Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Volume 39, Article 11, 2018

Preparing Your Students for the Future: Undergraduate Research Experiences with Rapid-Cycling *Brassicas*

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Undergraduate research experiences are valuable for all students, both science majors and nonmajors. Engaging in undergraduate research experiences provides unique problem-solving and innovation opportunities that can help build proficiency for professional excellence in any career. Wisconsin Fast Plants and other Rapid-Cycling *Brassica*s—with their short life cycles, genetic variation and phenotypic plasticity—are ideal organisms for both short- and long-term undergraduate research projects and independent studies. The Wisconsin Fast Plants Program staff and its research seed stock collection, the Rapid-Cycling *Brassica* Collection, are dedicated to supporting undergraduate research and education initiatives by supplying seed varieties of Rapid-Cycling *Brassica*s, background information, protocols, relevant existing data sets and on-line mentoring and support.

Keywords: undergraduate research, *Brassica*, plant breeding, plant growth and development, artificial selection, Fast Plants

Introduction

Presented in this paper are the background information, basic protocols, and materials/equipment descriptions that can be used to launch countless undergraduate investigations, using Rapid-Cycling *Brassicas* as a model organism. The intention for what is included here is to provide a combination of resources: 1) Brief descriptions of tried-and-true investigations that work well in undergraduate courses and research experiences. 2) Background information about seed stocks available for faculty and undergraduate students from the Rapid-Cycling *Brassica* Research Collection that may be used in designing and implementing original laboratory exercises or independent research.

Rapid-Cycling *Brassica* Origins

Professor Emeritus Paul H. Williams, in the Department of Plant Pathology at the University of Wisconsin-Madison, developed Rapid-Cycling *Brassicas*. Dr. Williams bred these Rapid-Cycling *Brassicas* as research tools that could be used for improving disease resistance of cruciferous plants. In order to speed up the genetic research in the crucifers, he began breeding *Brassica rapa* and six related species from the family *Cruciferae* (aka *Brassica*ceae) for shorter life cycles. The end result: petite, quick growing plants known as the Rapid-Cycling *Brassica rapa*—or Wisconsin Fast Plants®.

Dr. Williams continued to select plants that had characteristics most suitable for laboratory and classroom use, such as:

- Rapid development—short time from planting to flowering; rapid seed maturation with no seed dormancy
- High fecundity—able to produce many seeds from a single plant
- Small plant size—petite plants requiring minimal support for growth
- Ease of growth—ability to grow under continuous fluorescent lighting in a standard potting mix

For further reading, see Williams and Hill (1986) and Tomkins and Williams (1990).

Rapid-Cycling Brassica Collection (RCBC)

The Rapid-Cycling *Brassica* Collection (RCBC) is a gene bank and an international source for diverse seeds stocks of Rapid-Cycling *Brassica*s. Researchers from universities and industries around the globe use Rapid-Cycling *Brassica*s as both model organisms in research and as diverse seed stocks for agricultural applications.

The RCBC is committed to producing, maintaining, and distributing, viable and true-breeding

Brassica seed stocks for researchers. In so doing, our mission is to promote greater understanding of plants and the interactions that plants have in the environment to benefit basic biological sciences and agricultural practices.

The RCBC strives to represent the UW College of Agricultural and Life Sciences and the Department of Plant Pathology as a leader in agronomy research and practices by maintaining a high-quality seed bank that preserves with integrity its distinct Rapid-Cycling *Brassica* seed lines.

Brassicas include a diverse group of crop and research plants. Crop varieties of *Brassica*s are used in the production of condiment mustard; stored, processed, and pickled vegetables; seed oils for margarine, salad oil, cooking oil, biofuels, plastic and industrial use; animal fodders; and green manures for soil rejuvenation (Williams & Hill, 1986). While crop varieties of *Brassica*s have great economic value worldwide, Rapid-Cycling *Brassica*s are used primarily in research as model organisms.

The RCBC focuses on these six main species of Rapid-Cycling *Brassicas*:

- *Brassica rapa (campestris)*
- *Brassica nigra*
- *Brassica oleracea*
- *Brassica juncea*
- *Brassica napus*
- *Brassica carinata*

These *Brassica* species (developed by Dr. Paul Williams at UW-Madison) are extremely valuable because the life cycle of these plants is roughly 40 days, making them an effective research and teaching tools for genetics (Wendell & Pickard 2007); host-parasite relations, cellular biology, and molecular biology (Cardoza & Stewart, Jr. 2004), plant biochemistry, population biology, plant breeding and plant pathology (Tomkins & Williams 1990); and bioremediation of heavy metals in soils (Page &Page 2002).

