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A Tragedy Exposed? Clear Growth Medium Reveals Competing Roots

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Faculty Mentor, Dr. Nicholas McLetchie: This work represents a critical preliminary effort to quantify the Tragedy of the Commons (ToC) in plants growing in translucent growth medium. To the best of our knowledge, the application of translucent growth medium to ToC root studies pioneered here is completely novel. This development should allow future ToC studies of root competition to directly assess the effects of root competition on the spatial structure of root systems in real time throughout the life cycle of the developing plants. This will, in turn, inform the future development of spatially explicit simulation models of root growth. We believe that the groundwork laid in this study will ultimately transform the contemporary understanding of the nature of the Tragedy of the Commons in root competition. The growth protocol developed here represents extremely high quality work on Chris's part, which easily warrants publication.

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Introduction

The Tragedy of Commons (ToC) was first described by Garret Hardin in 1968 as an explanation for the conundrum of unrestrained population growth. Hardin applied it to a wide array of environmental phenomena. He described it as any circumstance in which multiple individuals have open access to an open resource which causes the selfish exploitation of that resource by all the individuals. This selfishness leads to a tragic collapse of the resource pool (Hardin 1968). The commercial fishing industry exemplifies a ToC because open-access to ocean fisheries has driven up competition and subsequently devastated fish populations. The ToC is also responsible for many other environmental issues including overgrazing, water and air pollution, excessive energy consumption, excessive waste-production, deforestation and over-fertilization. For environmental issues like air pollution, individuals exploit the commons by externalizing (sharing) costs instead of overutilizing common benefits; examples include polluting with toxic waste, excessive fertilizer and greenhouse gases. Applying game theory to explain a ToC requires looking at the benefits to the individual exploiting the commons as plus one unit of resource, since the individual gets the entirety of that benefit. In contrast, loss of one unit of resource from the common supply is distributed among all the members sharing the commons. Therefore the exploiting individual receives the entire benefit but pays only a fraction of the cost (Hardin 1968). Given this incentive, each individual will maximize net benefit by

exploiting as much of the resource as possible. However, the resource pool may not be able to sustain exploitation. If this happens the system deteriorates and environmental tragedy occurs. In order to avoid a ToC, there must be mutual avoidance of any selfish behavior that jeopardizes the common resource.

In the natural world many social organisms have mastered this mutually beneficial solution to the Tragedy of the Commons (Nowak 2008). This is exhibited best by the members of bee, ant, and termite colonies that consistently limit personal resource gain for the good of the colony. Plants may seem like a stark contrast in sociality to the aforementioned organisms; however, recent research finds plants capable of sophisticated communications and complex interactions (Bais et al 2004; Beiler et al 2010; Gruntman and Novoplansky 2004). Conspecific plants have even been found to recognize and help each other through these complex interactions. (Callaway 1995; Hauggaard-Nielsen H and Jensen ES 2005). However we do not know if the plants have harnessed these abilities to help solve the ToC and cooperate when acquiring shared soil nutrients.

In 2001, Gersani et al published " Tragedy of Commons in Plant Root Competition," a landmark paper that presented the theory behind the ToC in plant root scramble competition and sparked extensive plant root ToC research. However, more recently others have challenged Gersani's ToC paper bringing to question the prevalence of Hardin's model to plant roots (Semchenko et al 2007).

This study seeks to further test the ecological model of the ToC by pursuing the main research question: Does the Tragedy of the Commons exist in *Brassica rapa* plant root competition. In order to quantify a Tragedy of the Commons we used classic methods as described by Gersani and novel methods. Gersani found a ToC when root mass increased, a consequence of more intense competition. As this intensified competition drove a resource pool to depletion, the plants reduced their reproductive mass and thus their reproductive success. We hypothesized that plant root competition in *Brassica rapa* would increase root mass and reduce reproductive mass in competing plants. Additionally we used photographic analysis to quantify root architecture. Although root architecture has been successfully used for other applications this will be the first time is applied to showing a Tragedy of the Commons. (Lynch 1995; Dolordot et al 2007; Iyer-Pascuzzi et al 2010)

Our goal in this study was to reveal the roots' response to nutrients and spacing as it relates to reproductive mass yield in *Brassica rapa*. This information would be valuable for agricultural applications of *Brassica rapa* or canola, Canada's largest grossing cash crop, which generated profits of 2.8 billion in 1999 (Kershen 2000). This study could be used to improve *Brassica rapa* crop spacing techniques in order to maximize seed production per acre in future agricultural systems.

