Studies on Host Selectivity by *Cuscuta gronovii*

by

Caroline Snow Joseph Jesudoss

An Undergraduate Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Honours Bachelor of Environmental Management

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ABSTRACT

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Key words: *Cuscuta*, dodder, host selection, parasitism

The host selectivity of the plant parasite *Cuscuta gronovii,* and its growing patterns were investigated among pairings of corn, bean, and tomato plants in various combinations. These pairings were set up in pots, in a greenhouse environment. Seeds of *C. gronovii* were germinated and a single seedling was placed between each pairing. Observations were made over a two week period. It was observed that only 11% of *Cuscuta gronovii* seedlings made a choice with regards to host selection, the remaining 89% failed to make a choice as they either died, showed inhibited growth or non-directional growth. Suggestions are made to explain why these results deviated from those found previously in the scientific literature.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Hutchison for all his guidance, sourcing the seeds used in this experiment from Manitoba, providing laboratory equipment, and his constructive feedback. I would also like to thank Dr. Meyer for providing me with the sulphuric acid required for this experiment and being my second reader. A special thanks to Ryan Willkie, who has helped me gain greenhouse access, soil, and potting supplies.

INTRODUCTION

This thesis deals with an investigation of the parasitic vine called dodder (*Cuscuta gronovii* Willd.) and whether it has the ability to choose between hosts; this process involves the germination and release of dodder seedlings into an environment that incorporates the element of choice. For the purpose of this thesis, tomato, bean and corn were used as potential hosts.

Dodder or *Cuscuta* refers to a group of obligate parasitic plants that directly attack the vascular system of its host. The seed reserves of *Cuscuta* only provide the bare minimum energy for its germination and search for a host; unable to photosynthesize on its own for continual survival, *Cuscuta* invades the host plant with the help of haustoria which grants access to the host vascular system. *Cuscuta* can only grow 7 to 10 cm in search of a host (Dawson *et al*., 1970). Failure to acquire a host within the target range results in death from lack of resources.

There are approximately 170 species of *Cuscuta* and they are cosmopolitan in nature (Garcia *et al*., 2014). The wide range of dodder makes it an internationally significant species, as it is an invasive weed that reduces the quality of many economically significant crops. The U.S. Department of Agriculture has classified dodder in its list of the 10 most noxious weeds (Pennisi, 2006). Physical features that are common to the genus *Cuscuta* include the absence of roots and spiraling vines that grip onto the host plant, as shown in Fig 1.

Figure 1: *Cuscuta gronovii* parisitizing wild raspberry at Hogarth plantation.

Dodder has the ability to choose among potential hosts. Early studies speculated that these choice mechanisms involved growing towards a species that is of higher nutritional value (Kelly, 1992). More recent studies show that dodder seedlings grow towards volatiles produced by specific plants such as tomato. The phenomenon of volatile organic compounds attracting dodder to its host was first investigated by Runyon *et al*. (2006), who found that *Cuscuta pentagona* Englem. seedlings differentiated between the volatiles of wheat (*Triticum aestivum* L.) and tomato (*Lycopersicon esculentum* Mill.). It was found that dodder grew towards tomato as opposed to wheat. The exact methods of host selection are not known for the multitude of dodder species present. However, volatiles have been shown to have an effect on the directional growth of dodder, specifically, *C.pentagona.*

Objective

The objective of this thesis is to investigate the selection factors involved in dodder host selection for the species *Cuscuta gronovii* Willd. This is achieved with the help of an experimental design that provides *C.gronovii* the choice between pairings of potential hosts. The Hogarth plantation in Thunder Bay will serve as the primary source of dodder seeds used in this experiment. *Cuscuta gronovii* was found exclusively parasitizing goldenrod (*Solidago canadensis* L.) and raspberry plants (*Rubus idaeus* L.); these seeds will be germinated, and seedlings placed into pots. *Cuscuta* will be given the option to choose between host pairings within each pot. The host plants of interest are volatile emitting plants such as tomato, dill (*Anethum graveolens* L.), mint (*Mentha spicata* L.), marigold (*Tagetes erecta.* L.) and low-level volatile emitting plants such as corn (*Zea mays* L.), green bean (*Phaseolus vulgaris* L.) and pumpkin (*Cucurbita pepo* L.). However, due to early host mortality (explained in the discussion and excluded plants section), the results were only obtained from pairings between corn, bean and tomato.

Hypothesis:

Cuscuta gronovii will grow towards tomato plants since it has been shown that *C.pentagona* prefers the volatiles of tomato plants (Runyon *et al*., 2006). Bean and corn plants will be avoided due to their low levels of volatile emissivity.

LITERATURE REVIEW

Geographic range

Species of *Cuscuta* are generally annuals in temperate regions and perennials in tropical parts of the world (Dean, 1955). There are approximately 170 species worldwide and the species is seen in almost every continent. It is so cosmopolitan in nature, that it can even be found in Greenland (Kuijt. 1969). Common dodder or *C. gronovii*, used for this experiment, is common in temperate parts of the world and is known for its wide range of host selection; this includes most vegetables and flowering ornamentals (Dean, 1955).