In addition to providing quality seed and information for researchers the RCBC is dedicated to supporting undergraduate research and education initiatives by supplying seed varieties of Rapid-Cycling *Brassicas*, background information, protocols, relevant existing data sets and on-line mentoring and support.

Laboratory Objectives

Three research projects that were used as examples in the workshop are described briefly in this paper: 1) Investigating the effects of nutrition on plant growth and development, 2) Investigating the effects of a combination of population density and nutrition on plant growth and development, and 3) Investigating the effects of selective breeding for the polycot phenotype. Together, these three investigations provide opportunities for investigating:

- Structures, functions, and processes during the flowering plant life cycle, using Rapid-Cycling *Brassicas* as a model organism.
- Limiting factors and plant nutrition.
- Resource competition.
- Inheritance, evolution, and the interplay of genetics and environmental factors in determining phenotype.

Once familiar with the Rapid-Cycling *Brassica* life cycle, the protocols and other materials included in this paper are intended to support a wide variety of potential investigations that could be designed easily and implemented in an appropriate time-frame, using these fast-growing model organisms.

Background Information

<u>Timing</u>

The level of difficulty and time required to prepare, set up, and engage students with investigations that utilize Rapid-Cycling *Brassicas* can range from two or three days to a full semester. For example, an investigation that involves germinating seeds on moist paper toweling in a Petri dish requires minimal preparation time and approximately 72 hours for germination. Alternatively, an investigation into the effects of selection necessitates growing at least two generations of Rapid-Cycling *Brassicas*, requiring periodic tending over approximately 55-60 days.

The entire life cycle takes 35–45 days, from planting to harvesting seed. From day to day, the amount of time required varies, depending on the task and data collection required for the investigation being conducted. For example, some days may involve tending, simple observations, and checking the water levels with only a few minutes needed. Other days, such as those when students plant, collect quantitative data, pollinate, or harvest seed, may require 30 minutes or more. After flowers are pollinated, the plants require little care (except for watering) until the day the seeds are harvested.

Timing key events in the life cycle to fall on days when students have class is an important consideration. For example, planting, thinning, and pollinating can be accomplished by students on a weekly basis if need-be, though some supplemental tending will be necessary--particularly as plants grow larger and require more frequent watering. A "Grower's Calendar" is included in Appendix B as a generalized guideline for the timing of life cycle events from planting through seed development and harvest.

Environmental Considerations

Perhaps the most critical component for growing Fast Plants is 24 hours of intense fluorescent lighting. Fast Plants will grow poorly and may not reproduce if grown with the light available on a windowsill or in a greenhouse without supplemental lighting. High intensity lighting is very important in order to grow strong, healthy Rapid-Cycling *Brassica* plants that complete their growth cycle in the anticipated time. Fast Plants can be grown under commercially available fluorescent light banks, Compact Fluorescent light bulbs, or LED grow lights. Materials lists and procedures for building an inexpensive and effective fluorescent light bank or a Plant Light House (constructed from a box or plastic milk crates lined with aluminum foil with a 150 Watt equivalent CFL bulb) are available on the Fast Plants website (www.fastplants.org).

Temperature is the other environmental factor that has the greatest impact on Rapid-Cycling *Brassica* growth and development. Temperatures that stay consistently (day and night) in the range of 65–80°F are optimal for growth and development that aligns with the timing given (e.g. number of days to flower) for each Rapid-Cycling *Brassica* variety.

Troubleshooting any challenges that arise during the life cycle is much like growing any plant--it gets easier with practice. Included in the *Notes for the Instructor* section is a basic troubleshooting guide. In addition, staff who work with Wisconsin Fast Plants and the Rapid-Cycling *Brassica* Collection are quick to respond to email inquiries. Writing to info@fastplants.org is the easiest way to reach their assistance.

Ordering Seed From the Rapid-Cycling *Brassica* Collection (RCBC)

Seed Stocks and other resource are available for credit card purchases by university and college researchers via the online store at: rcbc@plantpath.wisc.edu The staff of the RCBC are accessible via email (rcbc@wisc.edu) and scheduled phone / video calls to assist researchers and others utilizing the seed stocks and resources of the Collection.

