (a.) and palisade (b. and c.) layers of cells in a typical seed of the family Cuscutaceae (Adapted from Gaertner, 1950)

The seeds of Cuscutaceae are normally scarified before germination trials to increase their germination rates. Scarification of seeds is achieved by abrasion with sand paper (Hutchison and Ashton, 1979; Rao and Reddy, 1991), pricking with a pin (Rao and Reddy, 1991), or by exposure to a specific concentration acid (Gaertner, 1950; Hutchison and Ashton, 1979; Rao and Reddy, 1991). However, in this experiment, artificial scarification of seeds may have interfered with the normal process of germination, making it impossible to tell if the seeds were germinating because of the presence of a chemosensory mechanism or simply because the seed coat had been broken down. To determine whether the seed coat has become impermeable or not, future studies should test this by imbibing seeds before experimentation using techniques described by Baskin and Baskin (2001).

Directional Growth by Seedlings of Cuscuta gronovii

It was shown that seedlings of *C. gronovii* may possess a chemosensory mechanism capable of detecting hosts in their vicinity. With 76% of seedlings showing directional growth towards host seedlings, this appears to be the case. Due to time constraints, further statistical analysis could not be conducted to determine whether a chemosensory mechanism was present in *C. gronovii* seedlings.

The presence of such a mechanism was first tested by Gaertner (1950). She observed seedling growth towards and attachment to particular host plants; she also determined that seedlings of *C. gronovii* had an affinity toward a particular host species. Further research supporting the existence of a chemosensory mechanism has been described only recently by De Moraes *et al.* (2006). The parasite *C. pentagona* is able to detect host plants and show host preference using volatile chemicals that had been emitted from the host plant. The exact mechanism by which this detection operated is still unknown and requires research at the molecular level.

Results from my experiment, however, cannot sufficiently support a chemosensory host detection mechanism in the seedlings of *Cuscuta* spp.

There may be a survival advantage for seeds of parasitic plants to have developed a chemosensory mechanism to detect host seedlings. However, it may be that in *Cuscuta* spp. such a mechanism did not receive sufficient selective pressure. It is known that the seeds of certain parasitic species, such as *Striga* spp. and *Alectra* spp., require a chemical stimulus from the host to germinate (Baskin and Baskin, 2001). These seeds, however, are usually small 'dust' seeds and do not survive long after germination without host attachment because of the relative lack of resources within the seed (Williams, 1961). In the case of *Cuscuta* spp.,

the embryo inside the seed is large and has chlorophyll, enabling it to search for a suitable host over a longer period of time. It has been shown by others that seedlings of *Cuscuta* spp. have the ability of detecting host seedlings and plants, although it was not statistically proven in my study. The seeds of *Cuscuta* spp. are equipped with chlorophyll, and may not require such a mechanism to germinate as they already possess a post-germination host detection technique.

Conclusions

- The germination requirements of *Cuscuta gronovii, Plantago major, Melilotus* spp., *Trifolium arvense, Phleum pratense* and *Poa pratensis* were determined for the temperature regime of 14 hours of light at 25°C and 10 hours of dark at 15°C in a germination chamber. The germination of *Odontites verna* and *Laportea canadensis* was not successful in this experiment due to difficulty in breaking their strong dormancy mechanisms. More research needs to be conducted and published on the specific germination requirements of *L. canadensis* to increase our ecological knowledge of this species.

- The Kruskal-Wallis analysis of data failed to reject the null hypothesis that seedlings had no influence on the germination of *C. gronovii* ($P = 0.776$), thus there is no influence of host seedlings on the germination rates of *Cuscuta gronovii*. Seeds of *Cuscuta gronovii* likely do not possess a chemosensory mechanism to detect host seedlings that have germinated in the vicinity. To reduce the possibility of experimenting with seeds that have dried, fresh seeds of *Cuscuta gronovii* should be tested for permeability by conducting an imbibition test.

- 76% of seedlings of *Cuscuta gronovii* grew towards host seedlings. Directional growth was observed in all treatments. There may be a chemosensory mechanism present in seedlings of *C. gronovii*. This, however, requires further statistical analysis. Recent publications do support the presence of such a mechanism in other members of the Cuscutaceae.

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Appendix I

Raw data for the germination pretrial

Parasites:	Sept.29	Oct.2	Oct.4	Oct.6	Oct.9	Oct.11	Oct.13	Oct.16
Cuscuta gronovii	0	1	1	1	0	0	0	0
Odontites verna	0	0	0	0	0	0	0	0
Hosts:								
Laportea canadensis	0	0	0	0	0	0	0	0
Melilotus spp.	36	10	STOP					
Plantago major	0	24	11	2	2	0	0	0

	Oct.6	Oct.9	Oct.11	Oct.13	Oct.16	Oct.18	Oct.20
Phleum pratense	0	49	STOP				
Poa pratensis	0	0	0	8	9	3	0
Trifolium arvense	14	34	STOP				

After 30 Days Stratification:	Nov.12	Nov.15	Nov.17	Nov.20	Nov.22
Odontites verna	0	9	1	0	0
Laportea canadensis	0	1	0	0	0