Characteristics

Species of *Cuscuta* are difficult to identify since their vegetative morphology have high levels of similarity between species (Kuijt, 1969). When the seeds germinate, it lacks roots or leaves and emerges as a singular stem (Mishra, 2009). The stem is threadlike and slender; usually yellow or orange. However, colours can range from purple, white or red depending on the species (Lee and Timmons, 1958). As the seed germinates and grows towards its host in a thread like manner, *Cuscuta* forms a tight spiraling twine around its host plant. The haustoria that emerges from the stem and into the host plant, results in a parasitic bond. Upon germination, *Cuscuta* requires a host within a few days otherwise it will result in its death. *Cuscuta* has little to no chlorophyll and is unable to be photosynthetically active; this is why they are obligate parasites as all species of *Cuscuta* need a host to complete their life cycle. (Kaiser *et al*., 2015). Dodder seedlings can grow towards a living host and a water source (Heide-Jørgenson, 2008).

The species of dodder collected for this thesis is *C.gronovii* (Figure 2). It has greenish-yellow stems in its ripened stage and dark brown stems in its latter stages as it overwinters. *Cuscuta gronovii* is able to regenerate from pieces of attached haustoria found on the stem on its host. *Cuscuta gronovii* can also regenerate at the shoot tips while the other end of the dodder vine may die (Heide-Jørgensen, 2008). *Cuscuta granovii* causes the host stem to swell when attacked and is also seen to produce more seeds on a host that is a weed (Sandler, 2010).

As dodder vines spiral around the host stem, they adhere onto the host by secreting polysaccharides that act like a glue that hardens when dry (Heide-Jørgensen, 2008). In order to penetrate the host cell, dodder seedlings produce and grow a gland. As the cells of the gland divide and multiply, they release pectolytic enzymes which degrade the pectin in the host plant cell (Heide-Jørgensen, 2008). With the pectin of the host degraded, it allows for easier penetration into the host cells.

Figure 2: *Cuscuta gronovii* in its early stages after germination, searching for a host.

Germination

In the natural environment, dodder seeds in temperate regions are likely to germinate the next season after they fruit; however some seeds can remain dormant in the soil for years (Saeed & Zaroug, 1989). Germination of the seeds takes place very close to the surface of the soil (Mishra, 2009). Most dodder seeds are similar in morphology and it is difficult to differentiate between the different species.. The structure of dodder embryos makes it apparent that it was tailored to be a parasitic plant; this is seen through its lack of cotyledons (Kuijt, 1969) . Dodder seeds can germinate on non living surfaces, however the haustoria can only be produced on a living surface (Heide-Jørgensen, 2008). When pressure is applied onto the elongation zone on the growing dodder stem, it can facilitate the formation of haustoria. Naturally, this pressure would occur when dodder shoots comes into contact with its host plant.

Seeds of *Cuscuta* have two palisade layers and an epidermis layer (Kuijt, 1969). These later differentiate into sclereids which are thick structural support cells. The tough outer seed coat of dodder makes it difficult to germinate in the laboratory and requires pretreatment to end its dormancy period. Successful methods of germination for *Cuscuta japonica* Choicy in the laboratory include pretreatment with 98% concentrated sulphuric acid or physical abrasion, usually done with the help of sandpaper or pricking the seeds with a sharp blade (Saeed & Zaroug, 1989). Seeds pretreated with sulphuric acid had a 98% germination rate at 30 degrees C (Saeed & Zaroug, 1989).

It has been shown that for some species such as *Cuscuta europea* L., cold storage can also aid in breaking dormancy (Kuijt, 1969). Not all species of dodder have a strict protocol for germination, as it can vary from species to species (Kuijt, 1969). For example, in the natural environment, dormancy in *C. japonica* is broken with the help of grassland fires, and dormancy in *Cuscuta campestris* Yunck. is broken through overwintering (Saeed & Zaroug, 1989). Dodder seeds can also pass through the gut of an animal unharmed and germinate after dispersed (Sandler, 2010). Dodder seeds are dispersed haphazardly and do not depend on a specific mechanism (Kuijit, 1969).

Environmental, social and economic significance:

Cuscuta has the ability to alter the balance of natural ecosystems as it takes over the hosts; this can be done either on a small or large scale depending on the species and hosts available. Legislations in 25 different countries declare it a noxious weed and deny its entry into their country (Mishra, 2009). Controlling dodder is still posing some serious challenges and causes significant economic loss in the agriculture industry. As of 2005, 25 crops suffered severe damage and losses due to *Cuscuta* in 55 countries (Lanini and Kogan, 2005). *Cuscuta gronovii* commonly attacks both woody and herbaceous plants; mint, potato and sweet potato being common hosts among them. It also has the ability to reduce cranberry yield by 80% (Sandler, 2010). One of the main difficulties of eradicating dodder seeds in crops is its two stages of dormancy. Because of this, dodder seedlings germinate after the host plant is at a suitable stage for the dodder seedling to take advantage of (Benvenuti *et al*, 2005).