Student Outline

Requirements in Brief

- Planting containers – plastic multipot cell packs
- Watering – constant water availability via capillary wicking system with reservoir
- Fertilizer – slow release pellets, or liquid fertilizer solution
- Growing medium – soil-less, finely ground seedling starter mix, based of peat moss
- Lighting – constant 24-hour lighting of ≥200µmol/m^2/s

Planting Protocol for *Brassica rapa*

- Planting container & growing system
  - recommended: plastic cell packs of 10cc's per cell for *Brassica rapa*
    - Rapid-Cycling *Brassica rapa* can complete its life cycle in this cell pack
  - if a larger plant is desired, larger soil volumes (and more fertilizer) can be used
  - recommended: position cell packs on capillary wicking system with reservoir
- Choosing a fertilizer
  - option 1: slow release fertilizer pellets will supply nutrients throughout life cycle of Rapid-Cycling *Brassicas*; use a balanced NPK ratio
    - use 3-4 fertilizer pellets per plant per 10cc's of soil
  - option 2: prepare a liquid fertilizer solution with a balanced NPK ratio
    - recommended: for "full growth" the solution should be ~100ppm N
    - apply 2-3ml per plant at seeding and at 3, 7, and 10 days after planting
- Preparation of growing medium
  - recommended: soil-less, finely ground seedling starter mix, based of peat moss
  - optional: course vermiculite can be added (up to 1:1 for peat-moss:vermiculite) to reduce compaction of growing media
  - mix growing media until damp and fluffy, adding small amounts of water if necessary; media should feel lightly moist, but remain loose and fluffy
- Planting seeds
  - fill cell packs loosely with moistened growing media until half full
  - if using slow release fertilizer pellets, add the pellets now
  - fill the remainder of the cell packs with growing media
  - place 1-2 seeds per cell on surface of media
  - cover seeds with thin layer (~0.5cm) of course vermiculite or growing media
  - sprinkle water to hydrate the thin layer of vermiculite/media over your seeds
- Position your growing system
  - place cell packs on capillary wicking bed with reservoir and fill water reservoir
  - place growing system under adequate lighting (constant, 24 hours, ≥200µmol/m^2/s)
    - keeping growing surfaces of plants within 5-10cm of lights helps ensure sufficient light intensity

Maintenance and Tending for Brassicas

- Watering – Under crowded conditions of cell packs, plants will use large amounts of water, especially as they reach flowering size.
  - Use of a capillary wicking system with reservoir allows constant, consistent availability of water to growing plants.
  - Ensure that water reservoirs stay sufficiently full, especially over weekends and during flowering.
  - Capillary action between soil and wicks can be re-established by gentle watering over the top of the soil.
- Thinning – When several seeds are sown in a single cell pack, emerging seedlings should be thinned to a single plant as soon as possible.
- Staking – When grown under sufficient lighting, most RCBC stocks are sufficiently sturdy to support themselves and a good load of seed pods. Certain mutant stocks may require staking for extra support. Bamboo skewers can be inserted into cell packs as a support structure. Lightweight twine can be used to manage plant support with bamboo skewers.
- Lighting – For sufficient lighting, ensure that growing tips of the plants stay within 5-10cm of lights. (This may not be necessary for High Intensity Discharge lamps including High Pressure Sodium or Metal Halide lighting).
  - Note that Rapid-Cycling *Brassicas* will not be successful when grown in a windowsill, when grown with normal overhead lighting, or when grown in a greenhouse without supplemental lighting.

- Fertilizing – If using a liquid fertilizer solution, remember to periodically fertilize the plants.
  - recommended: for "full growth" the solution should be ~100ppm N
  - recommended: apply 2-3ml per plant at seeding and at 3, 7, and 10 days after planting
- Pollination – Rapid-Cycling *Brassicas* do not self-pollinate; thus pollination is required to produce seeds.
  - recommended: pollinate with a beestick (a bee's thorax glued to a toothpick) by transferring pollen between flowers of different plants
  - to reach maximum potential seed yield, pollinate 2-3 times over 5 days, beginning when at least half of the population of plants has open flowers
- Harvesting – When seeds near maturity, they must be allowed to dry prior to harvest and replanting.
  - Stop watering and empty reservoirs 20 days after last pollination. Seeds are ready for harvest when both plants and pods are dried, browned, and crispy.
  - Seedpods can be broken up by crushing them inside a paper bag. Then, seeds can be separated from seedpod chaff by screening with a sieve.