Methods

Model Plant

Brassica rapa or fast plant is our model organism for root competition. Shoot competition is thought to be less significant than root competition (Donald, 1958). However, to further minimize shoot resource allocation we used a strain of dwarf *Brassica rapa* that were homozygous recessive mutants with gibberellin deficiency.   Our strain is a rapid cycling Fast Plant© distributed by Carolina Biological. The plants flower in 18 days and complete an entire life cycle in 35-40 days.

Growth Vessels

Growth vessels housed two plants and consisted of two experimental groups that manipulate competition. First group is growth vessels with impermeable barriers with one plant on each side. The barrier subdivides growth medium and 1.5 cm of the air above. The second group is without barriers with one plant on each side. 4.5 by 4.5 cm plastic squares placed vertically and sealed with pH neutral silicone sealer was used to create impermeable barriers that equally divide 100 ml graduated Biomex beakers. Growth vessels were gravity autoclaved after the silicone had cured.

Growing Medium

Distilled water was heated to just below boiling and was mixed in 2.5 g per liter of gellam gum and components of *Brassica rapa* Hoagland's nutrient solution (see Hershey 1992) were added. The solution was poured into growth vessels up to the 50 ml graduation. Using a Fischer Scoopula, symmetrical water wells were dug out of the Gellam Gum at the far ends of the growth medium. Below the Gellam Gum line beakers were wrapped in two layers of obscure paper towel which was covered by aluminum foil to insulate and block light.

Planting

        Seeds were not sterilized because of concerns of decreasing germination success. Seeds were planted half a centimeter from the vessel's center (perpendicular to barrier). If there was no barrier, the vessel was oriented as if it had one. Seeds were buried 1 mm below the surface. 124 seeds were planted. Each experimental group contained half of the total samples. 14 days later an additional 56 plants were planted; 28 in both experimental groups. This was done to sample two different plant maturities as Wilson argues is useful in plant competition studies (1988). After planting, a thin layer of water was applied to the top of the growth medium and the seeds germinated in full light.

Growth Conditions

        Five 20w cool white fluorescent bulbs were placed four inches from plants, and moved upwards as plants grew toward them. Lights were kept on continuously to allow the Fast Plants© to grow rapidly. Temperature was kept at a constant 24 degrees Celsius. Water wells were filled daily with distilled water. Once a week, the wells were filled with 0.5 ml of 10% nutrient solution in distilled water.

        Two trays housed the growth vessels. On the left tray, growth vessels with barriers were placed here and oriented toward the back of the growth chamber. On right tray, all the non-barrier growth vessels were housed and they all were oriented toward the back of the growth chamber. All vessels were under the same layer of plastic wrap to minimize evaporation of growth medium. Any plants that grew mold were taken out as soon as discovered.

        Our dioecious *Brassica rapa* were thoroughly hand fertilized using dried honey bees upon maturation of the flowers. Hand cross-fertilization was performed within experimental group and continued until apoptosis of stamen or pistil.

Harvesting

        At the end of the 40 days, vessels were photographed along their meridian and from directly below. A Nikon D50 SLR using an 18mm-55mm lens with manual focus captured macro images of a uniformly located growth vessel placed on top of a light board. To avoid glare and lighting effects, the light source was only directly below the growth vessel and direct light was blocked from the camera's lens. The camera was mounted directly below the mounted vessel for photos from underneath. The growth vessel was illuminated from two sides with two

20 w white light fluorescents. Light was uniformly directed so that it only illuminated the vessel from the sides and direct light was blocked from the camera's lens. A circular open space in the vessel mount's bottom allowed the camera to visualize the roots from directly below.

124 plants were harvested after 40 days (full life cycle) and 56 plants were harvested after 26 days in order sample effects of competition on less mature plants. After harvesting the growth vessels were individually microwaved for 15 seconds to liquefy the soil medium. The root systems were then cleaned with distilled water and individual plants were separated if necessary. If the roots' owner was unidentifiable it was attributed to the pot's total roots. Plants were briefly dried on a paper towel. The longest tap root length was measured. Then, the plants were then cut into root, shoot and reproductive parts and dried in a drying oven at 60 degrees Celsius for five days. Samples were then weighed to the nearest milligram. And then the seeds of each plant were counted. Only samples who had a successful competition (dry mass >.007 and reproductive organs) were counted towards the data set

Analysis

We analyzed mass of root, shoot and reproductive organs as a gross estimate of the plants' allocation strategy. We ran one-tailed student t-test on the homoscedastic values of root mass, reproductive mass and tap root length. We also pursued ANCOVA analysis of proportion of root mass versus reproductive success as well as proportion of reproductive mass versus total mass, but these are not included due to lack of significance (P>.4 in all cases). Ideally mass would be analyzed along with root architecture values (total root length, root direction, root diameter, root volume). We obtained root architecture values using the semi-automated quantitative root analysis program SmartRoot an open-source plugin for ImageJ software (Rashband, 1997; Lobet et al 2011).