Host Selection

Most species of dodder have a wide range of host selection. Monocotyledons can generally avoid being infected due to their arrangement of vascular bundles, making it difficult for the haustoria to penetrate and extract resources; an exception to this rule is

Cuscuta australis R. Br. (Dawson *et al*., 1994). Other species of dodder such as *C.campestris* can be driven by factors such as phototropism (Benvenuti *et al*., 2005). In a study conducted by Alers-Garcia (2005), it was found *C.gronovii* preferred taller plants that were non-mycorrhizal over shorter plants. Dodder has been known to grow towards leaf extracts or individual leaves (Kuijt, 1969). Dodder's search for a host can include a variety of different variables, a majority of which are not confirmed. Below is a list of relevant studies that examined the directional growth of dodder.

Studies based on dodder host selection

Phototropism and dodder growth

Host plants that have high amounts of chlorophyll and are producing many nutrients through photosynthesis give off far-red light. Benvenuti *et al.*, (2005) found in a study that dodder seeds exhibit phototropism and grow towards far-red light. These host plants are ideal for dodder seedlings to latch onto. Dodder seedlings reacted towards this far-red light given off by host plants when the host plant was within 6-8cm of the seedling. Any further and the seedling did not display phototropism toward the host plant's far-red light (Benvenuti *et al*., 2005).

Volatiles as a means for host selection

Volatiles influence host selection, giving dodder the ability to select its own hosts based on preferred volatiles. The only main piece of research done on this topic (focusing on tomato and wheat plants) was by Runyon *et al.* (2006). Runyon *et al.*

(2006) found that dodder seedling growth is directional and favours certain host plants over others. In order to determine which direction to grow towards and which host plant to latch onto, dodder seedlings react to volatiles released by potential host plants. Dodder seedlings were placed in a chamber with two host plant scenarios on either end, further than what normally occurs in nature. By measuring the growth in both directions, the host plant that the dodder seedlings favored was determined. Through this experiment, b-phellandrene, b-myrcene, and a-pinene were all volatiles found in tomatoes that dodder seedlings preferred and grew towards. Conversely, hexenyl acetate, found in wheat, was a volatile that repelled dodder seedlings. The study done by Runyon *et al.* (2006) served as the main inspiration for this thesis. However, it is important to note that the method of determining dodder preference in the study was determined solely by measuring dodder growth towards the host plant, as opposed to recording the number of plants it actually parasitizes and forms haustoria within (which is the method followed in this thesis). However, there is still a gap in research regarding the spectrum of volatiles that dodder prefers and the plants that can help dodder make the choice of host selection.

Plants selected as hosts for the purpose of this thesis

Tomato

According to the study conducted on dodder and its volatile interaction with tomato, the main volatiles emitted include " b-phellandrene, b-myrcene, and a-pinene" (Runyon et al., 2006). The study revealed that dodder favored tomato volatiles and grew towards rubber septa treated with tomato volatiles as it would towards whole tomato plants. Runyon et al. (2006) were able to direct the growth of dodder towards tomato volatiles. Tomato was selected as a high volatile emitting plant that dodder is expected to grow towards.

Corn

Corn is on the lower end of the volatile releasing spectrum, especially when compared to tree species and similar plants (De Gouw *et al.*, 2015). Higher temperatures result in the release of greater amounts of terpenes; ideal temperatures for releasing volatiles is between 22°C and 27°C, however, this was only the case for damaged or externally induced corn plants (Gouinguené & Turlings, 2002). Damaged corn releases volatiles that tends to attract hymenopterous parasitoids and release a variety of terpenes (Pare & Tumlinson, 1999). These terpenes attract parasitic wasps that prey on the caterpillars found feeding on corn plants. Terpenes are released by most plants during various stages of photosynthesis or development. In undamaged and healthy corn plants, the biogenic volatile organic compounds that are released as a result of plant growth and development are not significantly affected by light intensity or temperature (Gouinguené & Turlings, 2002). However, induced volatile emissions were greater when undamaged corn was grown in dry soil (Gouinguené & Turlings, 2002). Corn was chosen as a low volatile emitting plant. It is possible that the terpenes released by corn may affect the directional growth of dodder, and will be investigated in this thesis.

Green bean

The most common volatiles found in green beans or French beans are 1-Octen3-ol and trans-3-hexene-1-ol, however, the volatiles associated with odour is found in low or trace concentrations and are difficult to pinpoint in the volatile blend (Chatterjee *et al*., 2009). Therefore, this plant can be associated with low volatile emissivity when compared to the others. It is expected that dodder would grow towards high volatile emitting plants such as tomato as opposed to bean.

Excluded Plants

The list of plants below were expected to be included in the experiment. Due to over-watering and plant mortality, they could not be included for the purposes intended. Though unconfirmed, below is a list of high volatile emitting plants that may have the potential to attract dodder. Pumpkin was also excluded in the experiment; however, it is classified under low volatile emissivity.