After 60 Days Stratification:	Dec.26	Dec.28	Dec.30	Jan.2	Jan.4	Jan.5	Jan.8
Odontites verna	0	0	0	0	0	0	0
Laportea canadensis	0	0	0	4	1	1	1

Gibberellic Acid Treatment:	Jan.8	Jan.10	Jan.13	Jan.15	Jan.17	Jan.19
Odontites verna	0	0	1	0	1	0
Laportea canadensis	0	1	0	0	0	0

Appendix II

Raw data for germination trials of *Cuscuta gronovii*

Control	Jan. 13	Jan. 15	Jan. 17	Jan. 19	Jan. 21	Jan. 23	Jan. 25	Jan. 27	Jan. 29	Jan. 31	Feb. 2	Feb. 4	Feb. 6	Feb. 8	Feb. 11	Feb. 13	Feb. 15
T 1	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0
T 2	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
T 3	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0
T 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day	0	0	0	0	2	2	1	1	1	0	1	1	0	0	0	0	0
Cumulative	0	0	0	0	2	4	5	6	7	7	8	9	9	9	9	9	9

Sweet Clover	Jan. 15	Jan. 17	Jan. 19	Jan. 21	Jan. 23	Jan. 25	Jan. 27	Jan. 29	Jan. 31	Feb. 2	Feb. 4	Feb. 6	Feb. 8	Feb. 11	Feb. 13	Feb. 15	Feb. 17
T 1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
T 2	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
T 3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
T 4			0	0	0	0	1	0	0	1	0	0	1	0	0	0	0
Day	0	0	0	2	1	1	1	0	0	1	0	1	1	0	0	0	0
Cumulative	0	0	0	2	3	4	5	5	5	6	6	7	8	8	8	8	8

Red Clover	Jan. 15	Jan. 17	Jan. 19	Jan. 21	Jan. 23	Jan. 25	Jan. 27	Jan. 29	Jan. 31	Feb. 2	Feb. 4	Feb. 6	Feb. 8	Feb. 10	Feb. 12	Feb. 14	Feb. 16
T 1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
T 2	0	0	1	0	0	0	0										
T 3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
T 4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day	0	0	2	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Cumulative	0	0	2	2	3	3	3	4	4	4	4	4	4	4	4	4	4

Common Plantain	Jan. 17	Jan. 19	Jan. 21	Jan. 23	Jan. 25	Jan. 27	Jan. 29	Jan. 31	Feb. 2	Feb. 4	Feb. 6	Feb. 8	Feb. 11	Feb. 13	Feb. 15	Feb. 17	Feb. 19
T 1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T 2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
T 3	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0
T 4	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day	0	0	4	0	0	1	0	0	0	1	1	0	0	0	0	0	0
Cumulative	0	0	4	4	4	5	5	5	5	6	7	7	7	7	7	7	7

Timothy Grass	Jan. 17	Jan. 19	Jan. 21	Jan. 23	Jan. 25	Jan. 27	Jan. 29	Jan. 31	Feb. 2	Feb. 4	Feb. 6	Feb. 8	Feb. 11	Feb. 13	Feb. 15	Feb. 17	Feb. 19
T 1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
T 2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T 4	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Day	0	0	3	0	0	1	0	0	0	0	0	1	0	1	0	0	0
Cumulative	0	0	3	3	3	4	4	4	4	4	4	5	5	6	6	6	6

Kentucky Bluegrass	Jan.25	Jan.27	Jan.29	Jan.31	Feb.2	Feb.4	Feb.6	Feb.8	Feb.11	Feb.13	Feb.15	Feb.17	Feb.19
T 1	0	0	1	0	1	1	0	0	0	0	0	0	0
T 2	0	0	0	0	0	0	0	0	0	0	0	0	0
T 3	0	0	2	0	0	0	1	0	0	0	0	0	0
T 4		0	0	0	0	0	1	0	0	0	0	0	0
Day	0	0	3	0	1	1	2	0	0	0	0	0	0
Cumulative	0	0	3	3	4	5	7	7	7	7	7	7	7

Wood Nettle (plant tissue)	Jan.17	Jan.19	Jan.21	Jan.23	Jan.25	Jan.27	Jan.29	Jan.31	Feb.2	Feb.4	Feb.6	Feb.8
T 1	0	0	0	1	1	0	0	0	0	0	0	0
T 2	0	0	0	0	0	0	0	1	0	0	0	0
T 3	0	0	0	2	0	0	0	0	0	0	0	0
T 4	0	0	0	0	0	1	0	0	0	0	0	0
Day	0	0	0	3	1	1	0	1	0	0	0	0
Cumulative	0	0	0	3	4	5	5	6	6	6	6	6

Appendix III

Kruskal-Wallis analysis of data from germination trials of *Cuscuta gronovii*.

Species	N	Mean Rank
Control	4	18.13
Melilotus spp.	4	16.75
Trifolium arvense	4	9.00
Phleum pratense	4	14.00
Plantago major	4	15.13
Laportea canadensis (plant tissue)	4	13.50
Poa pratensis	4	15.00
Total	28	

	Data
Chi-Square	3.257
df	6
P -value	.776