Additional information about planting and tending *Brassica rapa* is available on the Wisconsin Fast Plants website (www.fastplants.org) and on YouTube: https://www.youtube.com/user/FastPlants

Protocol for Growth of non-rapa* Rapid-Cycling *Brassica Species as a Control

In general, the growth requirements for *Brassica rapa* can be applied to other Rapid-Cycling *Brassica* species, including *B. nigra, B. oleracea, B. juncea, B. napus, B. carinata,* and *Raphanus sativus.*

Protocol Modifications for Non-rapa Species:
- planting containers: plastic cell packs of 30-50cc's per cell (compared to 10cc's for *B. rapa*)
- fertilizer: slow release fertilizer pellets are recommended, use 5-8 pellets per plant
- development of plants: non-*rapa* species exhibit slower development (see table below)

Characterization of Development of Rapid-Cycling *Brassica* and Radish Populations			
Species	Genome	Days to flower	Days for life cycle
B. *rapa* (campestris)	Aaa	16	36
B. nigra	Bbb	20	40
B. oleracea	Ccc	30	60
B. juncea	ABaabb	19	39
B. napus	ACaacc	25	55
B. carinata	BCbbcc	26	56
R. sativus	Rrr	19	48

Non-*rapa* species may be maintained/tended in the same manner as *Brassica rapa*.

Sample Investigation #1
Investigating Plants' Nutrient Needs with Brassica rapa

1. Predict what you think the answer to the following questions may be. Be sure to explain your answer.
   a. If nutrients (provided in the form of fertilizer) are important for *Brassica rapa* to grow, develop, and reproduce, is there some particular amount of fertilizer that is **best** for *Brassica rapa?*
   b. Do you think that there is a minimum amount of fertilizer that *Brassica rapa* must have to grow, flower, and produce seed that is healthy?
      - How would you find out that amount?
   c. If fertilizer is good for *Brassica rapa*, is more fertilizer always better?
      - How would you find out?
2. Read and discuss the Protocol for Growth of Rapid-Cycling *Brassica rapa.*

   a. Using the given protocol for the control, design experimental procedures that will likely develop data that can be used as evidence for determining if there is a particular amount of fertilizer that is "best" for *Brassica rapa*. Be sure to define how everyone participating in the experimental work will define and will quantify "best."
3. Brainstorm ideas about meaningful measurements that can be made while plants grow and develop through their entire life cycle. Be sure to include *plant height, number of days to flowering*, and *number of seeds produced per plant* in your observations.
4. Develop a data table for recording the observations and measurements that you will take as your *Brassica rapa* grow. Include in your data table the measurements and observations you make for plant height, number of flowers, number of seeds, and any other observations you and your colleagues decide to record as the *Brassica rapa* grow to determine which plants are doing the *best*.
5. Follow your experimental design to plant your *Brassica rapa* seeds.
   - Work with your team to **carefully add the assigned amount of fertilizer to each cell as you plant.**
   - Be sure to **carefully label your planting container (and cells)** so that you know which planting container is yours, the amount(s) of fertilizer in each cell, and can identify each individual cell while making observations.
6. Implement your experimental design, recording observations as planned in your data table.
7. Aggregate the data gathered by all and use evidence from those data to develop explanations for the original questions (those that you made predictions about at the start of this investigation):
   a. If nutrients (provided in the form of fertilizer) are important for *Brassica rapa* to grow, develop, and reproduce, is there some particular amount of fertilizer that is **best** for *Brassica rapa*?
   b. Do you think that there is a minimum amount of fertilizer that *Brassica rapa* must have to grow, develop, flower, and produce seed that is healthy and will grow?
      - How would you find out that amount?
   c. If fertilizer is good for *Brassica rapa*, is more fertilizer always better?
      - How would you find out?
8. Look for patterns and evaluate the aggregated experimental results. For each of the traits observed during the life cycle:
   a. Which are regulated by genetics? Explain your answer, and cite specific experimental evidence to support your claim.
   b. Which were influenced by the environment? Explain your answer, and cite specific experimental evidence to support your claim.
9. Reflect on your learning: How have your answers changed as a result of the experimental evidence your class collected? What new questions do you have about nutrition and plant growth and development?