Validation of SmartRoot

We attempted to validate the results of the semi-automated SmartRoot plugin. Two wire models were pre-measured and then constructed to model actual root systems. They were then placed in a barrier growth vessel in normal growth medium and photographed and analyzed following the same methods.

Results

A total of 29 out of 62 forty day growth vessels had at least one unsuccessful competitor that invalidated the growth vessel by removing competition. Additionally 12 out of 56 of the twenty six day growth vessels have a least on unsuccessful competitor and were thrown out of the results. Experimental groups were uneven due to unsuccessful plants being more prevalent in the no barrier experimental group. Also plant #64 had 86 seeds. That is over half the seeds in the no barrier group. This caused the no barrier average to appear higher.

Generally 26 day old plants had either none or just a few immature seeds. This group was not massed but was photographed because it was the only group simple enough to undergo photographic analysis but lacked significant enough size to be accurately dry massed. 26 day old plants could be accurately separated from competitor and measured for length. All successful 40 day old plants were massed but were not measured for length due entangled root mats forming. If this occurred, the entire root system of both competitors was measured together and analyzed as a growth vessel instead of an individual plant.

Photographic analysis failed to be validated. Hand-measure diameter values were all

	n	Reproductive Mass	Root mass	Seeds Count	Longest Root Length

2mm for both models and SmartRoot consistently returned values of 4mm. Additionally hand measured lengths of both models were 9.1 cm. SmartRoot returned values of 7.1 cm and 5.3 cm. Basic root topography analyses were accurate as smart root successfully identified primary and secondary roots.

Root mass varied significantly between experimental groups ($p = .014$) allowing us to reject the null hypothesis that difference in longest root length exist between competitor and thus favors the alternative hypothesis that longest root length changes if competition is allowed. The other values in table 1 were insignificant; thus we were unable to reject our null hypothesis. Additionally the no barrier group has a substantially higher standard error in all counts. This made it impossible to find a statistically significant regression line for the no barrier group

			Average	Standard error	Average	Standard error	Average	Standard error	Average	Standard error
40 day old plants	Barrier	30	28.7 mg	2.4	17.0	1.9	1.5	0.5		
	No Barrier	22	32.0 mg	6.8	19.0 mg*	5.5	5.9	3.9		
26 day old plants	Barrier	26							8.8cm	0.69
	No Barrier	18							11.3 cm	0.81
t-test			.473=t		0.6= t		1.13=t			
P-value			p> .30		p> .25		p>.15		P=.014	