Dill

Dill is a commonly used culinary herb associated with high volatile emissivity. The most significant odour releasing compounds in dill are α-phellandrene and benzofuranoid (Huopalahti *et al*., 1985). Dill is quick to lose these volatiles when it desiccates. Dill's α-phellandrene has a citrusy peppery odour and is found in many spices and herbs with dill, eucalyptus and turmeric being common emitters (Zuzarte, & Salgueiro, 2015). There is limited research on dodder being attracted to dill plants.

Spearmint

Keytones and terpenoids are the most abundant type of volatiles in mint. The main volatiles responsible for the distinctive minty aroma includes menthone, carvone, limonene, eugenol, piperitone, and isopiperitone (Cirlini *et al.,* 2016). *Cuscuta gronovii* has been noted to commonly attack mint plants (Parker & Riches, 1993).

Marigold

French marigold (*Tagetes patula* L.) produces a range of terpenes and fatty acids. The main volatiles released in the vegetative stage of marigold include "carveol, 3-Hexen-1-ol, acetate, (Z)-terpinolene and linalool" (Foti *et al.*, 2017). French marigolds are also known to have a positive effect on the prevention and duration of parasitoid attacks (Dardouri *et al.* 2017; Foti *et al.*, 2017). This is a possible factor that might cause dodder to avoid marigolds. However, it is unknown whether prevention of parasitoid attacks is a result of volatile production.

Pumpkin

Pumpkin leaves release "(2E)-hexenal , benzaldehyde, (E)-β-damascone, trans-αbergamotene, (E)-β-ionone and nonadecane" (Leffingwell *et al.*, 2015). Pumpkin is associated with low levels of volatile emissivity, however, there is limited research on the volatiles released by pumpkin shoots as most studies focus on volatiles released by the edible parts of pumpkin and pumpkin products. It is possible that dodder would prefer more high volatile emitting plants when compared to pumpkin, however this could not be tested in this thesis.

METHODS AND MATERIALS:

Dodder seed collection:

Seeds of *Cuscuta gronovii* were collected at the Hogarth plantation, Thunder Bay, in October 2019. The berries were found on dodder vines entangled in goldenrod and raspberry plants. The berries to be selected are dark brown, dry and easily separated from the vine. The berries were brought to the lab and slightly crushed and rolled on a white surface (for visibility) to bring out the seeds within them. The seeds and crushed berries are shown in Figure 3. The seeds were then picked up individually with the help of fine forceps and were placed in a dry Petri dish.

Figure 3: Dodder seeds are obtained by crushing dried berries.

Germination of Dodder seeds:

Three hundred dodder seeds were placed in an empty 100ml beaker. While wearing gloves and protective eye gear, 98% concentrated sulphuric acid was slowly poured over the seeds to submerge them (Figure 4). This whole process was done under a fume hood, in the Forest Pathology Research Lab (BB1046), located at Lakehead University. After 30 minutes had passed, the seeds were moved separately to a 1L beaker containing water. This mixture of dodder seeds and water was then passed through a sieve to collect the seeds.

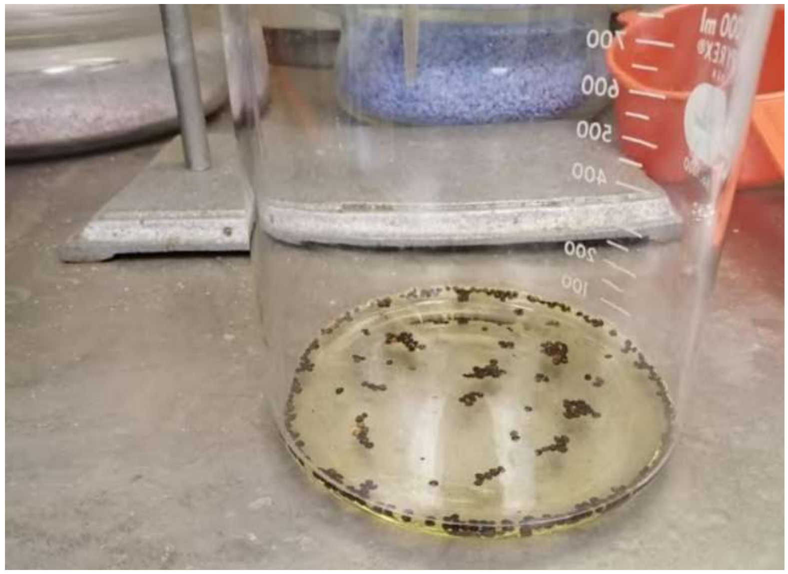

Figure 4: Dodder seeds submerged in 98% concentrated sulphuric acid.

The seeds were then dispersed equally into 15 Petri dishes that were lined with moistened filter paper treated with a fungicide (1% benomyl: 50% wettable powder). These Petri dishes were covered with their glass tops and placed in an area with a direct light source, at around 24°C. The Petri dishes were regularly moistened with fungicide treated water whenever the filter paper showed signs of drying (every 1 – 2 days). Seeds germinated in 5 to 7 days (Figure 5) and were transplanted immediately after germination.