Materials

Protocols for growing Rapid-Cycling *Brassica* that are included in the Student Outline describe one method that uses the following materials:

- Planting containers – plastic multipot cell packs
- Watering – constant water availability via capillary wicking system with reservoir
- Fertilizer – slow release pellets, or liquid fertilizer solution
- Growing medium – soil-less, finely ground seedling starter mix, based of peat moss
- Lighting – constant 24-hour lighting of ≥200µmol/m^2/s

However, a myriad of options for planting containers were developed by educators and researchers over the past 25+ years, and many of these planters employ recycled materials. Common to all good planting containers for Rapid-Cycling *Brassicas* is a water reservoir and wicking system that maintains a consistent moisture level in the growing medium. Two options for do-it-yourself planting containers are described on the Fast Plants website (www.fastplants.org) with associated planting instructions that can be given to students in print or video formats. Other examples can be found easily by searching online for "planting Fast Plants." Choices made regarding the type of growing container to use are integral to the question asked, experimental design, desired student-group size, and available materials.

Notes for the Instructor

Additional Sample Research Projects

Included in the Student Outline is a sample investigation that involves modifying the given growing protocol to design an experiment about the affects of fertilizer availability on *Brassica rapa* growth and development. This basic fertilizer experiment can be expanded upon and made more sophisticated by adding a population density variable in addition to nutrient availability. For more information, see "The Population Explosion" in the digital library on the Fast Plants website (www.fastplants.org). Both of these sample research projects primarily focus on how environment impacts the variation in traits that we observe in a population.

The next sample undergraduate research project explores more deeply the genetic component of variation. In this example, students selectively breed for a trait that appears not to be influenced by environment--number of cotyledons or seed leaves--to investigate how the frequency of a trait in a population can change over generations.

Sample Undergraduate Research Project: Selection for Polycotyledon Frequency

Background

Developmental plant mutants serve as useful materials to unravel the mechanisms necessary to better understand early grow and development of the embryo stage of organisms. Polycotyledon phenotype in Rapid-Cycling *Brassica rapa* is one such phenotype. A polycotyledonous phenotype (production of more than two cotyledons) is not limited to the *Brassica*ceae family. Polycots have been observed in the Asteraceae, Solanaceae, and Cactaceae families (and likely others) as well. However, there is a surprising lack of scientific literature on polycots. In general polycotyledon phenotype is not widely covered in extant publications (less than 200 results for a scholarly search of “polycotyledon”) leading to exploratory/observational papers.

The polycotyledon phenotype is an easily observable trait that is visible both at the seedling level and also during the development stages of the embryo via embryo dissection.

Figure 1. Preliminary results. The polycotyledon phenotype, three or more cotyledons, was identified in a number of Rapid-Cycling *Brassica* Collection research and educational varieties at different ratios (Table 1). Research stock 1-059 had a ratio of 1:250 plants. This stock is in a petite background and was initially developed for space research.

Table 1. Frequency of polycots in various rapid-cycling *Brassica* seed stocks.

Stock ID	# polycots	# total plants	% frequency	probability
1-133	12	14500	~0.00083%	~1 per 1,208
1-059	4	1000	~0.00400%	~1 per 250
1-145	4	3000	~0.00133%	~1 per 750

1-107	2	2800	~0.00071%	~1 per 1400

Plants expressing the polycotyledon phenotype from this seed stock were intermated and harvested separately. Progeny were observed, and recurrent selection continued for plants that expressed the polycotyledon phenotype. After four generations, we were able to stabilize a population with a 50% polycotyledon phenotype. Upon further selection and breeding we have produced populations with up to 80% expression of the phenotype. In addition, to better understand the inheritance of the trait, reciprocal crosses were made between plants with normal two cotyledons and plants with three of more cotyledons.

Table 2. Frequency of polycots in selected seed id lots of the 1-059 seed stock.

Generation	Stock ID	# polycots	# total plants	% frequency	probability
0	1-059	4	1000	~0.004%	~ 1 per 250
1	1-2275, 6, 7	23	250	~0.092%	~ 1 per 11
2	1-2290	48	273	~0.176%	~ 1 per 6
3	1-2502	127	415	~0.306%	~ 1 per 3.3
4	1-2528	206	384	~0.536%	~ 1 per 1.9

Conclusion

The polycotyledon phenotype appears to be a heritable trait that we have stabilized at different levels of expression in different Rapid-Cycling varieties. This includes varieties expressing 30% up to 80% polycots with in the populations.

Success with selectively breeding for the expression of the polycotyledon phenotype, increasing the trait frequency from one generation to the next in a relatively predictable manner, leads us to believe there may be one major gene controlling the expression of the phenotype, while many different genes may control the overall frequency of the expression in a population.