Table 1 Dry Masses, Seed Count and Taproot Length

* 40 day, no barrier root systems data was analyzed per growth vessel instead of individual (n= 11) (see **Results**)
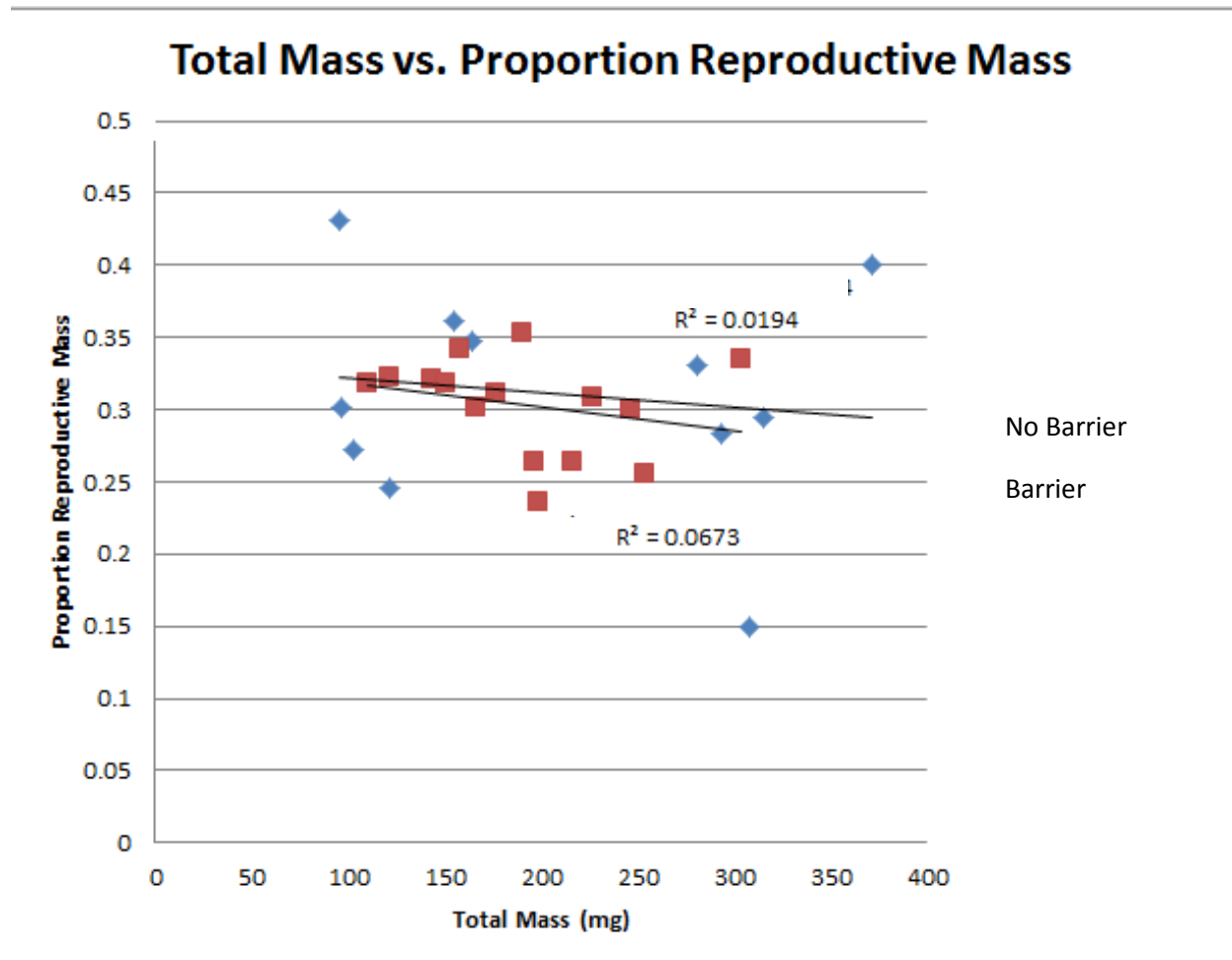
Figure 1 shows each individual pot's total root mass versus total seed mass. The graph shows high variation and plant # 64, the outlier that caused the open experimental group to have a higher seed count.

Figure 1   Dried Root Mass Versus Dried Reproductive Mass
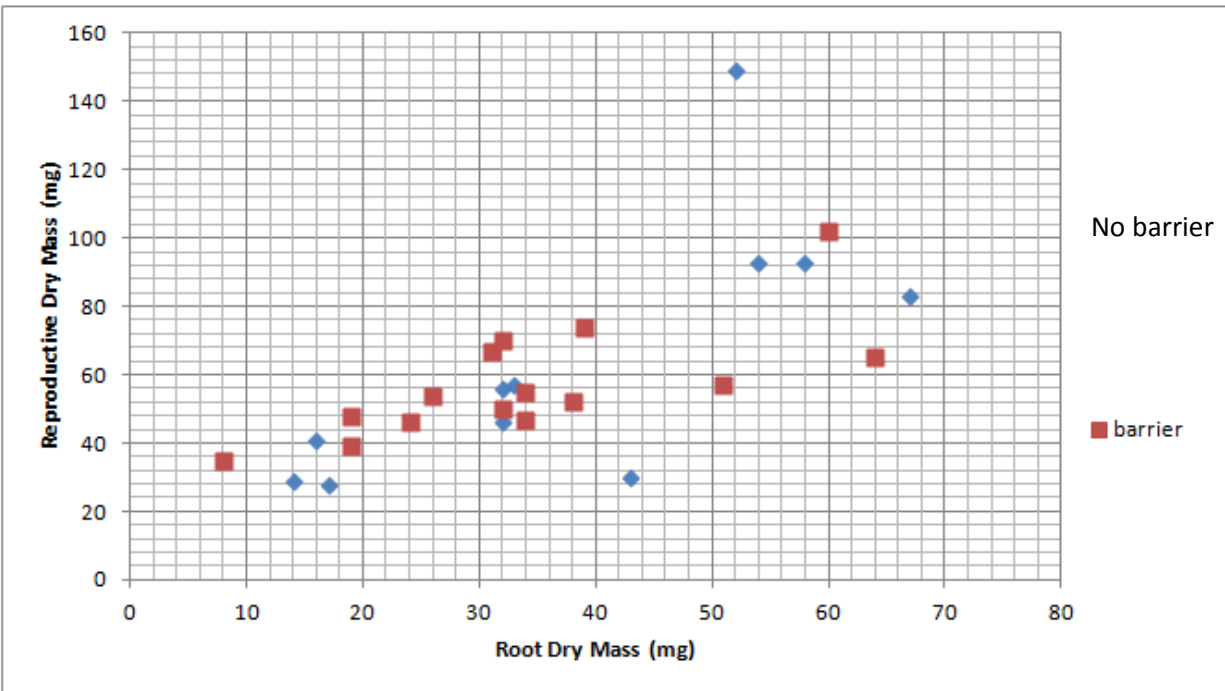
Lines of best fit were insignificant for Figure 2. Proportion of reproductive mass was plotted against total plant mass for both experimental groups. Notice low r^2