Germination of Volatile and non-volatile plants:

All plant seeds in this experiment were germinated in the Lakehead University greenhouse at the end of November, 2019. The seeds included corn, bean, pumpkin, tomato, mint, dill and marigold which were planted in 312 small starting pots placed in larger trays (Figure 6). Close to 50 of each type of plant seed was set up for germination (with the exception of pumpkin seeds; only 18 were planted).

Figure 5. Germinated dodder seeds. On the left, the dodder seeds are in their initial stages of germination at 5 days. The picture on the right shows dodder shoots that have elongated further at 7 days.

Figure 6: Dill, pumpkin, marigold and tomato seeds seen germinating on seed starting trays.

The starting pots were filled with a standard soil mix and leveled. The seeds were placed at the appropriate depth depending on their size. For example, tomato and dill seeds were placed at a shallower depth than corn seeds and bean seeds which were significantly larger. All pots are watered regularly every two days or appropriately, according to the moisture content of the soil. Seeds were germinated after 2 weeks and are ready for transplantation.

Experimental design:

After the germination of the volatile seeds, the experimental set up began in the greenhouse. Figure 7 shows the different pairings that were to be transplanted into pots. Each specific set of pairings was represented by 10 pots.

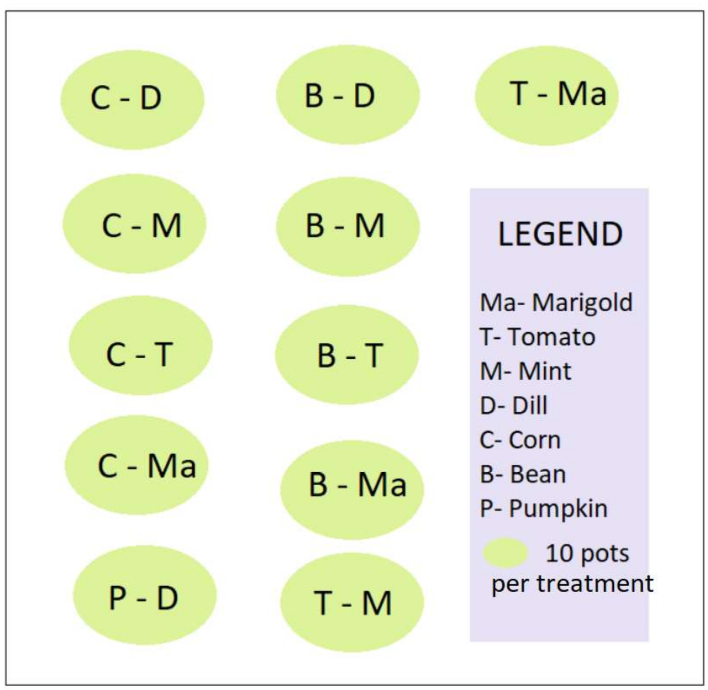

Figure 7: Experimental set up for seedling pairings in pots.

Each single pot contained two sprouted seedlings transplanted 10 cm apart (Figure 8). A single germinated dodder seedling was to be placed on top of the soil between the two plants using fine foreceps. There were to be replicates of 10 for each pairing as shown in the Figure 7. There were to be a total of 130 combination pots with a dodder seedling transplanted between them; for a total of 130 dodder seedlings employed.

Figure 8: Vegetable seedlings transplanted into larger pots in respective pairings.

Monitoring Dodder movement and growth:

Dodder growth was to be recorded every day and pots were watered every other day or as needed to ensure that the seedlings did not dry out. The final results were to be tabulated at the end of a 7 – 10 day period where the dodder parasite presumably, will have chosen its host between the two plants that it shares a singular pot with. This selection will be determined when the dodder plant attaches haustoria and spirals around the host plant (Figure 9).

Figure 9: Dodder parasitizing a bean plant as it begins to spiral around the host stem.

RESULTS:

Experiment size reduction due to overwatering:

The experiment could not be carried out according to the original design provided in Figure 7, due to the death of a large number of the volatile producing plants. Marigold, pumpkin and mint plants were wiped out due to oversaturation of their soils between December 17th, 2019 and January 3rd, 2020. This was due to a poorly set up self-watering system that over watered the plants. The plants could not be monitored during this time which may be another factor contributing to the high mortality rate. The number of bean and corn plants had also been reduced due to the same reason, but not to the same extent as marigold, pumpkin, and mint. Tomato plants survived at a greater rate than any of the other plants.

The experiment was then redesigned to be limited to corn, bean, and tomato. New corn and bean seeds were germinated on February 7th, 2020. The scale of the experiment was reduced significantly due to time constraints, seed availability, plant survival, and varying germination rates. The new experimental design consisted of 15 pairings of tomato and corn, 20 pairings of tomato and bean, and 10 pairings of bean and corn. This also meant that the original hypothesis could not be tested accurately since the experimental design was dramatically scaled back.