Further research has continued on the inheritance of tricots and tetracots separately as well as selfing polycot expressing plants and specific crosses to rule out of other inheritance models. For more information on these research areas contact the Rapid-Cycling *Brassica* Collection at: rcbc@wisc.edu

Sample Undergraduate Completed Research Project Abstracts

Phenotypic plasticity between two stocks of Wisconsin Fast Plants in a wind environment

Edler, J[1], Anibas, C[2], Hetue, J[3], Morales, R[1], Lauffer, HB[3], and de Leon, N[2].

[1] Wisconsin Fast Plants Program, student researchers; University of Wisconsin-Madison
[2] Dept. of Agronomy; University of Wisconsin-Madison
[3] Wisconsin Fast Plants Program, staff; University of Wisconsin-Madison

Edler J. 2016. Phenotypic plasticity between two stocks of Wisconsin Fast Plants in a wind environment. via Dept. of Agronomy, UW-Madison. 0:1-4.

Abstract - Plants with homologous genomes usually react similarly to different environmental variables they are faced with. The challenge undergone in this study was to obtain one environmental variable that would affect two different stocks of Wisconsin Fast Plants in different manners. This would indicate phenotypic plasticity within the different subpopulations. The environmental variable settled upon was wind disturbance. The two subpopulations were chosen due to the differences each stock displays during its lifecycle. Rosette was selected for its small sturdy stature, mimicking a plant that might be seen in an alpine environment. The growth is stunted due to a mutation that restricts gibberellin hormone production. Yellow Green Leaf was selected due to the fact it was a standard (with respect to gibberellin) growing plant, and possesses a mutation that leads to a pale yellow green color. The goal of the study was to search for different reactions to the same environmental stressor.

The effect of gibberellins on cell development in Brassica rapa

Hetue, Jackson[1,2] Stephens, Patrick[1], and Flesher, Tony[1]
[1] Botany 300, Plant Anatomy, Independent Project
[2] Wisconsin Fast Plants Program, student intern

Hetue J, Stephens P, and Flesher T. 2011. The effect of gibberellins on cell development in *Brassica rapa*. via Dept. of Botany, UW-Madison. 2011:0:1-4.

Abstract -Gibberellins are plant hormones that play a role in regulation of plant growth including stem elongation and flowering. The effects of gibberellins on cell elongation and cell division were measured in the model organism, Rapid-Cycling *Brassica rapa*, RCBr. The RCBr stocks, Ein, Rosette, and Standard have excess, deficient, and wild type production of gibberellins, respectively. Hypocotyls of these stocks were sectioned and cells were counted and measured. Gibberellins were found to affect both cell division and cell elongation in the measured stocks.

Inheritance patterns of polycotyledons in Brassica rapa

Pankratz L. 2014. Biology 152, Introductory Biology, Mentored Research Project.

Pankratz L. 2015. Inheritance patterns of polycotyledons in *Brassica rapa*. via Dept. of Biology, UW-Madison. 2015:0:1-16

Abstract - The Wisconsin Fast Plants Program develops programs for in-classroom learning as well as providing live plant material for research on the subjects of plant anatomy, genetics, and evolution. A novel phenotype was observed involving polycotyledony in *Brassica rapa,* the model plant organism used by the Fast Plants Program. However, the inheritance pattern behind polycotyledony in these organisms is unknown and must be more fully understood. In order to investigate the inheritance patterns behind polycotyledony, a drift population was utilized as a control group and was inter-pollinated at random between members of all cotyledon numbers. The frequency of tricots and tetracots in this drift population was compared to the frequencies in the offspring of tricot-tricot pollination and tetracot-tetracot pollination. In this way, certain mechanisms of inheritance could be ruled out and a probable mechanism arose. While our research gave us some insight into the inheritance and development of the polycot phenotype, more research is still needed in order to rule out more possible mechanisms and narrow the list down to the most probable inheritance mechanism. However, our research provides evidence to suggest that polycotyledony is indeed a heritable trait and can be increased with selection.

Troubleshooting and Tips For Growing Rapid-Cycling Brassicas

The *Brassica rapa* Grower's Calendar (Appendix B) outlines the anticipated timing for the life cycle of B. *rapa* that is grown under standard environmental conditions. As noted in the Background Information section, this timing is particularly influenced by temperature—with standard temperature requirements ranging between 65–80°F.