Figure 2

Total Mass vs. Proportion Reproductive Mass

$R^2 = 0.0194$

$R^2 = 0.0673$

No Barrier

Barrier

Proportion Reproductive Mass

Total Mass (mg)

Conclusions

We found the competitive plants tended to have longer tap roots than the non-competitive plants (p=.014). Typically the *Brassica rapa's* tap root would grow straight down to the bottom of the growth vessel then circle the bottom edges repetitively. The difference in tap root length could be due to the increased circumference for the no barrier plants' tap roots to circle. However this result is also consistent with Gersani's model, given that the barrier-less group had increased competitive effects according to this unreliable measure.

The differences between the other categories of measurement (root mass, seed mass and seed number) were all insignificant with p values of p>.25, p>.30 and p > .15 respectively. Statistical analysis revealed that the no barrier group's values were neither in Gaussian distribution nor considered homoscedastic.

An explanation for our insignificant results is that an excessive abundance of soil nutrients might have attributed to the lack of competition effects. Researchers have found that root competition increases in harsher conditions, including in poorer soil (Cahill 1999; Pugnaire & Luque 2001)

Another explanation is to attribute the plants' lack of a reproductive cost to their mutual cooperation. Our strand of *Brassica rapa* was highly inbred due to the Carolina Biological breeding methods for creating fast growing plants. This breeding method might have created enough genetic relatedness between the competitors to facilitate kin selection which is common in other inbred organism like naked mole rats and social insects (Freeman, 2007). Research has shown that kin selection facilitates sibling plants to minimize competitive costs (Lambin et al, 2001; Nakamura, 1980). This is consistent with other research which observed positive below-ground interactions between related plants (Callaway 1995; Hauggaard-Nielsen & Jensen 2005). In *Rhapanus sativus,* (Brassicaceae) increasing genetic relatedness from half sibling to full sibling was shown to decrease interference competition (directly affecting a competitor with chemicals) (Karron and Marshall, 1993). If the genetic relatedness of our *Brassica rapa* strain lowered competition between siblings, than it might result in experimental groups that don't vary significantly without a more sensitive measure. To test for the role of genetic relatedness in mitigating competition effects we could set up a study using the highly inbred *Brassica rapa* (Wisconsin Fast Plants©) as well as a wild-type *Brassica* found growing wild in Lexington, Ky. We would set up three different experimental groups. The first would be inbred vs. inbred which we'd hope to have the least competition. The second would be wild-type vs. wild-type and these might have intermediate signs of competition effects since they are conspecific but not drastically inbred. Thirdly we would have wild type vs. inbred which we would expect to have the far greatest competition effects since they are only related by genus.

The failed validation of our imaging analysis platform is believed to be largely due to blocking effects of roots in the foreground. Additionally the 2x magnification of the root diameter is thought to have been caused by the gel and the curved sides of the beaker. The magnification could be accounted for by multiplying all the data by a factor of 0.5. However to validate the imaging platform, we would have to take images from multiple angles around the circumference of the beaker in order to eliminate the blocking effects. The rendering of a 3-d

computer model from pictures at every 6 degrees of the circumference has been proven effective for finding accurate root architecture values (Iyer-Pascuzzi, 2010).

Acknowledgements

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