Dodder Behaviour

The total number of *C.gronovii* seedlings that successfully selected a host was only five out of forty five (Table 1, Figure 10). This low number does not allow for a meaningful statistical analysis of *C.gronovii* host preference. Out of the 45 pairings, *C.gronovii* chose tomato four times and bean one time.

Table 1: *Cuscuta gronovii* behaviour when placed into pots with various pairings between tomato, bean, and corn.

Pairing and number of pairings	Dodder Behaviour over 2 weeks				
	C.gronovii choices		Number of *C.gronovii* seedlings showing Non-Directional growth	Number of *C.gronovii* seedlings showing inhibited growth	Number *C.gronovii* seedlings dead before choosing a host
	Choice	Number of choices			
15 Tomato x Corn	Tomato	2	2	3	8
20 Tomato x Bean	Tomato	2	6	4	7
	Bean	1			
10 Corn x Bean	-	0	2	2	6
Total 45 Pairings	4 tomato 1 bean	5	10	9	21

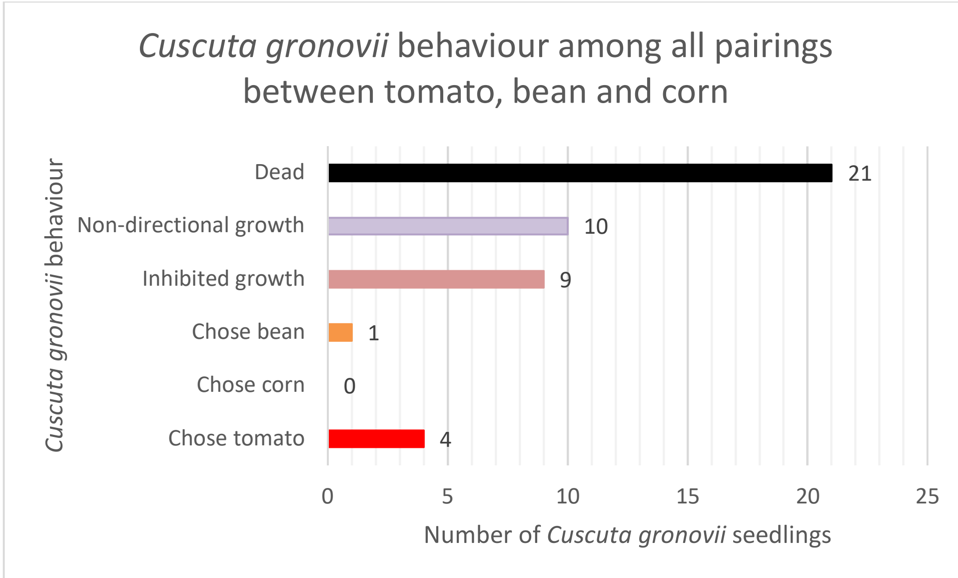

Figure 10: *Cuscuta gronovii* behaviour among pairings between corn, bean, and tomato

Qualitative Observations:

It was evident that *C.gronovii* seedlings did not behave as expected; typical upward growth in the vertical plane was rare. It was observed that the majority of *C.gronovii* seedlings did not choose a host and quickly died. Other *C.gronovii* seedlings were observed to show either inhibited or non-directional growth (Figure 11). Inhibited seedlings showed slow growth, usually in the horizontal plane; the thickness of the emerging stem decreased over time and host selection was not pursued over the two week time period observed. Seedlings that died early on showed similar behaviour.

Figure 11: A *Cuscuta gronovii* seedling placed between a tomato and bean pairing showing inhibited growth after two weeks.

A significant amount of *C.gronovii* seedlings showed unexpected non-directional growth. This could arguably be called a variation of inhibited growth. In this case, the emerging stem either spiraled downwards into the soil (Figure 12) or showed extended growth in the horizontal plane while avoiding the host (Figure 13).

Figure 12: *Cuscuta gronovii* spiraling inwards without a target direction.

Cuscuta gronovii would often grow past the host without selecting it. A common observance for almost all host pairings was that *C.gronovii* shoots thinned out significantly over time, losing the same level of shoot thickness and vigor observed during initial germination. The Appendix includes more pictures of the *C.gronovii* shoots in the host environment (Figures 16-23).

Figure 13: *Cuscuta gronovii* grows long distances horizontally and in unexpected directions as it avoids hosts over a two-week period.

Unexpected behaviours upon successful host selection

It was observed that the dodder shoots that took over the host did not survive long after host selection. The dodder stems shriveled up upon latching themselves onto the host (Figure 14).

Figure 14: *Cuscuta gronovii* stem shriveling upon parasitizing a tomato plan

DISCUSSION:

Dodder host preference among tomato, corn, and bean could not be determined accurately because 89% of *C.gronovii* seedlings either died, showed inhibited growth, or non-directional growth. Dodder seedling's inability to choose led to the rejection of the hypothesis since it could not be tested to the degree envisioned. Therefore, it is uncertain if dodder prefers tomato over corn and bean since the sample size of successful host selection was < 5. It is more certain, in the given circumstances, *C.gronovii* was unable to choose a host.