A small measure of attention to tending plants during each stage of growth and development will increase plant vigor and reproductive success. For example, thinning during the first five days after seedlings emerge reduces competition during the first critical phase of stem development. Those plants that remain after thinning develop stockier stems and are better able self-support throughout the plant's life. Learn more about tending Rapid-Cycling *Brassicas* from the Fast Plants website: www.fastplants.org.

The following troubleshooting guide is provided here and also on the Fast Plants website as a resource regarding challenges that occasionally arise when growing Rapid-Cycling *Brassicas* and their potential causes and/or remedies:

Poor Germination (No Seedling Emergence)

- Seeds planted too deeply or seeds washed out during watering.
- Planting mix compacted or too wet.
- Not watered sufficiently after planting.
- Fertilizer pellets were planted instead of seeds (it happens!).
- Room temperature below 15.5˚C (60˚F).
- If seedlings do not appear by Day 4, start over.

Plants Growing Slowly

- Lower temperature in growing area than normal on weekends and holidays.
- Insufficient light. Plants not grown under recommended lighting conditions.
- Plants growing at lower temperature due to location near window in winter.
- Poor capillary action between plants and reservoir.

Plants Look Spindly

- Lights too far away from plants. Plant growing tips should be 5–10 cm from lights.
- Wrong number of fertilizer pellets or seeds placed too close to fertilizer pellets.
- Poor capillary action between plants and water reservoir.

Plants Wilt

- If the worst happens (e.g. you forgot to fill the reservoir) and the plants are wilting but not yet crispy, you may be able to save them.
    - Water the plants gently from above for several minutes.
    - Be sure that the wicks are dripping and the planting mix is moist.
    - If the planting mix has completely dried, it may be difficult to remoisten.

Plants Die

- Wicks not placed correctly in bottom of planting medium.
- Water in reservoir ran out over weekend (always check water on Fridays!).
- Plant damaged during thinning (handle gently).
- Plant damaged during movement (as plants grow taller, stake and secure them with twist ties or a small piece of tape).

No Seed Production

- Pollination not adequately performed.

Too much heat in the growing environment during pollination period. When temperatures are above 29˚C (85˚F), Fast Plants will lose the capability of producing pollen.

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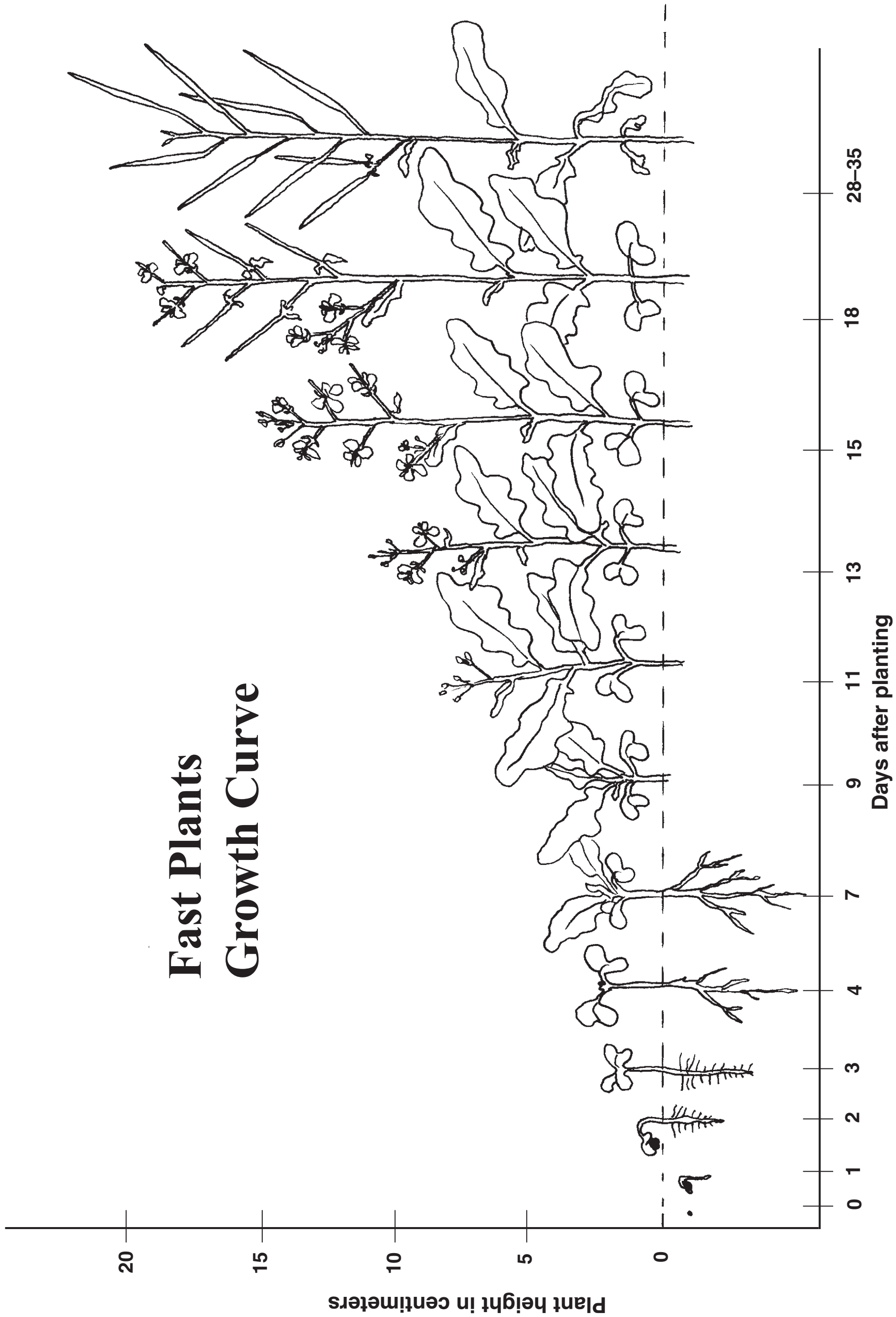
Acknowledgments