Only five plants, out of the 45 pairings, were chosen as hosts by *C.gronovii*; these included four tomato plants and one bean plant. There was no host selection made in the group that included pairings between bean and corn. Therefore, since only five hosts were selected in total, the data collected was insufficient to conduct a statistical analysis that determined the host preference of dodder seedlings.

While *C.gronovii* seedlings did choose tomato (4 times) in the rare occurrence that it was able to choose a host, the number of dodder seedling deaths and their inability to choose was far greater (40 occurrences in total). Therefore, it cannot be assumed that *C.gronovii* prefers to parasitize tomato plants in this experiment. It is important to note that Runyon *et al.* (2006) who saw tomato volatile preference in their study, conducted their experiment with *C.pentagona* seedlings, which may have a different response than *C.gronovii* seedlings.

The unexpected behaviours of dodder stems on the chosen tomato and bean plants can be questioned because *C.gronovii* quickly shriveled on the host stems after colonizing them. Therefore, it is uncertain whether dodder successfully 'chose' these hosts when considering their inability to make use of the host's vascular system to survive.

Factors that may have contributed to the death, inhibited, and non-directional growth of *C.gronovii* seedlings.

While there is no one definitive reason to attribute to the death of *C.gronovii* seedlings and their unexpected responses in the host environment, below is a list of possible reasons. Each factor also has recommendations included, if the experiment were to be repeated.

Unnatural Hosts

The *C.gronovii* seedlings collected at Hogarth plantation were seen exclusively parasitizing goldenrod and raspberry plants. The parasitizing vines found on the raspberry and goldenrod plants were thick and healthy with a uniform coil arrangement pattern. The parasitizing vines of *C.gronovii* are expected to coil around the host stem three times within five hours after latching onto the host (Dean, 1937). In contrast, the parasitizing dodder vines on tomato and bean plants were thin, shriveled, and on the verge of dying. This suggests an unnatural or unfamiliar host environment that *C.gronovii* seedlings were unable to adapt to. It is possible that tomato, bean, and corn were hosts that *C.gronovii* would avoid in their natural environments. *Cuscuta gronovii* is known to have a large host range of over 175 species and is most commonly known to parasitize cranberry, mint, grapevine, and citrus (Costea & Tardiff, 2006). However, it is possible that even *Cuscuta* species with a wide host range can be incompatible with certain hosts (Costea & Tardiff, 2006). Tomato and bean plants are well known to be parasitized by *C.campestris* (Costea & Tardiff, 2006). Therefore, if the experiment were to be repeated, host plants that are more commonly parasitized would be used and tested first, before the mass germination of host plants.

Atypical germination

Dodder seed germination was implemented as discussed in the methods section, however, it was initially implemented without the antifungal agent benomyl; this was because the original study conducted to germinate dodder in a laboratory setting by Saeed & Zaroug (1989), did not use a fungicide agent. The first and second batch of *C.gronovii* seeds showed germination rates of approximately less than 4%, this was far lower than the expected germination rate of 98% demonstrated by Saeed & Zaroug (1989). Both the first and second trials for germinating the seeds suffered contamination from *Rhizopus* and mould as shown below.

Figure 15: An unsuccessful batch of *C.gronovii* seedlings contaminated by mould.

The third batch of seeds was treated with an antifungal agent and was successfully germinated. These new seeds had a germination rate of approximately 20%, which was still far lower than the expected germination rate. Saeed & Zaroug (1989) used *C.japonica* seeds to determine dodder germination rates when submerged in concentrated sulphuric acid. While this method may have worked for *C.japonica* seeds, the results were significantly different for *C.gronovii* seeds. It is also possible that the strong concentration of sulphuric acid (98%) may have inhibited the growth of seedlings or caused a difference in their natural ability to respond to hosts. Naturally, *C.gronovii* seeds would overwinter at cold temperatures below freezing and germinate at shallow depths the next season. If the experiment were to be conducted again, freezing the dodder seeds as a means to break dormancy can be implemented. There should also be a control group for seeds germinated without sulphuric acid so that the results can be compared for efficacy.

Soil Characteristics

The soil that the germinated dodder seeds were exposed to may have also inhibited their growth. The soil medium used for planting the host plants was nitrogen rich and consisted mainly of compost and organic material. The soil at Hogarth plantation was dry, sandy and acidic. Hogarth plantation is a red pine plantation, therefore, dead organic matter was far less prevalent when compared to the experimental host environment which used compost and nitrogen. A recommendation to improve soil type in the host environment would be to use less compost and organic material and try to mimic the natural soils as closely as possible. In this case, excess nitrogen should be avoided and sandy soils can be mixed in, given that the host plant can survive these conditions. Therefore, it is also important to select host plants that can survive drier soils with less organic matter.

Allelopathic effects

Many plants are known to produce compounds that are inhibitory to the germination of seeds and growth of seedlings. Hassannejad & Ghafari (2013) demonstrated that various members of the mint family could impact seed germination and seedling growth of *C. campestris*. Perhaps something similar was happening with *C. gronovii* in the current experiment where allelopathic chemicals were being released by the roots of tomato, corn and bean into the soil, thus inhibiting growth.