The work presented in this paper is a synthesis of research and experiences from the Wisconsin Fast Plants Program and Rapid-Cycling *Brassica* Collection at the University of Wisconsin, Madison. We are guided by and grateful for the many thousands of educators, researchers and student-learners (of all ages) who have experimented and shared ideas freely with our global Rapid-Cycling *Brassica* learning community.

About the Authors

Dan Lauffer has been a researcher and educator with Wisconsin Fast Plants at the University of Wisconsin-Madison since 1991. Paul Williams has been a professor in the Department of Plant Pathology at the University of Wisconsin-Madison since 1962. Hedi Baxter Lauffer has been researching teacher professional development and supporting science education with Wisconsin Fast Plants since 2003. Jackson Hetue first began working with Williams and the Lauffers as an undergraduate researcher in 2012 and has since continued working with the Rapid-Cycling *Brassica* Research Collection at the University of Wisconsin-Madison.

Appendix A


Fast Plants
Growth Curve
Days after planting
0
1
2
3
4
7
9
11
13
15
18
28–35
Plant height in centimeters
0
5
10
15
20

Appendix B
Brassica rapa Grower's Calendar

Day of the life cycle	Activities
Preparations Before Planting	Assemble/Prepare lights. Prepare all planting materials.
Friday **Day 1**	Plants Seeds
Monday-Friday **Days 2-5**	Observe growing plants, and record evidence about growth daily.
Thursday or Friday **Day 4 or 5**	Thin plants according to experimental design/growing system used. Check water levels in the reservoirs before the weekend.
Monday-Friday **Days 8-13**	Observe growing plants, and record evidence about growth daily. Keep checking the water levels in the reservoirs.
Thursday or Friday **Day 12 or 13**	Make beesticks (see Fast Plant website for options). Flower buds begin to open. Check water levels in the reservoirs before the weekend.
Monday-Friday **Days 16-21**	Pollinate when flowers are open for 2-3 days. Observe growing plants, and record evidence about their growth and development. Check water levels in the reservoirs before the weekend!
Monday-Friday **Days 16-21**	Observe seed pod development Continue monitoring water levels.
Day 36	20 days after the last pollination, empty water reservoirs and let plants dry for 5 days minimum.
Day 40	Harvest seeds from dry pods and observe. Plant next generation as needed. Seeds can be stored and kept viable if kept cool and dry (e.g. sealed in a Ziploc bag or Mason jar--ideally with silica gel--kept in the freezer).

Mission, Review Process & Disclaimer

Citing This Article

Lauffer D, Williams P, Lauffer HB, Hetue J. 2018. Preparing Your Students for the Future: Undergraduate Research Experiences with Rapid-Cycling *Brassicas*. Article 11 In: McMahon K, editor. Tested studies for laboratory teaching. Volume 39. Proceedings of the 39th Conference of the Association for Biology Laboratory Education (ABLE). **http://www.ableweb.org/volumes/vol-39/?art=11**