Humidity Levels

It is possible that the thinning of the dodder vines may be a result of dehydration. Under natural conditions, dodder seedlings would germinate from a shallow depth under the soil. In this experiment, the *C.gronovii* seedlings were placed at the surface level due to the nature of germination and the methods used by Runyon *et al.* (2006), who placed dodder seedlings at surface level to determine dodder's attraction to tomato volatiles. However, the environment in the greenhouse was dry even with constant watering/misting every day. The moisture level at the surface was easily dried out due to the presence of an overhead fan installed in close proximity to the host plants. Humidity levels should have been improved by placing a breathable plastic layer to enclose the host plants. This way, moisture can be trapped in the host environment and *C.gronovii* seedlings will not be affected by surface level moisture loss.

Temperature

The temperature in the greenhouse may not have been optimal for the growth of *C.gronovii*. Under natural conditions, dodder would germinate in the spring after it overwinters in much colder temperatures. Spring temperatures in Thunder Bay can range from -3 to 15 ° C, depending on the year. Therefore, the host environment may have been too warm for the *C.gronovii* seedlings. A consistent and colder atmosphere may have supported better growth of *C.gronovii* seedlings.

It is also possible that the difference in the temperatures at which the seedlings were germinated in (27°C) and the temperature at which they were introduced into the greenhouse (21°C), caused an inhibited response from *C.gronovii* seedlings. Therefore, it is recommended that the seedlings are germinated at the same temperature as their host environment, to maintain consistency.

Day length

Dodder growth was observed during winter. Normally, during winter, *C.gronovii* seeds would remain dormant in the soil as it overwinters and germinates in the spring. Days in the spring receive more intense sunlight, for longer periods, when compared to the days in the winter. Since the experiment was carried out in winter, this could have affected *C.gronovii's* ability to grow adequately. The intensity and duration of sunlight during the day may have been insufficient to exhibit its natural growth patterns.

Maturity of Host Plants

The maturity of tomato plants was far greater than the maturity of corn and bean plants. This is because tomato plants survived the oversaturation phase and did not require additional germination to replenish their numbers. However, corn and bean plant numbers were replenished through additional germination. Several corn plants were older, and some were younger. The age of bean plants remained consistent. Tomato plants used were over 3 months old, corn plant ages varied between 1- 3 months and bean plants were 1 month old. Therefore, it is possible that the varied maturity of plants was unsuitable or unfamiliar to *C.gronovii.*

Resistance in Tomato Plants

Even though host selection was limited, the older tomato plants may have been preferred because of their maturity and greater volatile release. It is impossible to say for certain that *C.gronovii* prefers mature tomato plants. However, it is also possible that the mature tomato plants repelled or interfered with *C.gronovii's* natural ability to select a host. It has been noted that several species of tomato, specifically Heinze tomato varieties, were resistant to dodder parasitism (Goldwasser *et al.,* 2001). *Cuscuta pentagona* seedlings, when grown in a greenhouse environment, would latch on to tomato hosts and die shortly after, as a result of the haustoria's inability to penetrate the stem (Goldwasser *et al*., 2001). The behaviour of *C.pentagona* was very similar to the behaviour of *C.gronovii* seedlings that parasitized tomato plants in this experiment. Therefore, it is possible that the variety of tomato used was a resistant type. While it is a possibility, it is not a certainty that the variety of tomato used was resistant, due to limited research availability on the topic.

CONCLUSION

In conclusion, the host preference of *C.gronovii* could not be determined among corn, bean, and tomato plants. The reason behind *C.gronovii'*s inability to successfully choose and parasitize corn, bean, and tomato plants is uncertain. Although the results do not show that tomato, corn, and bean plants are viable hosts, the numerous external factors that impacted this experiment call into question the validity of the results. In addition, the possibility that C. *gronovii* may select hosts based on volatile sensitivity cannot be excluded, as the results of this experiment are inconclusive. Due to the reduction in the number of volatile plants and several failed seed germination attempts, the scale of this experiment is severely lacking to make an accurate judgment on the host preference of *C.gronovii*. It was observed that most *C.gronovii* seedlings died, showed inhibited growth, or non-directional growth; a variety of environmental factors could be the cause. Future studies could be done in a controlled environment that can mimic the natural setting, and hosts of *C.gronovii,* as closely as possible.

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APPENDICES

Figure 16: Corn and bean pairings

Figure 17: Example of a corn and tomato pairing

Figure 18: Dodder vines shriveling several days after parasitizing a bean plant.

Figure 19: Dodder seedling chooses tomato plant over corn

Figure 20: Dodder seedling choosing tomato over corn during its initial stage of selection (zoomed in)

Figure 21: Non-directional growth of dodder seedlings in a pairing between bean and tomato (zoomed out)

Figure 22: Non- directional growth of dodder seedlings in a pairing between corn and bean (zoomed out).

Figure 23: Non directional growth of dodder seedlings zoomed out.